Published in final edited form as:
Chem Rev. 2011 November 9; 111(11): 6603–6782. doi:10.1021/cr100242s.

Artificial receptors for the recognition of phosphorylated molecules

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1. Introduction

Phosphate anions are one of the most important constituents of living systems. Together with heterocyclic bases and sugars, phosphates make up DNA, the hereditary element of living systems.[1,2] In addition, phosphate ions and their derivatives play pivotal roles in energy storage and transduction in biological systems. Protein phosphorylation is a key mechanism for signaling, and phosphorylated proteins are a significant source of ingested phosphate. Furthermore, phosphates are industrially important components of both medicinal drugs and fertilizers. Pollution from phosphate and phosphorylated compounds is in part responsible for the eutrophication of natural water sources, leading to a dangerous increase in toxic algal blooms.[3,4]

Phosphate also has important medicinal implications. Normally, excess phosphate is cleared through the kidneys; however, controlling the blood phosphate levels of patients with kidney failure is a difficult problem for which there is no adequate solution. Hyperphosphatemia, defined as an abnormally high concentration of serum phosphate levels, is a condition that affects nearly all hemodialysis patients.[5,6] The adverse effects of hyperphosphatemia include the development of hyperparathyroidism, soft tissue calcification, cardiovascular complications resulting from the development of metastatic calcifications at the cardiac level, and increased morbidity and mortality.[7-18]

Given the central role phosphate plays in the environment and in biology, it is perhaps not surprising that efforts have been made to achieve the selective binding of phosphate and phosphorylated molecules for almost 100 years. In 1914, Taylor and Miller developed a colorimetric method for the estimation of phosphorous in biological material.[19] This method, now much improved,[20-34] relies on a colored molybdenum(IV) phosphate complex to assay inorganic phosphate. This complex is produced from sodium molybdate and a strong reducing agent and involves a procedure that is often time consuming. It also generates toxic metal waste and suffers from interference. Other traditional assays for phosphorylated molecules have involved derivatization-based protocols[35-44] or biological recognition elements, such as enzymes, that often require special handling or that suffer from poor stability and complicated, costly production procedures.[45-51]

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The investigation of synthetic (abiotic) phosphate receptors seeks to provide improved methodologies for the detection, extraction, and transport of biologically, chemically and environmentally important phosphates. While it has been extensively pursued, the sensitive and selective binding of phosphorylated molecules remains a challenging area of research in supramolecular chemistry. The large size of the phosphate anion and its high hydrophilicity place it near the bottom of the Hofmeister[52] selectivity series, which is based on the Gibbs free energy of hydration. In the case of monobasic anions, for instance, the hydration energies (given in parentheses) increase as follows: ClO_4^- (-205 kJ/mol) > I^- (-275 kJ/mol) > CN^- (-295 kJ/mol) > NO_3^- (-300 kJ/mol) > Br^- (-315 kJ/mol) > Cl^- (-340 kJ/mol) > HSO_4^- (-330 kJ/mol) > HCO_3^- (-335 kJ/mol) > OAc^- (-365 kJ/mol) > > $H_2PO_4^-$ ≈ F^- (-465 kJ/mol).[53] These values increase substantially as the charge of the anion increases. For instance, the hydration energy of PO_4^{3-} is -2765 kJ/mol. As a result, phosphate binding is generally inefficient. The design of phosphate receptors is further complicated by the acid-base properties of phosphate anions. The pK_a values of inorganic phosphate in water are 2.12, 7.20, and 10.9, meaning that two dominant anionic protonation states (monohydrogenphosphate and dihydrogenphosphate) are present at neutral pH.[54] Receptor deprotonation by phosphate anions must also be considered, particularly at hydrogen bond donor sites and in organic media. In addition to the more common anion-hydrogen bond donor interactions, protonated phosphate anions can also interact with hydrogen bond acceptors. This property can be exploited to impart selectivity for inorganic phosphate over other tetrahedral anions such as sulfate that are not protonated near neutral pH; however, the dual donor-acceptor nature of phosphate anions can also lead to anion-anion interactions in less polar media. This behavior has been manifested in some cases through the binding of phosphate aggregates by organic hosts.[55,56] More recently, Gale and co-workers reported anion-anion proton transfer between dihydrogenphosphate species in the presence of a high affinity phosphate receptor in mixed organic / aqueous media.[57]

Given these challenges, a variety of recognition interactions, such as hydrogen bonding, electrostatic interactions, van der Waals forces, π-surface interactions, shape complementarity, and metal coordination, have been employed alone or in concert to generate phosphate receptors.[58-62] Such phosphate receptors have been developed for several different applications, including phosphate ester hydrolysis, ion selective electrodes and membrane transport. The goal of this review is to provide a comprehensive summary of these efforts with an emphasis on the fundamental molecular recognition processes involved rather than the potential or realized applications.

This review will specifically focus on small molecule phosphate receptor systems. Such systems offer several advantages over enzymatic and inorganic-based recognition and sensing protocols. In spite of these advantages, which will be highlighted in the sections that follow, a thorough review of the major progress in this area has not yet appeared. Although several recent works cover general aspects of anion binding and the associated recognition motifs,[54,62-78] only a few reviews have specifically examined synthetic phosphate receptors,[61,79-82] with these examples focusing on selected aspects of phosphate binding. Here, we have attempted to survey synthetic phosphate receptors reported through 2009 in order to codify the most relevant results within a single reference. Efforts have been made to include both advances in sensing methods and progress in the area of binding, providing an up-to-date survey of the basic subunits used to achieve phosphate recognition.

A list of the full names, common names, abbreviations and structures of the commonly targeted phosphates covered in this review is shown in Table 1. Discussions will focus on phosphate and small phosphorylated molecules rather than macromolecules (e.g., DNA or phosphorylated proteins).

2. Detection Methods

The interaction of phosphate and phosphorylated molecules with artificial receptor systems has been monitored using several methods, the choice of which has been largely dependent on the nature of the receptor in question. These methods will be outlined here in order to facilitate the ensuing discussion of receptor design.

2.1. Nuclear Magnetic Resonance

The detection of host-guest interactions by nuclear magnetic resonance (NMR) spectroscopy relies on guest-induced ^{1}H NMR spectroscopic shifts of the synthetic receptor and/or ^{31}P NMR spectroscopic shifts specific for phosphorylated molecules. This method is particularly useful for hydrogen-bonding moieties and can be used to gain both structural and thermodynamic information. For example, the interaction of phosphate anions with amide hydrogen atoms can often be conveniently followed by monitoring changes in the ^{1}H NMR spectrum. The receptor can also be modified with groups such as aromatic moieties whose ^{1}H NMR signals are highly sensitive to changes in the local environment, or which can interact with additional structural elements present in the targeted phosphorylated compounds. These modifications often increase both the sensitivity and specificity of the system. Phosphorous NMR spectroscopic studies can also lend valuable insight into host:phosphate interactions. Such analyses are often straightforward due to the high abundance of the ^{31}P isotope and the high gyromagnetic ratio (17.235 MHz/T) of the phosphorous nucleus. Phosphorous chemical shifts, which are generally referenced to an external phosphoric acid standard, are often changed upon complexation to a host compound. In addition, the individual phosphorous atoms of nucleotide di- or triphosphates display characteristic signals that when monitored allow for the determination of which phosphate units participate most strongly in complexation. In well-behaved systems, the association constants corresponding to complex formation can often be elucidated using standard curve fitting programs, such as the EQNMR software.[83] Stoichiometries can often be deduced from the method of continuous variation (so-called Job plots). Thus, considerable insights can be obtained from NMR spectroscopic analysis. However, it should be noted that the utility of ^{1}H NMR spectroscopic methods is limited by the fact that relatively high concentrations (usually 10^{-2}-10^{-3} M) are often required to obtain well-resolved signals. This leads to rapid saturation of the response during the titration in the case of strong binding. As a consequence, the dynamic range accessible by NMR spectroscopic methods is generally limited to $K \leq 10^{4}$ M^{-1}. Further limitations to these methods occur when the exchange rate is slow. In such instances, it is necessary to integrate the signals for both the bound and unbound forms.

2.2. Optical Methods

Optical methods have several advantages compared to those based on NMR spectroscopy. While much lower concentrations are often required, a change in the spectrum is also needed. To achieve this change, systems containing a combination of substrate-recognition functionality (receptor) and optical-signaling capacity (chromophore) are often prepared. These moieties may be either directly linked or appropriately associated in a noncovalent manner. Such designs permit the detection of substrates via binding-induced changes in the absorption or emission properties. Increasingly, these generalized approaches have been used to effect both qualitative and quantitative analysis. As such, optical methods have been instrumental in the creation of colorimetric sensors (“chemosensors”), as discussed in subsection 2.2.1 below. Other common methods of detection, including fluorescence spectroscopy and isothermal titration calorimetry (ITC), will be described in later sections.

2.2.1. Colorimetric Sensing

2.2.1.1 Covalently Attached Chromophores: As noted above, many non-colored receptors have been functionalized with chromophores to produce covalent frameworks that undergo a pronounced color change when treated with an appropriate guest (Figure 1).[84] These color changes can have their origin in analyte binding-induced changes in the HOMO-LUMO gap or modification in key charge-transfer (CT) bands.[85]

2.2.1.2 Indicator Displacement Assay (IDA): Indicator displacement assay (IDA) is a competition method for the sensing of analytes (Figure 2).[86] The molecular ensemble employed consists of a recognition unit designed for selective interaction with a desired analyte along with an external indicator that associates with the recognition unit in the absence of the analyte. When the analyte is added, the indicator is displaced from the binding cavity, producing a measurable change in the optical properties of the indicator. This method exhibits several useful features. For example, it can be applied to a variety of receptors without the need for covalent attachment of a reporter group. The use of noncovalent indicators makes each system amenable to both fluorescence and UV-Vis spectrophotometry following the selection of appropriate indicators. Furthermore, the use of indicators with varying association constants allows tuning of the system to analytes with a range of binding affinities. Finally, several indicators may be used with the same host-guest system as a means of corroborating results.

2.2.2. Fluorescence Sensing—Fluorescence spectroscopy is an attractive analytical method due largely to its high sensitivity and submillisecond temporal resolution.[87,88] Reported fluorescence anion sensors have utilized competitive binding,[86] photo-induced electron transfer (PET),[89-91] electronic energy transfer (EET),[92] metal-to-ligand charge transfer (MLCT),[93] excimer/exciplex formation,[94,95] internal charge transfer (ICT),[96] and, less frequently, excited-state proton transfer (ESPT).[97,98] Many of the structural features that modulate fluorescence efficiency have been determined, including double-bond torsion, low energy n-π^* levels, “heavy” atoms, weak bonds, and the availability of subunits that allow for PET or EET.[87] As a result, many opportunities exist for modulating structural features at the molecular level in order to produce receptors that allow emission spectroscopic properties to be exploited in an analytically useful way.

2.3. Electrochemical Redox Activity

2.3.1. Cyclic Voltammetry (CV)—Another method for detecting the interaction between a receptor and a substrate involves monitoring changes in the redox properties of the system. Often the receptors themselves are not redox active, at least not within a useful electrochemical window. Therefore, redox-active groups (cobaltocenium, ruthenium(II) bipyridyl groups, ferrocenyl moieties, *etc.*) are often attached to receptors near the substrate binding sites.[99] Analyte binding then shifts the redox potential of the reporter group. For example, in the presence of an anion, the voltammetric behavior of a metallocenium moiety is shifted toward that of the corresponding metallocene. This cathodic perturbation is rationalized in terms of anion-induced stabilization of the positively charged metallocenium moiety relative to the metallocene form. However, often a poor correlation exists between the electroactive changes and the actual binding affinity; i.e., the largest cathodic perturbation does not imply the strongest association. This is because the redox changes include contributions from at least two oxidation states, only one of which reflects binding to the parent form of the receptor.

2.3.2 Ion Selective Electrodes (ISEs)—A variety of anion-selective electrodes have been introduced based on recent advances in the host-guest chemistry of anions.[100-110] While significant progress has been made, it remains a challenge to obtain systems that are

selective for the strongly hydrophilic phosphate species. This reflects in large measure the unfavorable standard free enthalpies of transfer of phosphate anions from aqueous milieus to ISE membranes. This energetic cost, which again reflects the Hofmeister bias, needs to be overcome by selective complexation. While workable phosphate-selective membranes can be achieved by using homogenously distributed ionophores, such as organotin, organovanadyl, polyamine, or guanidinium-based cations, in the polymer membrane, the resulting systems often suffer from poor stability or low detection limits.[111]

2.4. Isothermal Titration Calorimetry

In recent years, isothermal titration calorimetry (ITC) has emerged as one of the more powerful methods for studying anion-receptor interactions. ITC is especially attractive since, in well-behaved cases, it provides ready access to the individual thermodynamic parameters corresponding to the proposed binding interactions (i.e. ΔG, ΔH, and ΔS). Although temperature-dependent studies (e.g., NMR, UV-Vis) can be used to derive these same thermodynamic parameters, such analyses are generally laborious, insensitive, and error-prone. ITC, on the other hand, allows the dissection of the free energy of association into its individual enthalpic and entropic components via a measurement carried out at a single temperature. However, ITC does not provide direct insights into the underlying chemistry (in contrast to, e.g., NMR spectroscopy) and requires fitting the data to a presupposed binding model. In other words, because calorimetric measurements reflect all processes occurring in solution, it is important to find and study simple systems whose host-guest interactions can then be extrapolated to more complex systems. Even then, great care needs to be exercised lest an incorrect assessment of the underlying chemistry be made.

3. Phosphate Recognition in Nature

A number of design principles for selective synthetic phosphate receptors can be derived from an examination of naturally occurring phosphate receptors.[54,80] Over half of all proteins in living systems are thought to bind phosphorylated guests, particularly protein kinases and phosphatases, which regulate a large range of inter- and intracellular signaling processes. Analysis of phosphate binding sites has been made possible from a number of X-ray diffraction structural studies. For example, Quiocho and co-workers have examined a periplasmic phosphate binding protein that binds inorganic phosphate with high affinity and specificity over sulfate (Figure 3).[112,113] Both dihydrogenphosphate and monohydrogenphosphate were found to be bound through an extensive hydrogen bonding network as well as through the formation of a salt-bridge with the guanidinium side chain of an arginine residue (Arg 135). The specificity of this protein against sulfate was attributed to a short hydrogen bond between the protonated oxygen of the phosphate guest and the carboxylate side chain of an aspartate residue (Asp 137) within the binding pocket.[114,115] Specifically, it has been suggested that other anionic guests, such as sulfate, would be not be able to participate in this interaction and would thus be repulsed based on charge-charge interactions. The mutation of the Arg 135 and Asp 137 residues led to decreased selectivity but little change in the binding affinity of this protein.[116] It was thus proposed that the strength of phosphate:protein binding was dominated by hydrogen bonding or local dipolar interactions.[117]

Diederich and co-workers recently examined the Protein Data Bank for structural elements common to proteins that bind inorganic phosphate and other naturally occurring phosphorylated molecules.[80] Over 3000 phosphate-binding protein structures were identified, and several trends were reported. For example, nearly all protein binding sites contained a large number of glycine residues located in loop motifs that allowed the phosphate guest to be encircled by a number of electrostatic or hydrogen bonding groups. This recognition element was compared to the encapsulation of a phosphate guest by

flexible synthetic macrocycles. Hydrogen bonding interactions between the anion and the amide backbone or polar residues were also common. In fact, nearly one third of the studied protein structures relied solely on these interactions (i.e. without assistance from metal chelation or electrostatic interactions). Of the remaining structures, approximately 50% utilized electrostatic interactions with basic lysine or arginine residues, and approximately 20% of the studied proteins incorporated metal chelation interactions. Furthermore, cationic charges were found largely on the exterior of the binding pocket, while neutral polar residues predominated the interior of the cavity. It can thus be concluded that encapsulation, hydrogen bonding, and electrostatic interactions are critical for successful phosphate recognition in nature. As will be seen in the ensuing discussion, these themes are also common among synthetic phosphate receptors.

4. Major Phosphate-Binding Functionalities

In this section, the most common binding subunits employed to create phosphate selective receptors are introduced with the aim of providing a general overview of the motifs that are emanating from this area of research. While we have attempted to organize receptors according to their main binding moiety, it should be noted that many recent receptors combine multiple recognition subunits to increase functionality in complex systems, which makes their classification less than straightforward. It is also important to note that the association constants discussed in this review are taken directly from the referenced reports, and the accuracy of these values will depend heavily upon the detection method and mathematical model employed. While a thorough discussion of these limitations is beyond the scope of this manuscript, we encourage the reader to evaluate critically the methods used for binding constant determination in papers of particular interest.

4.1. Charge-Charge Interactions

4.1.1. Polyammonium Systems—The first synthetic receptors for phosphate to be described in the literature were polyammonium cations. Such systems, first reported over 30 years ago, owe their efficacy in large measure to strong electrostatic interactions generated between the negatively charged phosphates and the protonated polyammonium systems near neutral pH. Nevertheless, it is important to appreciate that systems containing closely spaced nitrogen atoms are not always fully protonated at pH 7. The protonation constants are often greatly reduced, presumably due to charge-charge repulsion. Such effects may explain why amine nitrogen atoms are often separated by 3 or 4 methylene units in naturally occurring systems, such as spermine and spermidine; presumably this spacing ensures maximum protonation near neutral pH. A careful determination of the degree of protonation for a given polyammonium receptor is critical to a discussion of its binding efficacy. Generally, this is accomplished through careful pH titrations. Such titrations are also used to determine binding constants, since the effective pK_a of an ammonium site generally shifts in the presence of a bound anion.

In addition to the important role of electrostatic interactions, amine hydrogen bonding can also affect complex stability and selectivity.[118] Other relevant factors include macrocycle formation, macrocycle size, nitrogen methylation and metal coordination (a factor discussed in greater detail in Section 4.3). The addition of other receptor moieties, such as aromatic rings and amide groups, can further increase selectivity between various phosphorylated species.

Interest in polyammoniums and their analogs as phosphate binding systems first arose from the discovery that biologically important acyclic polyamines, such as putrescine (**1**), spermidine (**2**), and spermine (**3**), bound 5′-adenosine monophosphate (AMP), 5′-adenosine diphosphate (ADP), and 5′-adenosine triphosphate (ATP).[119] Early ^{1}H and ^{13}C NMR

spectroscopic studies of the spermidine (**2**):5′-AMP complex supported a binding mode in which the amine ligand interacted with both the anionic phosphate group and the adenine base.[120] In studies of the interaction of spermine (**3**) with AMP, ADP, and ATP, potentiometric titrations and ^{1}H, ^{13}C, and ^{31}P NMR spectroscopic experiments led to the conclusion that hydrogen bonding interactions between the host and the adenine ring of the nucleotides were critical to complex formation.[121,122] On the other hand, similar studies with putrescine (**1**) and 5′-uridine monophosphate (UMP) revealed interactions only with the phosphate group of the nucleotide.[123] The apparent affinity constants for the interaction between these three naturally occurring polyammonium cations and 5-phosphoribosyl-1–pyrophosphate and 2,3-diphosphoglyceric acid (2,3-DPG) were determined using anion affinity chromatography.[124] Values for K_1 ranged from 300 to 2700 M^{-1} and increased as the number of amine units increased. Spermidine (**2**) and spermine (**3**) were also shown to bind pyrophosphate with association constants of 6.4×10^2 M^{-1} and 2.7×10^3 M^{-1}, respectively, at pH 7.5, as inferred from resin competition experiments.[125] Calorimetric studies yielded standard Gibbs energies of formation of -15.4 and -20.0 kJ/mol, respectively, for the complex formed between amines **2** and **3** and tetra-anionic pyrophosphate at this pH.[126] Single crystal X-ray diffraction analysis of the **3**:pyrophosphate complex revealed extensive hydrogen bonding interactions between the NH units of the host and the oxygen atoms of the guest.[127]

Felcman and co-workers later compared the ATP binding ability of spermidine (**2**) with other small linear polyammonium receptors (**4** - **6**).[128] In potentiometric titrations, the diprotonated derivatives of these receptors were found to bind ATP in the order **6** > **2** > **5** ≈ **4**. The stability constants were found to correlate with the number of nitrogen atoms available for electrostatic and hydrogen bonding interactions. These receptors were also found to form ternary complexes with ATP and copper(II), but these complexes were generally less stable than the binary receptor:ATP complexes.

In the early 1980's, the Kimura and Lehn research groups reported monocyclic polyamines, such as **7** - **11**, that interact with biologically important polyanions, such as inorganic phosphate, AMP, ADP, and ATP, in aqueous media and at neutral pH.[129,130] A wide range of macrocyclic amine receptors were used for these and later studies. Many contain regularly spaced nitrogen atoms. These will hereafter be referred to as [*n*]aneN$_m$, where *n* represents the number of atoms in the ring and *m* the number of nitrogen atoms (e.g., **8** can be represented as [15]aneN$_5$). In the Kimura systems, polarographic methods and ^{1}H NMR spectroscopic shift measurements were used to provide support for a 1:l host:guest stoichiometry for receptors **7-11** and the polyanionic forms of several phosphates.[129] In such cases, phosphate complex formation was found to be governed mainly by electrostatic forces. Thus, more negative nucleotides were bound more strongly by the protonated forms of these polyamine receptors. For instance, the stability sequence was found to be ATP^{4-} > ADP^{3-} > AMP^{2-} for systems **7-11**, with the corresponding association constants falling in the range of 10^2 - 10^6 M^{-1}. The strongest association occurred between ATP and receptor **7**, the only macrocycle that was found to bear four positive charges at neutral pH. Smaller polyamine macrocycles containing only two protonated nitrogen atoms at neutral pH did not bind appreciably to these test phosphates. Despite the fact that AMP and inorganic phosphate bear the same negative charge at pH 7, AMP was found to form complexes with **9** and **11** ([18]aneN$_6$) that were approximately 10-100-fold more stable than those formed with inorganic phosphate. The added stability was thought to be due to an additional interaction involving the adenine base. Proton NMR spectroscopic shifts were observed that are consistent with the base bending back to complex the macrocycle, lending support to this hypothesis.

Receptors **9** and **11** were also found to solubilize $Ca_3(PO_4)_2$, $Ca(C_2O_4)$, and human calculi in acidic solution, presumably as a result of their anion chelating properties.[131] At pH 4.4, polyamine **11** was found to dissolve calculi better than EDTA, a system commonly used to treat calculi. Alkyl functionalization of **11** led to receptor **12**, which was found to dissolve $Ca_3(PO_4)_2$ more effectively than EDTA or **11**, displaying optimum activity at pH 7.0.[132] In a separate study, macrocycle **9** was functionalized with a lengthy alkyl chain (**13**) to give a derivative that was then used as the active component of an ATP selective electrode.[133] The electrode obtained in this case was found to function from pH 3.0 to 7.0, with a dynamic range of 10^{-7} to 10^{-3} M.

Compounds **14-16** were introduced and studied by Lehn using pH-metric methods. These measurements revealed that the fully protonated forms of these compounds formed stable complexes with the nucleotide anions AMP, ADP, and ATP. Log stability constants (log K_S) were found to range from 3.4 to 9.1.[130] As with Kimura's systems (i.e. compounds **7-11** discussed above), the stability of the complexes formed by a given receptor increased with the charge of the anion. Likewise, for anions of a given charge, the ion-receptor complex stability was found to increase as the degree of protonation (and hence the positive charge) of the receptor increased. Receptors **14** - **16** were studied in their fully protonated forms. In addition to 1:1 complexes, the hexaprotonated receptor **14** ([24]aneN_6) was found to form 1:2 host:guest complexes with ADP, and ATP. Likewise, the octaprotonated system **15** ([32]aneN_8) was found to form 1:2 complexes with AMP, ADP, and ATP. Although receptors **14** and **16** both contain six protonated nitrogen atoms in their fully protonated forms, the ether-derivative **16** was generally found to bind nucleotide substrates more effectively than compound **14**. This finding was attributed to the higher local charge density permitted by the ethylene spacers as compared to the corresponding propylene systems. Later on, ^{31}P NMR spectroscopic studies were used to probe further these stoichiometries.[134-136] Interestingly, these studies revealed that the [18]aneN_6 (**11**) and the acyclic polyamine **17** formed mixtures of 1:1 and 1:2 host:guest complexes when treated with ATP near neutral pH. In contrast, the [24]aneN_6 macrocycle (**14**) was found to form 1:1 complexes with ATP and ADP near neutral pH, while the [32]aneN_8 (**15**) formed 1:2 complexes. The oxygen-containing macrocycle **18** (commonly named OBISDIEN) was found to form stable 1:1 complexes with AMP, ADP, ATP, and pyrophosphate. The corresponding log K_s values ranged from 2.85 to 11.00 as inferred from potentiometric measurements. The anion binding properties of OBISIDEN have also been extensively studied in the case of its metal complexes, as discussed in Section 4.3. Based on this early work, both Lehn and Kimura suggested a “macrocyclic effect on anion binding,” a conclusion supported by the fact that macrocyclic analogues of naturally occurring polyamines **1** - **3** were observed to bind anions one to two orders of magnitude more strongly than the linear systems.

Hosseini expanded the chemistry of these systems by covalently attaching receptor **18** to polystyrene beads.[137] The resulting solid-supported systems were found to interact with fluorescent N^6-ethenoadenosine-5′-diphosphate (εADP) and N^6-ethenoadenosine-5′-triphosphate (εATP) in solution at pH 4 where the hexamine is fully protonated. At pH 11 the receptor was no longer protonated and released the guests. The best guest interaction was achieved between 1-3 minutes after polymer immersion. In addition, the expected preference for ATP was observed, as inferred from competitive experiments.

In addition to the biologically important analytes discussed above, these simple macrocycles were also found to bind nicotinamide adenine dinucleotide in its oxidized form (NAD) and the oxidized form of nicotinamide adenine dinucleotide phosphate (NADP).[138] In the case of tetraprotonated [21]aneN_7 (**19**), potentiometric studies revealed log K_s values of 4.27 and 4.75 for these two substrates, respectively. While the differing basicities of NAD and NADP

complicated the analyses, competition studies showed that the NADP:**19** complex was indeed more stable over a wide pH range. Molecular dynamic simulations involving the two complexes in question gave rise to a minimum energy structure in which the NADP guest is bent, presumably to allow strong electrostatic interactions between all three phosphate groups and the four charged nitrogen atoms as well as to permit the formation of 11 hydrogen bonds.

Wilson and Williams examined the phosphate binding affinities of both acyclic triethylene tetraamine (trien, **20**) and its cyclic variant [12]aneN$_4$ (**21**) using ^{31}P NMR spectroscopy.[139] The linear trien was found to bind pyrophosphate, triphosphate, ADP, ATP, and hexametaphosphate (HMP) in D_2O over a range of pH values. The highest association constants were found in the case of trien interacting with HMP, giving a log *K* value near 6 when triprotonated at pH 8.0. A similar value (within error) was found for the cyclic [12]aneN$_4$ (**21**) and HMP, though at a pH (8.5) where the receptor was only monoprotonated. These results provide yet additional support for the conclusion that significantly higher association constants are seen for ring systems as compared to linear systems. It was also found that polyammonium receptors must contain four protonated nitrogen atoms in order to compete with Mg^{+2} and Ca^{+2} ions for phosphate complexation in biological systems.

A closer look at the protonation states of polyamine receptors revealed important guidelines for obtaining an optimal charge density at neutral pH.[140-144] These guidelines reflect several key structure-function features of polyamines. In particular, the protonation constants for the macrocyclic systems were generally lower than those of linear systems, presumably due to higher charge density. Similarly, smaller, conformationally constrained macrocycles often had lower pK_a's than larger rings. While ethylene spacing gave higher charge density than propylene spacing, pK_a's for adjacent groups between ammonium residues were often far below 7 (ranging from, e.g., <1 in the triamine macrocycle, [9]aneN$_3$, to 4.09 in [18]aneN$_6$ (**11**)).[141] This effect has the consequence that a full supplement of positive charges is only obtained at a lower pH. Fortunately, it can be mitigated somewhat by separating individual ethylenediamine units by non-basic spacers, as in for instance hexamine **16**. In this latter system, where ether oxygen atoms “replace” an NH moiety, the final three protonation constants were 6.80, 5.65, and 5.55.[140] The use of propylene spaced systems also served to alleviate much of this problem; in this case the amine sites displayed higher pK_a's (i.e., between 6-7).

At a given protonation state, the effects of differences in charge density can be significant. A binding study of a series of larger ethylene spaced rings, such as [18]aneN$_6$ (**11**), with ATP, inorganic phosphate and pyrophosphate revealed that, for a given protonation state, smaller macrocycles generally bound anions more strongly.[144] For example, both [21]aneN$_7$ (**19**) and [24]aneN$_8$ (**22**) were tetraprotonated at neutral pH, but in this state the former bound ATP with a log K_s of 4.54 while the latter displayed a log K_s of 3.74. This effect was attributed to the reduced charge density present in the larger rings systems.

Electrostatic analyses and structural studies involving simple polyamine receptors opened the door for the synthesis of what may be considered as tunable polyamine systems. For example, it was found that the basicities of simple polyammonium systems could be modulated through nitrogen methylation, a modification that generally served to lower the protonation constants of polyammonium macrocycles.[142,145,146] Methylation also led to charge localization, especially in partially methylated polyammonium receptors, such as **23-25**. In macrocycle **23**, for example, NMR spectroscopic studies revealed that the triprotonated species contained protons on alternating nitrogen atoms (one tertiary and two secondary).[146] In the corresponding tetraprotonated species, four protons are present on

secondary nitrogen atoms, which leads to significant charge localization. In the case of macrocycle **24**, all protons of the triprotonated species were found to be located on secondary nitrogen atoms. Because of the symmetry of the compound, this selective protonation served to divide effectively the macrocycle. Crystal structure analysis of several complexes between pyrophosphate and the macrocycles **23** and [18]aneN_6 (**11**) revealed these two receptors to be nearly planar in the solid state, with **23** adopting an almost elliptical shape.[147]

Methylation patterns can also affect the anion binding properties of polyammonium receptors. In preliminary competition studies, the tetramethylated macrocycle **25** was found to bind to ATP more strongly than the corresponding unsubstituted [18]aneN_6 (**11**).[148] Further studies revealed that the methylation pattern on **23** led to stronger ATP binding than is seen in the case of **24, 25**, [21]aneN_7 (**19**), and [18]aneN_6 (**11**), at least above pH 5.3.[146] Macrocycle **24** was found to be the weakest receptor. Similar trends were observed for the interactions of **23** and **24** with AMP, ADP, and pyrophosphate.[149] The strong binding of tetraprotonated **23** was attributed to the localization of the cationic charge on the secondary nitrogen atoms, where as the charge was found to be delocalized around the ring in unsubstituted [3*k*]aneN_k systems.

In an effort to explore the properties of more rigid systems, the protonation and phosphate binding behavior of methylated macrocycles **26** and **27** were analyzed. Receptors **26** and **27** contain only tertiary nitrogen atoms. Thus, it is of interest that in **26** protonation occurs first at the four benzylic nitrogen atoms, followed by the methylamine nitrogen atom between the piperazine rings.[150,151] A similar pattern of protonation, made simpler by its symmetry, was observed in the case of **27**. This latter system was found to interact with ATP and ADP as inferred from potentiometric and NMR spectroscopic methods.[151] The NMR spectroscopic studies led to the suggestion that the main driving force for the formation of ATP and ADP complexes with **27** was electrostatic in nature, with the contribution from hydrogen bonding and π-surface interactions being minimal. An examination of structures from single X-ray diffraction analysis led to the suggestion that the conformational requirements of the piperazine rings in **26** act to orient the nitrogen atoms on opposite sides of the ring, thus decreasing the effective local charge. NMR spectroscopic studies of the substituted macrocycles **23, 24, 25**, and **27** as well as [21]aneN_7 (**19**) and [18]aneN_6 (**11**) provided support for the notion that, while all macrocycles in the series bind inorganic phosphate and pyrophosphate in a 1:1 manner, no significant redistribution of charge takes place upon binding of the anion.[147] Interestingly, these studies led to the conclusion that the charges in unsubstituted macrocycles remained delocalized on the NMR time scale when bound to these test anions. No obvious trends in stability were observed for the binding of inorganic phosphate and pyrophosphate in this series of macrocycles.

In a study that can be considered as a variation of investigations involving the alkylated polyammonium macrocycles discussed above, the anion binding properties of receptor **28**, containing aminoethyl groups as substituents, were analyzed.[152] This receptor was found to be tetraprotonated near neutral pH, with NMR spectroscopic studies leading to the inference that the first four protonation events involve the primary amines. Potentiometric studies provided support for the conclusion that ATP, $P_2O_7^{4-}$, $[Fe(CN)_6]^{4-}$, and $[Co(CN)_6]^{3-}$ are bound to this receptor, with the strongest anion-receptor interactions being present at pH 6. Under these latter conditions, the 1:1 H_6(**28**) $^{6+}$:ATP complex was considered to be the predominate species in solution. Comparisons of this macrocycle to [24]aneN_8 (**22**), which would also be tetraprotonated near neutral pH, revealed that **28** is a less effective anion binding agent at an equivalent protonation state. This difference was attributed to the effective higher charge density of [24]aneN_8 (**22**) compared to **28**.

To avoid the complexities associated with protonation, Bianchi and co-worker prepared a series of quaternary ammonium receptors. These systems are a departure from earlier receptors in that the net charge was pH independent. However, the nitrogen atoms, being quaternized, were no longer able to participate in hydrogen bonds. Despite its high charge density, macrocycle **29** was found to have no appreciable interaction with ATP, as inferred from potentiometric measurements.[153] These results were consistent with the hypothesis that the hydrogen bonding ability of the protonated 2° and 3° amine moieties played a major role in regulating the interactions of these receptors with ATP and like species. On the other hand, it proved difficult to make a direct comparison between **29** and various protonated ammonium receptors. For instance, it was noted that [12]aneN$_4$ (**21**) did not to interact with ATP, even though it is of similar ring size to **29**; however, receptor [12]aneN$_4$ (**21**) was also found to be only doubly protonated near neutral pH.[129] It thus makes a poor reference. In contrast, tetraamine **30** was found to be tetraprotonated near neutral pH and did interact with ATP when studied in aqueous media.[153] However, the larger ring size of **30** compared to **29** could be responsible for the enhancements in anion binding. Thus, it too represents a poor reference for **29**.

In the case of the quaternary ammonium cage compounds of general structure **31**, effective binding with phosphate and nucleotides was achieved.[154-156] Partial inclusion complexes with 1:1 stoichiometry were also inferred for inorganic phosphate, glucose-1-phosphate, glucose-6-phosphate, AMP, ATP, and NAD through CPK modeling.[156,157] Dissociation constants with **31a** were measured potentiometrically via bromide ion displacement, giving log K_D values ranging from 2.0 to 2.5. Dissociation constants for **31b** were measured spectroscopically via displacement of 2,4-dinitrophenolate, giving log K_D values from 0 to 1.4. Both receptors exhibited little selectivity among the phosphates. The lower values observed for the larger receptor (**31b**) were attributed to the greater distance between the positively charged ammonium centers. This greater distance is expected to lead to a reduced positive electrostatic potential within the cavity. In general, the increased rigidity and lack of hydrogen bonding interactions in the methylated polyammonium systems dominate over the effect of charge density. Although the latter is increased through methylation, the net effect is a reduction in stability and selectivity.

Related to the problem of developing effective quaternary polyammonium receptors is the application of tetraquaternary 1,4-diaza[2.2.2]-bicyclooctane (DABCO) derivatives (**32-40**) as nucleotide phase transfer agents. Compounds **32-34** bear lipophilic alkyl chains that were meant to impart phase transfer properties, leading in some cases to systems capable of effective transport of nucleotides from an aqueous phase into an organic phase. Early efforts in this area were carried out by Tabushi, et al., who found that attaching stearyl chains to DABCO gave rise to a system (**32**) that allowed transport of AMP and ADP from aqueous solutions into chloroform with efficiencies that were much improved over traditional phase transfer and micellar reagents (trioctylmethyl ammonium chloride and stearyltrimethylammonium chloride, respectively).[158] Transport across a chloroform membrane was up to 40-fold faster for ADP than AMP and could be driven by both pH and salt (NaBr) gradients.[159] While ATP was extracted to the greatest extent at equilibrium, transport of ADP occurred at the fastest rate.[160] The transport rate of ATP only matched the level of ADP in the presence of a coordinating cation (Na^+ or K^+), presumably due to the creation of a neutral species such as would be expected from **32**:ADP. Similar trends in exchange efficiencies were observed for guanosine and uridine phosphates; however, transportation rates were much slower for uridine and cytidine phosphates as compared to the corresponding adenine systems.

Diederich, et al. investigated the structural optimization of DABCO carriers for the transport of dideoxynucleotide triphosphates (3′-azido-2′-deoxythymidine triphosphate (AZTTP) and

dideoxythymidine 5′-triphosphate (ddTTP)). This was done by preparing and studying compounds **33-40**.[161,162] Transport studies were conducted in a standard U-tube cell with a chloroform liquid membrane. Receptor **33** proved to be insoluble in both organic and aqueous media, while receptor **34** was found to leak into the ATP-containing aqueous phase.[161] In both cases, transport was precluded. On the other hand, the branched compound **35** proved to be a highly effective carrier for ATP, 5′-cytidine triphosphate (CTP), ddTTP, and AZTTP, even when compared to the original system, **32**. Interestingly, compound **36** was found to be effective only for ddTTP under the conditions studied. Careful extraction studies suggested a 2:1 **35**:ATP binding stoichiometry, perhaps indicating a need for cooperation among four ammonium centers to effect nucleotide triphosphate transport. In line with this latter argument, receptors **34, 37, 38**, and **39** each were found to form 1:1 complexes with ATP.[162] However, only **39** gave rise to complexes that were sufficiently soluble in the organic layer to allow for transport. Even then, the transport rate proved to be an order of magnitude lower than that observed in the case of receptor **35**. In liposomal studies, carriers **34, 35**, and **39** largely acted as detergents. Specifically, these receptors were found to break up the liposomal structure, thus giving rise to nonspecific leakage from the liposomal interior. As part of a separate study, DABCO-based cyclophanes (**40)** were synthesized. This set of receptors was then analyzed in an attempt to correlate the intracavity encapsulation ability with receptor size.[163,164] In this case, a 1:1 binding stoichiometry was established for ATP with log *K*'s near 4.1 - 4.2 for all cavities. Such a finding is consistent with the fact that no evidence was found for encapsulation of the guest.

Smith, Duggan, and co-workers examined the effect of adding a boronic acid-based sugar-binding carrier (**41**) to the membrane transport system.[165] The boronic acid moiety present in **41** is known to interact only with *cis*-diols. In accord with expectations, the transport of ribonucleoside-5′-monophosphates (AMP, 5′-guanosine monophosphate (GMP), and UMP) was facilitated when **41** was used in combination with ammonium-based carrier **34** as compared to when transport was carried out using **34** alone. Carrier **41** did not effect transport when used on its own. Nor did it effect the transport of 2′-deoxyribonucleoside-5′-monophosphates when used in conjunction with **34**. The combination of **34** and **41** was found to transport 5′-GMP roughly 10-fold more effectively than either 3′-GMP or 2′-GMP. This study remains historically important because it highlights the benefit of combining multiple functional groups to tune selectivity and improve efficacy. Both are a recurring theme in supramolecular chemistry.

Direct attachment of ancillary binding motifs to polyammonium receptors has frequently been employed in attempts to both increase binding affinities and impart selectivity among other anions and within phosphate derivatives. Kimura and co-workers first incorporated amide groups, which are known to participate in hydrogen bonding, into polyamine macrocycles. Among the systems these researchers prepared are the diamides **42**. While macrocycle **42a** is only monoprotonated at pH 7.5, it was found to bind AMP, ADP, and ATP with affinities on the same order of magnitude as the naturally occurring polyamines **2** and **3**. Compound **42a** was also found to display a slight selectivity for AMP.[129] Derivatives **42b** and **42c**, however, did not interact with the phosphates studied, likely due to steric interference. In later studies by Carrey and Riggan, the N_3-cyclic amine **43** was prepared and found to be an effective ionophore for dibasic phosphate when employed in an ion-selective electrode. It proved more effective than other cyclic amine derivatives (i.e. the corresponding N_4, N_5, and N_6 analogues).[166] The electrode based on **43** exhibited submicromolar sensitivity and high selectivity. This finding was attributed to appropriate size and charge matching between the N_3-cyclic amine ionophore and the HPO_4^{2-} ions. Later, Ebdon and co-workers created an electrode with better stability and robustness by covalently linking ionophore **43** to the electrode.[167] The resulting electrode displayed a higher stability while exhibiting similar selectivity toward HPO_4^{2-}. This electrode operated

from pH 6 to 8 over a working range from 3.9×10^{-3} to 1.0×10^{-6} M with a detection limit of 1.0×10^{-6} M.

The acyclic and cyclic receptors **44-46**, which also combine amide and amine groups within their structure, were reported by Smith and co-workers.[168] Based on potentiometric titrations carried out in aqueous media, receptors **44-46** were found to have a high affinity for phosphate anions (i.e. log $K > 5$). In addition, these receptors were found to interact with inorganic phosphate anions more strongly than with organic phosphate anions. This preference was attributed to the higher charge density and the smaller size of the inorganic phosphate analytes relative to the organic congeners.

The functionalized chiral polyamines **47** and **48** were prepared by the Burrows group, who studied their interactions with ATP.[169,170] The triammonium macrocycle **47** was prepared to test whether a tripodal arrangement in a fully protonated macrocycle would provide a geometry suitably disposed to bind a trigonal oxyanion. However, analysis of the binding abilities of **47** and **48** revealed that tetraprotonated receptor **48** was more effective. Such observations further underscore the importance of charge-charge interactions in mediating the anion binding behavior of polyammonium receptors.

The Sessler group examined the combination of hydrogen bonding between complementary base pairs and the electrostatic interactions of polyammonium groups in an effort to enhance the affinity and selectivity of nucleotide recognition. This led to the synthesis of receptors **49-51**.[171,172] Their interactions with GMP were studied by ^{1}H NMR spectroscopy in DMSO-d_6 with the goal of analyzing both the strength of complexation and the relative contributions of each subunit.[171] As expected, the alkyl receptor **49d** formed 1:1 complexes of relatively low stability (i.e., with K values comparable to those displayed by simple guanine-cytosine base-pairs). On the other hand, the amine-containing receptors **49a-c**, ditopic systems ostensibly capable of stabilizing both base pairing and electrostatic interactions, displayed higher affinities than **49d**. In this latter case, ^{1}H NMR chemical shifts were consistent with the conclusion that the additional nitrogen site also served to strengthen the hydrogen bonding interactions between the guanine and phosphate moieties of GMP. Receptors **49** also displayed 2:1 host:guest binding stoichiometries, presumably as the result of amines from two different receptors binding to a single dibasic GMP. The binding constants increased with increasing alkyl chain length, with **49c** displaying association constants of 1300 M^{-1} (K_1) and 1200 M^{-1} (K_2). By comparing these values to that for triethylamine, which would interact with GMP only through electrostatic interactions, the contribution of hydrogen bonding effects to the overall affinity was estimated to be approximately 2.5-fold. A much stronger first association constant was observed for **50** and GMP as compared to **49c**, with the K for **50** (2.60×10^3 M^{-1}) being 20-fold higher than the first association constant and 17-fold greater than the second association constant for **49c**. Analysis of the NMR chemical shifts led to the suggestion that the outer two nitrogen atoms of **50** chelate the phosphate group of GMP while the base pair participates in hydrogen bonding. Comparisons with *N,N*-tetramethylbutyldiamine revealed that the enhancement afforded by hydrogen bonding (2.7-fold) was similar to that observed in the case of **49c**. Furthermore, substitution with long alkyl chains produced **51**, which was then used to generate an ion-selective electrode.[172,173] While little selectivity for phosphate or GMP was observed relative to other anions, the electrode was highly selective for guanosine nucleotides (5′-guanosine triphosphate (GTP) and 5′-GMP) over adenosine nucleotides (ATP, AMP) of the same charge. An electrode using a monotopic alkyl cytosine host (**52**) was found to have no response to nucleotides, while azamacrocycle **13** displayed no selectivity between nucleotide bases. This finding emphasizes the importance of using more than one binding mode. Consistent with this conclusion was the observation that selectivity

for GMP over AMP could be achieved when receptor **53** was used in combination with either **54a** or **54b**.

The introduction of aromatic moieties into polyammonium macrocycles gives rise to structures with increased rigidity relative to aliphatic analogues. It also produces systems capable of supporting charge-dipole, π-surface, and hydrophobic interactions, features that have proved particularly important for nucleotide recognition. One early system reported was the pyridine macrocycle **55**, which was investigated by Kimura.[129] Receptor **55** is doubly protonated near neutral pH and was found to bind AMP with moderate selectivity over ADP and ATP. However, the overall stability constants proved to be significantly lower than analogous simpler polyammonium macrocycles (log $K_S \approx 2.2$-2.5).

The related 1,4-benzo macrocycle **56** was shown to be triprotonated near pH 7 and to bind ATP and pyrophosphate with log K_S values of 2.58 and 3.32, respectively.[174] A comparison between **56** and the larger systems **57** and **58** revealed similar binding constants, with **57** showing the highest affinity for inorganic phosphate and **58** showing the highest affinity for pyrophosphate (log K_S from 2.60-5.79 over a range of protonation states).[147] A comparison between an aryl-containing macrocycle and a less rigid control was made using the 1,3-phenylene receptor **59** and the acyclic polyamine **60**. These systems exist in their tetra- and pentaprotonated forms near neutral pH.[175,176] Both receptors bound ATP > ADP > AMP, with macrocycle **59** showing greater stability constants than **60** (log K_S (ATP) = 5.2 and 4.6 for **59** and **60**, respectively). This finding was rationalized on the basis of ^{1}H NMR spectroscopic studies, which provided support for significant π-surface interactions between the phenyl unit of **59** and the adenine moiety of the guests. In the case of AMP and ADP, partial inclusion complexes were proposed wherein the adenine moieties participate in hydrogen bonding interactions with the benzylic nitrogen atoms. Further studies compared the binding of receptors **59** and **60** to the *ortho*- (**61**) and *para*-(**62**) substituted derivatives.[177] Similar selectivities were observed among the nucleotides studied (ATP > ADP > AMP). Receptor **61** was found to form the most stable complexes over a wide pH range, followed by receptors **59** > **62** > **60**. The stronger complexation of the *ortho*-derivative was presumably due to the formation of a more favorable conformation for nucleotide binding. Further studies compared the nucleotide binding of linear polyammonium receptor **60** with shorter derivatives **63** and **64**.[178] All receptors were found to form 1:1 adducts with ATP, ADP, and AMP, with the complex stabilities again being found to correlate with the charge of the guest. Interestingly, the ethylene-spaced receptors (**63, 64**) displayed more effective binding behavior than the propylene-spaced receptor **60** at the same pH (pH 2 - 10); presumably, this reflects the lower charge density of the latter receptor. Receptor **64** was also found to form ternary complexes with Cu(II) and AMP over a wide pH range.

The binding of phosphate to the bis-aromatic macrocycles **65** - **68** was investigated by Martell and co-workers. Comparisons to the ether-containing versions **18, 69**, and **70** were also carried out. In the case of **65**, a macrocycle prepared as a more rigid version of OBISDIEN (**18**), lower overall basicity was found for the furan-containing system, perhaps as a result of the electron withdrawing nature of the furan moieties.[179] Measured association constants with pyrophosphate, however, proved similar for the two receptors. A structural analysis of diffraction grade crystals of the **65**:pyrophosphate complex (grown at pH 3.5) revealed that the macrocycle adopts a bowl conformation. This provides a cavity that encloses one of the phosphate groups, which is ligated via hydrogen bonds to all three oxygen atoms. A hydrogen bond with the neighboring oxygen atom of the “outside” phosphate group is also reported. Macrocycle **66**, in which the furan rings are replaced with phenyl rings, is characterized by a basicity that falls between that of **18** and **65**; it was found to bind inorganic phosphate similarly to **18**.[180] The bis-phenyl macrocycle **66** was observed

to bind pyrophosphate more strongly than did the bis-furan macrocycle **65**, with triphosphate being complexed even more effectively. Single crystal X-ray diffraction analysis of the **66**:pyrophosphate complex revealed a twisted chair-type shape with the anion completely enclosed within the cavity. Interestingly, the oxygen atoms on each end of the guest formed hydrogen bonds with the benzylic nitrogen atoms. As a consequence, the guest was found to bind perpendicular to the plane of the macrocycle in contrast to the co-planar arrangement typically found with nucleotides. In the course of efforts to explore the ability of receptor **65** to interact with nucleotides, it was demonstrated by ^{31}P NMR spectroscopy and potentiometric studies that macrocycle **65** displays a preference for ATP over ADP and AMP; presumably, this reflects the large negative charge of ATP.[181] Detailed ^{31}P NMR spectroscopic studies provided evidence for ATP interacting with receptor **65** via insertion of the terminal phosphate group into the receptor cavity. As with the mono-phenyl macrocycle **59**, receptor **66** was found to bind ATP > ADP > AMP through π-surface interactions, as inferred from ^{1}H NMR spectroscopy.[182] However, receptor **66** did not bind the nucleotides better than their inorganic analogues, despite the presence of moieties that could provide for additional π-surface interactions. This finding can be rationalized in terms of the reduced basicity of the nucleotides. Replacing the ethylenic spacers of **66** with propylenic spacers gave receptor **67**. This latter system, as expected, proved a more basic macrocycle than **66**. However, it displayed similar binding trends.[183] Single crystal X-ray diffraction analysis of **67** with pyrophosphate revealed a facial interaction in which the macrocycle adopts a relatively planar arrangement with the phenyl rings perpendicular to the plane and facing opposite directions. While receptor **66** bound most phosphate anions more strongly than **67** at a given protonation state (presumably reflecting an increased charge density), the larger macrocycle (**67**) out competed **66** for phosphate anions near neutral pH. The binding of inorganic phosphate and pyrophosphate with macrocycle **67** was later compared to the *para*-substituted derivative **68**.[184] These receptors displayed similar binding affinities for inorganic phosphate; however, receptor **67** was found to bind pyrophosphate more strongly than receptor **68** at equivalent protonation states. This trend was attributed to a better geometric complementarity between the smaller ring of receptor **67** and pyrophosphate.

The phosphate binding properties of this class of macrocycle were further compared with those of the expanded OBISDIEN (**18**) derivatives. Among the latter, the ether-containing system **69** is analogous to **67**, while **70** can be considered as an intermediate between **18** and **69**.[185] Both **69** and **70** proved to be more basic than their phenyl counterparts. While similar general trends were observed within the ether-containing series, competition experiments between **69** and **67** revealed that the ether-bridged macrocycle **69** bound inorganic phosphates more strongly than the phenyl-containing macrocycle **67**. On the other hand, this latter macrocycle was found to bind nucleotides more strongly than **69**. The metal complexes of many of these macrocycles were also studied as phosphate anion receptors. This chemistry will be presented in a later section (4.3).

In subsequent work, Schneider and co-workers investigated cyclophanes **71-73**, which included, biphenyl, bipyridyl and *para*-benzyl bridging elements.[186] Proton NMR titrations, carried out in D_2O, provided evidence that receptor **71** interacts more strongly with nucleotide monophosphates than do either **72** or **73**. The increased affinity observed in the case of **71** was attributed to its larger cavity size, which allowed for more efficient π-surface interactions between the phenyl rings of the receptor and the nucleobase of the guest, as inferred from studies of molecular models. Receptor **71** displayed a preference for AMP over other nucleotides, binding this particular nucleotide with an association constant of ca. 2200 M^{-1} in D_2O. Macrocycle **71** also exhibits a selectivity for 5′-AMP over 3′-AMP and appears to form an inclusion complex with 5′- thymidine monophosphate (TMP). Macrocycle **73** was found to bind GMP in preference to other nucleotides, displaying an

association constant of 540 M^{-1} in D_2O. Proton NMR spectroscopic studies led to the inference that π-surface interactions were not the main driving force for binding in this latter instance.

Recently, Llobet and co-workers studied the anion-binding behavior of the anilinic compounds **74-75**.[187] In this case, ^1H NMR spectroscopic and potentiometric studies carried out in aqueous solution provided support for the conclusion that the receptors with more rectangular cavities, **74a-b**, bound triphosphate and ATP anions more strongly than **75**. This finding was ascribed to size and shape complementarity.

Recently, Kumar and co-workers demonstrated by means of UV-Vis studies carried out in acetonitrile that the mixed amine ether macrocycle **76** interacted with sodium dihydrogenphosphate in preference to other anions.[188] The incorporation of ionophore **76** into a polyvinyl chloride (PVC) membrane allowed for the creation of a phosphate-selective electrode with a dynamic concentration range of 2.1×10^{-7} to 1.0×10^{-2} M.

In 2004, Bowman-James and co-workers reported the anion binding properties of a simpler, acyclic phenyl-polyammonium receptor (**77**).[189] This tripodal receptor was based on a tris(aminoethyl)amine (tren, **78**) scaffold functionalized with benzyl units. Proton NMR spectroscopic studies in chloroform revealed a strong selectivity for dihydrogenphosphate and hydrogensulfate (log $K > 3$) over nitrate, chloride, and bromide (log $K < 2$). All anions were studied as the tetrabutylammonium (TBA) salts. Further studies of the **77**:$H_2PO_4^-$ complex were conducted through single crystal X-ray diffraction analysis (Figure 4). These experiments led to the conclusion that extensive hydrogen bonded networks were present in the solid phase. In addition, a 1:3 host:guest ratio was inferred on the basis of these analyses.

García-España and co-workers reported the AMP binding ability of a series of similar systems (**79**) in 2006.[190] In this case, potentiometric titrations revealed a number of host:guest stoichiometries in aqueous media. For example, receptors **79a** and **79b** were both found to form 1:2 and 1:3 host:guest complexes. However, only 1:1 stoichiometries were observed with receptor **79c**. Significantly higher stability constants were measured for the receptors containing aromatic substituents (**79b**,**c**) relative to the alkyl polyammonium receptor **79a**. This increase in binding was attributed to a combination of π-surface interactions and hydrophobic interactions between the aromatic units of the receptors and the adenine ring of AMP. The highest log stability constant among the hexaprotonated forms of these receptors was found to be 7.65 in the case of AMP and receptor **79b**. Interestingly, the presence of AMP was observed to facilitate metalation of this receptor by copper(II).

A variety of other receptors with extended π-surfaces and positively charged bridges have been used to bind nucleotides. Among these is macrocycle **80**. This system contains four quaternary ammonium centers along with long alkyl chains and displays a strong interaction with adenosine phosphates.[191-193] Proton NMR spectroscopic studies and theoretical calculations of the complex formed between **80** and ATP provided support for the nearly complete inclusion of the adenine base within the lipophilic macrocycle, as well as an electrostatic interaction between the phosphate group and a quaternary nitrogen. Presumably as the result of this combination of factors, the equilibrium constant (derived from ^1H NMR spectroscopic studies) proved to be on the order of 10^4 M^{-1} (ca. 26 kJ/mol) in D_2O. Equilibrium constants were consistently higher for phosphate derivatives relative to the corresponding unsubstituted nucleobases, with each electrostatic interaction contributing ca. 5 kJ/mol to the overall binding energy. Receptor **80** was found to have a preference (approximately five-fold) for adenosine derivatives over other common nucleotides derivatives, possibly due to the higher polarizability of the adenine base. The free energy of binding for the open, cleft-like receptor **81** interacting with AMP proved to be ca. 9.5 kJ/mol

lower than the corresponding value in the case of **80**. This reduction in binding energy could reflect a lower level of preorganization, the absence of lipophilic inclusion, or the presence of fewer positively charged binding sites.

The inclusion of polyaromatic heterocycles, including known DNA intercalators, has been shown to increase the binding affinity for nucleotides, presumably due to π-surface interactions between these moieties and the nucleobases. For example, the Lehn group studied the synthetic incorporation of acridine into the OBISDIEN macrocycle (**18**) to give the ditopic nucleotide receptor **82a**.[194] In this case, the addition of ATP, ADP, and AMP produced measurable changes in the ^{31}P NMR, ^{1}H NMR and fluorescence spectra of **82a** (studies carried out in D_2O or H_2O, respectively). The interaction of **82a** with nucleotides produced a significant fluorescent enhancement (>30% with ATP) and a slight bathochromic shift. At the same time, the addition of pyrophosphate and triphosphate to **82a** led to a modest quenching of the acridine fluorescence. Acridine units substituted with single amine groups (instead of full incorporation into a macrocycle) were found to undergo no change in emission upon the addition of ATP or ADP. Evidence for the presumed π-surface interactions between the π-surfaces of the nucleotide and the receptor was found through ^{1}H NMR spectroscopy. Furthermore, competition experiments led to the suggestion that **82a** bound ATP twice as strongly as **18**, and that the binding of **82a** with ATP as compared to simple triphosphate anion was also favored by a factor of two. Further studies revealed that the emission of **82a** is constant over the pH range 4.0 to 7.6, ruling out protonation as a cause of the fluorescence change.[195] Little to no change in fluorescence was observed with inorganic phosphates or simple nucleobases, confirming the necessity of the covalent linkage between the two units. An increase of the emission of **82a** was also observed with CTP, and a slight decrease was observed with GTP. These findings were interpreted in terms of the receptor having a degree of inherent selectivity. The binding of the fluorescent derivative εATP was also studied, and it was found to bind five-fold more strongly than ATP. The combined data supported a dual binding mode in which the phosphate group of ATP is bound to the polyammonium macrocycle while the adenine moiety interacts with the acridine substituent.

The binding of nucleotides was further studied using the bisacridine receptor **82b**.[196] In this case, both NMR and fluorescence studies provided support for the suggestion that the two acridine units interact closely in the absence of an analyte, leading to a slight decrease in fluorescence. Consistent with this notion, the emission increase upon the addition of ATP to **82b** was found to be more dramatic than the corresponding increase in the case of **82a**. While 1:1 complexes with GTP, CTP, and 5′-uridine triphosphate (UTP) were also produced in the case of **82b**, the emission increase was much smaller than with ATP. The complexation of dinucleotide phosphates NAD, NADH, NADP, and NADPH by **82a** and **82b** was also investigated. Proton NMR spectroscopic studies of **82b** and NADH led to the suggestion that receptor **82b** interacts with both the nicotinamide and adenine moieties of the guest. Both ^{1}H NMR and fluorescence spectroscopic studies revealed that positively charged guests NAD and NADP were bound significantly less well than their neutral counterparts. This result is rationalized in terms of charge-charge repulsion with the protonated acridine near neutral pH. At the same time, the phosphorylated species (NADP and NADPH) bound more strongly than the non-phosphorylated species. Indeed, NAD and NADH produced no change in the fluorescence spectrum of either receptor. Fluorescence titrations revealed stable 1:1 complexes of both receptors with NADP and NADPH (log K_S = 5.5 to > 8.4). The contributions to the overall stability of the complexes were investigated through competition experiments. In the context of these analyses it was found that the binding constants for **82a** and **82b** were similar for each analyte studied. However, a comparison with the unsubstituted receptor **18** revealed that the acridine units present in **82a** and **82b** served to increase the stability of the complexes by more than one order of

magnitude. On the other hand, ATP, triphosphate, and NADPH were found to bind to both receptors with similar stabilities. This is consistent with electrostatic interactions again being the most significant factor in terms of defining complex stability. Interestingly, a 600-fold increase in selectivity was observed for NADPH over NADP.

Lehn and co-workers further examined a series of cyclic and acyclic aromatic receptors based on acridine derivatives and a variety of other substituents (**83-85**).[197,198] A strong hypsochromic shift was observed in the UV-Vis spectrum upon addition of nucleotides to **83-85** in aqueous media, a finding consistent with the presence of π-surface interactions. Binding constants, determined by absorbance titration methods, revealed 1:1 binding stoichiometries and log K_S values ranging from 2.04 to 5.05 for the binding to AMP (aqueous media, pH 6). Monomeric receptors **83** were found to bind AMP 2-3 orders of magnitude less well than the corresponding dimeric analogues **84**. In addition, the acyclic dimeric receptors **84** were found to bind AMP one order of magnitude more strongly than the macrocyclic derivatives **85**, possibly due to the rigidity of the cyclic systems. No large differences in stability were observed for the different substitution patterns of each receptor. Interestingly, in the case of **85b** no significant differences in stability constants were observed between AMP, ADP, and ATP. This is consistent with electrostatic interactions playing only a minor role in terms of the overall binding energetics.

Similar studies focused on the use of phenanthridinium (ethidium), also a DNA intercalator, as a receptor subunit.[199] This led to the synthesis and study of compounds **86** - **88**. In all cases, fluorescence quenching was observed upon the addition of nucleotides, with the log K_S values ranging from 4-6. All complexes were determined to be of 1:1 stoichiometry, with receptor **86** proving to be the weakest receptor. The dimeric, acyclic receptor **87** proved to be a slightly weaker receptor than **88**. Apart from these general trends, little selectivity was observed. Specifically, for any given receptor no appreciable differences were observed among nucleotides. The lack of selectivity among differently charged phosphates led to the suggestion that electrostatic interactions may not have been significantly influential. Additional studies with this class of hosts as well as other intercalator-based receptors that were found to depend nearly exclusively on π-surface interactions were carried out but are not discussed further in this review.

Recently Piantanida and co-workers reported strong nucleotide binding by the phenanthridine-containing receptor **89** in aqueous media.[200] Binding constants were determined through fluorescence titrations performed at pH 5 and 7 in a 0.05 M sodium cacodylate buffer. Among the series of AMP, GMP, and 5′-cytidine monophosphate (CMP), stronger binding constants were observed with the purine nucleotides at both pH values. Higher selectivity was observed at pH 7 than at pH 5. This trend was attributed to a significant contribution from π-surface interactions (which are known to favor purine binding) that were expected to be more pronounced for complexes with the unprotonated phenanthridine (pH 7) than the positively charged phenanthridinium (pH 5). Greater stabilization was also observed upon an increase in phosphorylation of the nucleotide (AMP < ADP < ATP). This latter trend supported the contribution of electrostatic interactions to the binding affinity. The highest stability constant for this series was measured for the complex of **89** and ATP at pH 7 (log K_S = 3.67).

The previous studies by Lehn and co-workers led to the design of a series of inclusion receptors based on polyammonium macrocycles containing polycyclic aromatic moieties (**90-92**), as well as their acyclic analogues (**93, 94**).[201-203] Based on the measured pK_a values, the diethylenetriamine moieties of **90-92** were expected to be doubly charged near neutral pH, leading to tetraprotonated macrocycles. This high degree of protonation was expected to lead to strong electrostatic binding in addition to interactions involving the

aromatic subunits of the receptors and those present on various test nucleotides. The naphthalene in receptor **90** was used to explore nucleotide binding. This was done using both ^{1}H NMR and fluorescence spectroscopy in aqueous media, from which log K_S values ranging from 3-5 at pH 6 were inferred. As expected based on charge considerations, **90** was found to bind ATP > ADP > AMP. Moderate selectivity for purines was observed among different nucleotide monophosphates, with GMP > AMP > UMP > CMP. The singlet emission features of the acridine derivative (**91**) proved to be a sensitive indicator of substrate binding. Specifically, purine nucleotides were found to quench the fluorescence, while pyrimidine nucleotides enhanced the fluorescence.[202] Binding a pyrimidine substrate likely increases the distance between the two acridine units, giving rise to a strong fluorescence signal similar to that of the acyclic species **93**. An electronic interaction between the purines bases and the acridine subunits likely overrides this latter effect, resulting in overall quenching. Stability constants (log K_S) derived from these measurements were found to range from 3.8 to 8.4 at pH 6. Selectivities were similar to those obtained with **90**, with the combination of **91** and ATP displaying the strongest interaction. Nucleotide binding by **91** was generally stronger than that of the acyclic derivative **93**. This finding led to the suggestion that both hydrophobic and van der Waals inclusion interactions were critical for substrate binding interactions. Similar binding affinities and selectivities were inferred from emission studies involving the tris-acridine receptor **95**.[204]

Still larger aromatic systems, specifically the quinacridine receptors **92** and **94**, were designed with the expectation that the expanded aromatic system would allow for the binding of two nucleotides, possibly associated through traditional Watson-Crick base pairing.[203] Addition of nucleotides to **92a** produced a decrease in both absorption and fluorescence intensity. Complexes of 2:1 (host:guest) stoichiometry were observed with several nucleotide monophosphates. A strong preference for guanine bases was observed through potentiometric titrations in aqueous media (first stability constant, log K_{S1} = 4.1, second stability constant, log K_{S2} = 4.5), with a selectivity order of GMP > AMP > CMP > UMP ≈ 3′,5′-cGMP ≈ 2′,3′-cGMP. Such findings, evidence of cooperative binding, were rationalized in terms of the co-bound GMP entities being better able to interact with one another via hydrogen bonds. Complexes of 1:1 stoichiometry were observed for di- and triphosphates, with triphosphates exhibiting the highest binding constants, followed by diphosphates and monophosphates. The selectivity for guanine over adenine was retained for the higher phosphates. Interestingly, the K_{S1} values corresponding to the interaction of nucleotide monophosphates with **92a** were lower than the corresponding binding constants in the case of **91**. This was rationalized in terms of the cavity of **92a** being too large to bind efficiently a single nucleotide monophosphate. Receptor **92b** was found to display similar binding behavior. However, **92c** was found to have reduced binding interactions with monophosphates. While the side chain of **92c** provided an additional positive charge that was expected to enhance binding, on the basis of this finding it was concluded that the cavity size plays a dominant role in terms of regulating the anion binding affinities.

In the same report, the monomers **94** were described. These systems were found to be less effective nucleotide receptors than the corresponding dimeric macrocycles. Interestingly, the second association constant was found to be higher than the first association constant when GMP and 3′,5′-cGMP derivatives were tested as substrates. This result was rationalized in terms of strong G-G interactions within the complex. Indeed, no mixed ternary complexes were observed when mixtures of CMP and GMP were tested with **94**. In addition, GMP apparently acts to displace CMP from the cavity to allow for G-G binding. Similar trends in stoichiometry and binding trends were noted when electrospray ionization (ESI) mass spectrometry was used to analyze mixtures of this receptor and various putative substrates. It was concluded by the authors that combining π-surface interactions, especially those

associated with intercalation, and electrostatic interactions can greatly increase binding affinities and selectivities for nucleotides.

A similar insertion binding mode was inferred in the case of the polycationic pyrenophane receptors **96**, prepared by Inouye and co-workers.[205,206] In this case, preliminary studies with receptor **96a** in 3:1 water:ethylene glycol revealed a change in the absorption spectrum upon the addition of a variety of guests, including nucleotide monophosphates AMP, UMP, GMP, and CMP. Among this latter class of substrates, a modest selectivity was observed for GMP ($K = 3.5 \times 10^4$ M^{-1}). Improved water solubility was achieved with receptor **96b**. With this latter system, it was found that the addition of nucleotides led to a change in the receptor-based excimer emission spectrum in water. Receptor **96b** exhibited a higher response toward nucleotide triphosphates over the mono- and diphosphates. For instance, association constants of 1.9×10^3 M^{-1}, 5.3×10^3 M^{-1}, and 1.0×10^6 M^{-1} were seen for AMP, ADP, and ATP, respectively. This differential response was attributed to a combination of the large charge of the triphosphate guests and the inclusion of the guest into the cavity formed between the pyrenophane and the ammonium groups present on the crown ether portion of the receptor.

Bencini and co-workers have included phenanthroline heterocycles within polyamine frameworks to form receptors **97** - **100**. The interactions of **97** and **98** with ADP, ATP, pyrophosphate, and triphosphate were studied through potentiometric titrations.[207] In general, **98** was found to bind phosphates more strongly than **97** for a given protonation state, as seen previously for smaller macrocycles. The stability constants with pyrophosphate and triphosphate, however, were much lower than those with ADP and ATP, respectively, with no binding observed between **98** and triphosphate. While guest basicity must be considered, these receptors were found to bind the nucleotides over analogous inorganic phosphates in competition experiments over a wide pH range. These results led to the proposal that interactions between the adenine base and the phenanthrolenic receptor are important. Support for this suggestion came from ^{1}H NMR spectroscopic analyses. Specifically, chemical shift differences were seen that were consistent with the presence of strong π-surface interactions and partial inclusion of the guests. In addition, more dramatic shifts for ADP over ATP, as well as increased shifts for the larger cavity of **97** over **98**, were observed.

More recently, these researchers reported the nucleotide binding properties of larger phenanthroline macrocycles **99** and **100**.[208,209] Hexaamine receptor **100** displayed higher binding affinity toward nucleotide triphosphates (ATP, CTP, GTP, and 5′-thymidine triphosphate (TTP)) than the pentaamine macrocycle **99** at the same pH, as inferred from potentiometric titration studies. The increased binding affinity for the former receptor was attributed to the increased charge density of the larger macrocycle at a particular pH. This increased charge density would be expected to augment electrostatic interactions with anionic guests. The complex of receptor **100** with ATP was found to be the most stable as judged by potentiometric titration analyses as well as ^{1}H NMR and fluorescence spectroscopic methods. Both ^{1}H NMR spectroscopic studies and molecular modeling studies supported an increased π-surface interaction between the adenine base and the phenanthroline moiety in the **100**:ATP complex over the other studied complexes. Interestingly, ^{31}P NMR spectroscopic studies and single crystal X-ray diffraction analysis (Figure 5) provided support for a binding model wherein the triphosphate moieties of nucleotide guests were encapsulated within the macrocyclic cavity. Receptor **100** thus proved to be a highly selective sensor for ATP in aqueous media in the pH range of 4.5-7.

Bencini, Bianchi, Paoletti, and co-workers later investigated the phosphate binding properties of an acyclic analogue (**101**) of the previously described phenanthroline

polyammonium macrocycles.[210] As inferred from potentiometric titrations, stability constants were found to depend heavily on electrostatic interactions, with the strongest complexes found near neutral pH. The anion recognition properties of the bipyridine derivative **102** were also investigated. This latter receptor displayed a similar trend but slightly higher binding affinity than receptor **101**. Higher binding affinities for nucleotides relative to inorganic phosphates was seen for both receptors. Proton NMR spectroscopic studies provided support for the presence of π-surface interactions in the nucleotide:receptor complexes. The most stable complex in these studies was found to be H_4(**102**)$^{4+}$:ATP^{3-} (log K = 5.45). These researchers further investigated anion binding by a bicyclic derivative of receptor **102** (**103**).[211] Higher stability constants were observed with a greater degree of protonation of the receptor. At equivalent charge states, macrocyclic receptor **103** was found to bind pyrophosphate and triphosphate more strongly than ADP or ATP, respectively, in contrast to the binding behavior of receptor **102**. This reversed selectivity was investigated through molecular modeling, which supported a folded conformation of the macrocycle. The predicted conformation featured a cleft appropriate for the binding of smaller, inorganic phosphates over nucleotides. This finding was further supported by a lower degree of π-surface interactions with receptor **103** compared to receptor **102**, as inferred through ^{1}H NMR spectroscopy. The thermodynamic analyses of these interactions revealed a strongly favorable entropic contribution in the binding of anions by receptor **103**, which was also consistent with an encapsulation-type binding event.

The aminoanthracene unit has also allowed for sensitive fluorescent detection of anion binding. The Czarnik group reported anthrylpolyamine compounds (**104, 105**) that exhibited chelation-enhanced fluorescence (CHEF). Increased fluorescent intensity was observed for the binding of inorganic phosphate and ATP to receptor **104** at pH 6, giving log K_S values of 0.82 and 4.2, respectively.[212] It was proposed that, while the lone pair of the neighboring nitrogen atom quenched the anthracene fluorescence in the absence of an anion (presumably through PET), the change in protonation state of the nitrogen upon anion binding interfered with the quenching and led to the large enhancements in fluorescence. The expanded chemosensor **105** was found to bind pyrophosphate over 2000-fold more tightly than monophosphate.[213] As binding analyses can be complicated by the chelation of pyrophosphate to adventitious transition metals, these studies were conducted in the presence of cyclen (**106**). This azamacrocycle strongly chelates transition metals but does not interfere with phosphate binding.

García-España and Piña later investigated a series of cyclic and acyclic polyaminoanthracene derivatives as possible ATP chemosensors.[214] Receptor **107** was found to bind AMP, ADP, and ATP with log K_S values ranging from 2.9 to 7.2 according to potentiometric titrations, with a 600-fold selectivity for ATP over ADP being observed. Receptors **108a-108c** were found to have similar binding affinities. This series of receptors displayed guest-dependent changes in the fluorescence intensity below pH 4 (aqueous $NaClO_4$), with a decrease in fluorescence being observed for ATP and ADP across the full series **107** – **108c**. This quenching and inferred binding was attributed to interactions between the π-faces of the anthracene and the protonated adenine base of ATP. No change in fluorescence was observed when **107-108c** were treated with AMP, adenosine, or triphosphate under the same conditions. Receptor **109** did not show a change in fluorescence with any of the tested analytes. Further studies served to demonstrate that receptors **110-113** also bound ATP and that, in general, receptors that contained one anthracene unit interacted more strongly with ATP than those containing a naphthalene group.[215]

Anthryl-functionalized open-chain polyammonium-alkanes **114-116** were studied by the Martínez-Máñez group.[216] Due to solubility incompatibilities, in the case of **114** only the binding of inorganic phosphates could be studied by potentiometric methods (log K_S = 3.99

- 15.52 in 7:3 MeCN:H_2O). Both **114** and **115** could be studied using fluorescence methods. In both cases, the emission intensity was selectively quenched in the presence of ATP at acidic pH (MeCN/H_2O 7:3), although no stability constants could be calculated. The addition of other anions (bromide, inorganic phosphates, sulfate, ADP, GMP) did not change the emission intensity. Interestingly, in water the emission intensity was found to increase at neutral pH in the presence of ADP and ATP but not with any other anions studied. This result was considered consistent with the conclusion that hydrogen bonding interactions with the nitrogen atoms nearest the anthracene lead to a reduction in PET quenching and therefore an emission increase. At lower pH values and in a solvent such as MeCN/H_2O with a lower dielectric constant (where the degree of protonation relative to pure H_2O is higher), the electrostatic interactions between the positively charged receptors **114** and **115** with guest anions were thought to be more significant. Such increased interactions were expected to favor PET resulting in a quenching of the fluorescence as seen by experiment. The most significant quenching in the presence of ATP in mixed aqueous-organic media was observed below pH 4. In this pH range, both **114** and **115** were fully protonated, maximizing the expected electrostatic interactions. On the other hand, no change in the fluorescence intensity was observed upon the addition of anions to **116**, perhaps due to steric constraints imposed by the diazolidine moiety. All anions were present as the TBA salts in these studies. Further studies revealed that an anthrylmethylamine (**117**) anchored in mesoporous solids permitted the fluorescent sensing of ATP in aqueous media.[217,218] The addition of ATP to solids with low loading levels of this anthracene derivative led to a quenching of the anthracene signal with a micromolar detection limit being noted at pH 2.8.

In 2007, García-España, Alcarón, and co-workers investigated the ATP binding properties of polyammonium naphthalene receptor **118**.[219] Log binding constants in the range of 4-5 with ATP were determined through pH-metric titrations. The binding affinity was found to correlate with the number of electrostatic interactions. Despite this high binding ability, quenching of the naphthalene fluorescence was only observed below pH 5. This pH dependence was attributed to protonation of the adenine moiety of the guest under more acidic conditions. The protonated adenine ring was expected to be a more capable acceptor for PET from the naphthalene excited state. ATP-dependent quenching was achieved over a broad pH range (2-12) by immobilizing this receptor on a boehmite solid support (**119**).

The rigidifying properties of aromatic rings can been used to effect specific preorganization. These effects were studied by the Anslyn group who designed the polyammonium receptors **120** - **122** in order to determine the optimum binding cavity size and hydrogen bonding arrangement for the complexation of phosphoric acid diesters. This series of receptors also allowed for the determination of the strength of interactions arising from individual hydrogen bond donors and acceptors in the host systems.[55,56] Both **120** and **121** are cleft-like systems that contain three hydrogen bond donation sites and one hydrogen bond acceptor site. These receptors were designed to be complementary to the three oxygen atoms and one acidic hydrogen atom of a tetrahedral phosphoric acid diester. Compound **122** was designed to be a cleft-free control. Investigations by isothermal ^{31}P or ^{1}H NMR spectroscopy in chloroform led to the suggestion that mixtures of 1:1 and 1:2 complexes are formed in the case of receptors **120** and **122** with dibenzyl hydrogenphosphate, while receptor **121** exhibited strong 1:1 binding with this analyte. The complex equilibria of **120** and **122** were attributed to the dimerization propensity of phosphoric acid diesters in organic media. The dimerization constant of hydrogenphosphate derivatives was determined to be 6.5×10^4 M^{-1} through fluorescence titrations of dinaphthyl hydrogenphosphate with dibenzyl hydrogenphosphate. A lower substrate concentration was therefore needed to measure binding constants for receptor-phosphate interactions reliably in the absence of dimerization. In view of this need, optical methods were considered the most practical method of analysis. Using UV-Vis methods, 1:l binding constants of 7.8×10^3 M^{-1} and $8.9 \times$

10^4 M^{-1} could be determined in chloroform for the interaction of dibenzyl hydrogenphosphate with receptors **120** and **121**, respectively. Using fluorescence methods, a binding constant of 1.3×10^3 M^{-1} was measured for the interaction of dinaphthyl hydrogenphosphate and receptor **122**. On the basis of these analyses, it was concluded that the extent to which phosphoric acid diesters were complexed by this set of polyammonium clefts increased with both the number of hydrogen bonds formed (each hydrogen bond contributing roughly 1.2-1.7 kcal/mol) and the size of the cleft ($-\Delta G°$ being approximately 1.4 kcal/mol larger in the case of **121** than **120**). These trends were in line with molecular modeling experiments.

Benzene and pyridine units were used to preorganize the cyclen (**106**) units of receptors (**123** - **125**) in systems developed by Handel and co-workers.[220-222] The interactions of these receptors with inorganic phosphate, pyrophosphate, and triphosphate in aqueous media were studied through potentiometric and NMR spectroscopic methods. Only 1:1 anion:receptor complexes were found to be formed under these conditions. In studies with the bis-macrocycle systems **123**, the highest stability constants were observed with *ortho*-substituted receptor **123a** and the pyridine-substituted receptor **123c** over a large pH range (pH 2 - 8).[220] These trends were attributed to a greater degree of organization in the former case and to the additional binding site available in the latter case. In all cases, the three anions were bound with affinities that were seen to match their overall charge (inorganic phosphate > pyrophosphate > triphosphate). Interestingly, a reversal of this trend was observed for triphosphate at higher pH values (pH 9-12), and higher stability constants were reported for the *meta*- and *para*-substituted phenyl receptors **123b** and **123d**. It was proposed that these latter receptors were better organized for hydrogen bonding interactions, which would be expected to play a more important role under more basic conditions. Modeling studies of receptors **123a** and **123c** led to the suggestion that these receptors formed a cavity with cyclen-cyclen distances consistent with the size of the triphosphate anion. The more rigid tri-macrocycle systems **124** displayed generally higher stability constants compared to the bis-macrocycle systems **123**, particularly at slightly acidic pH values.[221] Significant changes in selectivity were also observed. For example, the *ortho*-substituted receptor **124a** was found to bind inorganic phosphate and pyrophosphate more strongly than the larger receptors **124b** and **124c**. These latter receptors strongly bound triphosphate, with the *para*-substituted receptor **124c** displaying the greatest selectivity for this anion. This selectivity was attributed to the large size of the receptor **124c** cavity compared to the other receptors. Phosphorous NMR spectroscopy and modeling studies of the various **124**:triphosphate complexes were consistent with a model in which the two terminal phosphate subunits were bound by the cyclen moieties while the central phosphate unit was forced outside the cavity.

The effects of pyridine incorporation were studied through a comparison of receptors **125** to receptor **124b**.[222] Pyridine receptors **125** displayed a stronger selectivity and affinity for triphosphate over inorganic phosphate and pyrophosphate, particularly at acidic pH values where the pyridine nitrogen is found to be protonated. The three studied receptors bound triphosphate in the order **125a** > **125b** > **124b** over the entire pH range studied (pH 2 - 12). It was proposed that the mono-pyridine receptor **125a** bound triphosphate most strongly due to a relatively higher charge density at the pyridine unit as compared to receptor **125b** (containing two pyridine units) at an equivalent protonation state. Phosphorous NMR spectroscopy and modeling studies yielded similar results as those for receptors **124**. These studies thus serve to underscore the importance of electrostatic interactions and geometric complementarity in receptor design.

Further studies compared the anion binding of receptor **123d** and the linear analogue **126**.[223] As with the previous examples, only 1:1 anion:receptor complexes were observed.

Interestingly, the more flexible linear analogue **126** displayed significantly stronger binding ability with triphosphate as compared to receptor **123d**, particularly near neutral pH. This finding was attributed in part to the greater degree of protonation in the case of the linear derivative at equivalent pH values. In addition, ^{1}H NMR spectroscopy and modeling studies provided support for a binding mode in which the polyammonium arms of receptor **126** were able to wrap around the anion for highly efficient electrostatic and hydrogen bonding interactions with the bound anions. In this particular case, increased receptor flexibility proved advantageous over preorganization.

These same researchers later reported the recognition of nucleotides (AMP, ADP, ATP) by receptors **123b** and **123c** under similar conditions.[224] As inferred from potentiometric titrations, only 1:1 host:guest complexes are stabilized. Within this general trend, receptor **123b** displayed a preference for AMP over ADP and ATP, while receptor **123c** most strongly bound ATP over a wide pH range. Proton NMR spectroscopy of the anion:receptor complexes were consistent with strong π-surface interactions near neutral pH. Phosphorous NMR spectroscopy and modeling studies of the **123c**:ATP complex provided support for a binding model in which the β- and γ- phosphate units were complexed to the cyclen moieties while the adenine ring participated in π-surface interactions with the pyridine ring. A similar binding mode was inferred in the case of the linear derivatives **127** and ATP.[225] Interestingly, similar binding affinities for both ATP and triphosphate were found with both of these receptors, a finding consistent with the conclusion that pyridine nitrogen atom did not play a large role in anion binding. A direct comparison between the cyclic and linear systems, however, was not made.

Simple pyridine receptors **128** - **131** were prepared by Araki and co-workers and found to interact with diphenylhydrogenphosphate in organic solvents of both low and high polarity.[226] The amino bipyridine receptor **128** was found to bind this substrate strongly in both cyclohexane and acetonitrile, with log *K* values over 7.1 as determined by fluorescence spectroscopy. Due to spectral similarities between the protonated form of **128** and the anion:receptor complex, binding was thought to occur largely through electrostatic interactions involving the protonated pyridine of **128** and the bound diphenylphosphate moiety. Proton NMR spectroscopic studies led to the suggestion that the amino groups are involved in simultaneous hydrogen bonding to the phosphate ester. On the other hand, triethylphosphate (lacking an acidic proton) and hexanoic acid produced minor changes in the optical properties of **128**. On this basis, it was concluded that these substrates are bound with binding constants less than 100 M^{-1}. Control receptors **129-131** also displayed optical changes when treated with diphenylhydrogenphosphate but to a much lower extent, despite the nearly identical basicities of all four systems in question. To the extent these optical changes reflect binding, the fully alkylated receptor **129** was found to bind this particular phosphate ester 10^{5}-fold less effectively than **128** in cyclohexane. In addition, the monopyridine receptor **130** was found to bind the phosphate ester 100-fold less effectively than **128**. These results provided support for the conclusion that the amino hydrogen atoms play an important role in regulating the binding process. These atoms were also considered to underscore the benefits that accrue from having a second pyridine subunit within the receptor, at least for binding studies carried out in cyclohexane. The effects of the amino protons and pyridine subunits were less pronounced in acetonitrile. For instance, in this latter solvent receptor **128** displayed affinities that were only four-fold higher than those of **129** and six-fold higher than those of **130**. This solvent effect was attributed to a reduction in the strength of the underlying hydrogen bonding interactions in the more polar solvent, a reduction that in turn was expected to mask the differences between the different receptors.

Receptor **132**, designed by the Anslyn group, relies on a rigid, conformationally locked benzene ring to orient both boronic acid and secondary ammonium groups within a well-

defined "pinwheel" cavity.[227] On the basis of both ^{1}H NMR spectroscopic analyses and UV-Vis indicator displacement assays, it was found that this system bound glucose-6-phosphate selectivly. Presumably, this selectivity was the result of boronic acid-diol interactions and electrostatic attractions to the protonated amines. Binding constants on the order of 10^3 M^{-1} for glucose-6-phosphate and receptor **132** were derived in 7:3 CH_3OH:H_2O at pH 7.4. Neither the addition of glucose nor the addition of sodium phosphate effected displacement of the indicator in competition-type indicator displacement assays, even when these latter species were present in a 100-fold excess relative to the receptor.

In a more recent example, the rigid scaffold of the natural macrocycle tetrandrine (**133**) was investigated for anion binding by Eliseev and Yatsimirsky.[228] Proton NMR spectroscopic experiments carried out in water demonstrated that while **133** interacted most strongly with dicarboxylate anions, it also bound nucleotides ATP, ADP, and AMP. In the case of ATP and ADP, 1:1 complex formation was observed, although a 1:2 host:guest stoichiometry was observed in the case of AMP. Binding constants were reported to be 110 M^{-1} for ATP and 40 M^{-1} for ADP, with a first association constant of 48 M^{-1} and second association constant of 55 M^{-1} being noted in the case of AMP. Interestingly, the binding affinity did not correlate with the charge on the anion. This was thought to reflect a strong contribution from donor-acceptor interactions in the recognition process.

In addition to aromatic moieties, the use of aliphatic spacers has been explored by Bowman-James, Alcock, and co-workers. [229] These researchers reported the binding interactions between the simple macrocycle **134** with inorganic phosphate, as inferred from potentiometric titrations and single crystal X-ray diffraction analysis. Stability constants determined by the former method for 1:1 complexes were found to correlate with electrostatic interactions. Near neutral pH, the strongest 1:1 complex was formed between the pentaprotonated form of macrocycle **134** and monohydrogenphosphate (log $K = 6.01$). Interestingly, 1:2 host:guest stoichiometries were observed in the pH range of 2-4, presumably reflecting the binding of one phosphate anion to each triamine binding site. The existence of ditopic binding modes at lower pH was supported by solid-phase studies.

Yet another effective structural theme involves the use of polymacrocyclic receptors. As a general rule, these kinds of receptors can provide additional binding sites as well as permit the encapsulation of phosphate guests. In the case of bis(macrocyclic polyamine) ligands **135** and **136**, Kimura and co-workers combined two smaller macrocycles through either a tether (**135**) or through direct fusing (**136**).[230] Both receptors were found to form stable 1:l complexes with AMP, ATP, HPO_4^{2-}, and other anions as observed through polarographic and potentiometric methods. The comparison of these association constants with those of the parent monomeric polyamines (**9** and **42a**, respectively) revealed that the attachment of the second polyamine moiety consistently enhanced anion encapsulation and did so by at least an order of magnitude despite an overall lower charge density (**135** being only tetraprotonated and **136** diprotonated near neutral pH).[129] The greater complex stabilities exhibited by the bismacrocycles were explained in terms of ditopic interactions, such as sandwich complex formation, where the donor anion is located between the two protonated macrocycles. Presumably, this allows for maximum electrostatic and hydrogen bonding interactions.

Bencini and Lippolis investigated the anion binding character of receptor **137**, which was found to be zwitterionic at neutral pH.[231] Potentiometric studies showed that receptor **137** bound phosphate anions with an affinity order diphosphate < triphosphate < ATP. While the diphosphate to triphosphate selectivity could be attributed to charge-charge interactions among the phosphate and the amine subunits, ^{1}H NMR spectroscopy led the researchers to suggest that π-surface interactions were involved in the binding of ATP. Molecular

dynamics calculations predicted that the two macrocycles would indeed act concertedly to chelate polyphosphates.

In 2009, a tris-macrocycle polyammonium system (**138**) was reported by Bencini, Giorgi, Handel, and co-workers.[232] This receptor was found to display both proton sponge and anion binding properties. Stable 1:1 complexes were formed between receptor **138** and inorganic phosphate, pyrophosphate, triphosphate, ADP, and ATP in aqueous media (0.1 M NMe_4Cl) as inferred from potentiometric studies. The strongest stability constants were observed in the pH range of 3.5 - 4.5. Complex stability was found to increase in the order of inorganic phosphate < pyrophosphate < triphosphate ≈ ADP < ATP. While the stability trend of the unsubstituted phosphate derivatives can be attributed to increased anionic charge, the stronger binding affinities displayed in the case of the adenosine derivatives at equivalent charge states led to the suggestion that the adenine base was also participating in binding interactions with receptor **138**. A weak π-surface interaction between the adenine base and the pyridine unit of the receptor was inferred on the basis of ^{1}H NMR spectroscopic analyses. Molecular modeling studies supported a binding model in which electrostatic interactions with the terminal one or two phosphate groups of each of the studied guests is the main contributor to complex stability.

As illustrated in part by the previous examples, cryptand-type polyammonium polycyclic receptors have been studied for their ability to form inclusion complexes with anions. An additional system that falls within this generalized receptor class is cryptand **139**. This receptor was studied as a three-dimensional version of OBISDIEN (**18**).[233] In its penta- and hexaprotonated ($pK_{a\,5}$ = 7.00, $pK_{a\,6}$ = 5.90) forms, **139** was found to form stable complexes with a variety of anions, including inorganic phosphate, pyrophosphate, AMP, ADP, and ATP, in aqueous media. While no conclusive data was obtained for the inclusion of inorganic phosphate and pyrophosphate, a comparison of the relative stabilities of the AMP, ADP, and ATP complexes revealed that the hexaprotonated form of monocyclic “control” receptor **18** binds these three nucleotides more strongly than does the hexaprotonated polycyclic receptor **139** as inferred from potentiometric titrations in aqueous medium. These results were rationalized in terms of both the partial exclusion of the anion from the cavity due to the steric bulk of the adenine moiety, as well as the relatively lower charge density present in **139** as compared to **18** given equal protonation states.

Another multidentate cryptand-type system is receptor **140**, which incorporates hydrogen bonding pyrrole units. This system was found to form high-affinity 1:1 complexes with inorganic phosphate as well as other anions near neutral pH.[234] The interaction with inorganic phosphate was studied by potentiometric and ITC methods, from which association constants of nearly 10^7 M^{-1} were calculated. Simple calculations of the electrostatic contribution predicted affinities lower than those observed. This was reconciled by considering a strong contribution from additional interactions, such as hydrogen bonding and possibly anion inclusion. Analysis of the thermodynamic parameters revealed that, while the binding was exothermic at 298.2 K, the large negative ΔH value was partially compensated by markedly negative ΔS values. These unfavorable entropic contributions, in turn, were attributed to the additional inclusion of water molecules in the anion/receptor complexes.

In 2009, Bianchi and co-workers reported the anion binding properties of simple tren (**78**), which was used as a scaffold in receptor **140** as well as a variety of other multidentate receptors (e.g., **77, 95**, and **139**).[235] These studies were intended to elucidate the contribution of this subunit to the anion binding affinities of more complicated systems. Stability constants determined through potentiometric titration generally correlated with the strength of electrostatic interactions, and a significant selectivity for triphosphate was

observed among the other anions studied (pyrophosphate, inorganic phosphate, nitrate, sulfate, tosylate) near neutral pH. Thermodynamic analyses of these binding interactions generally revealed favorable entropic changes and negligible enthalpic changes, as would be expected for electrostatic interactions; however, the binding of monohydrogenphosphate was found to display a strong favorable enthalpic component with an unfavorable entropic change. This unexpected result was attributed to a partial anion-to-amine proton transfer between monohydrogenphosphate and diprotonated tren (**78**). Studies of anion binding with tren (**78**) led to the conclusion that this unit can significantly contribute to the anion binding of larger structures.

Using very different design principles, Severin and co-workers reported the self-assembly of piperazine-based cryptand **141** in 2007.[236] Anion binding studies were conducted using ^{1}H NMR spectroscopy in D_2O at pD 6.6. This receptor selectively bound inorganic phosphate and acetate over chloride, bromide, iodide, nitrate, and sulfate in neutral aqueous solution. Significant sharpening of the piperazine methylene signals was observed while no changes were found for the pyridyl protons. Based on these results, the authors proposed that the anions were bound in the center of the cryptand though electrostatic and hydrogen bonding interactions with the piperazine nitrogen atoms. Further analyses revealed a binding constant of 950 M^{-1} and 1:1 binding stoichiometry for inorganic phosphate. The affinity for inorganic phosphate was found to be significantly stronger than that for acetate (270 M^{-1}), leading to the suggestion that this receptor has a geometry that is complementary to the phosphate anion.

The anion binding properties of a 2,3,6-triethylbenzene-based polyammonium cryptand (**142**) were reported by Delgado and co-workers in 2009.[237] Anion:receptor interactions were studied through potentiometric titrations in a 1:1 methanol:water mixture containing 0.1 M potassium tosylate. Significant interactions near neutral pH were observed with $H_2PO_4^-$, AcO^-, SO_4^{2-}, $S_2O_3^{2-}$, and SeO_4^{2-}. Overall complex stabilities were found to correlate with predicted electrostatic interactions between the receptor and anions. A strong selectivity for sulfate was observed below pH 5, and this anion was thus the focus of further studies. Similar studies were conducted with pyridine-containing cryptand derivative **143**.[238] The presence of hydrogen bond donor (ammonium) and hydrogen acceptor (pyridine) units was expected to impart selectivity for inorganic phosphate over other anions. Under conditions identical to those used for studies with receptor **142**, a strong preference for hydrogenphosphate over sulfate, acetate, nitrate, and chloride was observed near neutral pH in the case of receptor **143**. The selectivity for hydrogenphosphate over sulfate near pH 7 was also supported by competition studies carried out using ^{31}P NMR spectroscopy.

4.1.2. Guanidinium Systems—While by definition guanidinium-based anion receptors are polyamines, this functional group has several features that have led to it being categorized as its own unique anion recognition motif. It has been studied extensively in the context of phosphate anion recognition.[239-243] Guanidine is readily protonated to form the guanidinium ion, which is stabilized by resonance and charge delocalization. Guanidine groups are characterized by a pK_a value of 13.6, compared to a value of ca. 10.5 for a typical secondary amine. As a consequence, guanidinium residues remain protonated up to high pH values, and, when incorporated into suitable frameworks, the group can be used to broaden the pH range over which an anion receptor operates. The guanidinium moiety also presents two parallel hydrogen donor sites, a feature that leads to strong interactions with oxo-anions. However, the exploitation of guanidinium residues in host-guest chemistry is hampered by the fact that such species are highly solvated in water. These units are also characterized by a lower charge density compared to ammonium cations. Since binding affinity is often primarily electrostatic, polyammonium salts generally form more stable complexes (at a

given net charge) than do polyguanidinium salts. Nevertheless, an appreciable number of artificial guanidinium-based anion receptors have been reported in the last few decades.

Historically, the recognition that the guanidinium ion had a role to play in anion recognition can be traced back to 1964 when Watters and Matsumoto reported the interaction between the guanidinium ion (**144**) and tetraphosphate.[244] Cotton and co-workers published the crystal structure of a methylguanidinium:dihydrogenphosphate complex in 1973,[245] as well as that of a bis(methyl)guanidinium:dihydrogenphosphate complex in 1974.[246] Both structures confirmed the planarity of the guanidinium moiety as well as the two parallel hydrogen bonds between the guanidinium hydrogen atoms and phosphate oxygen atoms. Springs and Haake measured the binding constant of the guanidinium ion (**144**) with inorganic phosphate in 1977.[247] Relatively low association constants were obtained using potentiometric titrations in aqueous media. Although not particularly dramatic, these studies were among the first to demonstrate the potential utility of guanidinium motifs in phosphate anion recognition.

Lehn and co-workers were among the early pioneers using guanidinium subunits to create more elaborated receptor systems, including a series of cyclic and acyclic guanidinium analogues **145** – **150**.[248] In the context of this work it was found that the tris-guanidinium receptor **145** bound inorganic phosphate with the highest affinity in water within the series **145** – **150**, as determined via pH-metric titrations (log K_S = 2.4). As a general rule, the macrocyclic systems (**145, 146, 148**) exhibited greater affinities for phosphate than did their acyclic analogues (**147, 149, 150**, respectively). All recorded stability constants proved significantly higher than those for *N,N′*-diethylguanidinium or for guanidinium itself. These results were considered consistent with the presence of a macrocyclic effect similar to that described in the previous section, as well as specific chelation interactions, that served to increase the affinity for phosphate-type anions relative to other negatively charged species. As expected given the less competitive nature of the environment, stronger binding was observed in a methanol/water mixture than in water. This was a general trend, true across the whole series of receptors.

The Lehn group also developed a series of protonated amine and guanidinium receptors to permit a more direct comparison between these two anion recognition motifs.[249] The interaction of each receptor with inorganic phosphate and pyrophosphate was studied potentiometrically. As expected, electrostatic interactions proved to be the most important determinant of complex stability. Thus, receptors with amine substituents (**151b** – **157b**) consistently gave rise to stability constants that were higher than their corresponding guanidinium analogues. This effect was significant, with the difference being roughly one order of magnitude for the same overall charge. It was rationalized in terms of the higher charge density provided by protonated amines relative to guanidiniums. This increase serves to overcome the putative benefits provided by the two linear hydrogen bonds possible with guanidinium receptors. Among the guanidinium containing receptors (**151a** – **157a**), those with additional ammonium sites (and therefore a higher electrostatic charge) generally displayed the highest affinities. At the same time, increasing the spacing between the individual guanidinium subunits was found to decrease the propensity to bind phosphates (the log K_S order was as follows: **151a** > **152a** > **153a** and **156a** > **157a**) when studied at equivalent protonation states in aqueous medium. The most stable guanidinium complexes were formed with the closely packed triguanidinium receptor **156**.

Göbel and co-workers examined the binding of a series of guanidinium receptors (**158** - **163**) to catechol cyclic phosphate (tetramethylguanidinium salt) using ^{31}P NMR spectroscopy in DMSO-d_6.[250] Anion interactions were found to be much stronger with *meta*-substituted benzene receptors **158** and **159** ($K \approx 95$ and 75 M^{-1}, respectively) than monosubstituted

receptors **160-162** ($K \approx$ 15-20 M^{-1}). These differences in binding affinities could reflect the presence of a bidentate binding mode in the case of the former receptors. Although all association constants were calculated assuming a 1:1 binding stoichiometry, the authors report that some evidence of higher order complexes was found. Further details regarding this result were not discussed. A more complex scaffold, decalin, was explored to examine the effects of rigidifying the guanidinium receptors.[251] Little difference was observed between the two bisguanidinium receptors **158** and **163** (K = 110±15 M^{-1} and 95±10 M^{-1}, respectively).

Attachment of pyrene to a guanidinium residue led to the synthesis of receptor **164**, which was designed to act as a fluorescent sensor for pyrophosphate.[252] Proton NMR and fluorescence spectroscopic measurements by Teramae and co-workers provided evidence that **164** formed 2:1 host:guest complexes with pyrophosphate in methanol. These sandwich-like complexes were characterized by an enhanced excimer emission relative to the monomer emission. This change in emission behavior was not observed with other anions, including $H_2PO_4^-$, $CH_3CO_2^-$, SCN^-, Cl^-, and Br^-. At low concentrations of guest, the binding constant corresponding to the formation of the 2:1 receptor:pyrophosphate complexes was determined by fluorescence measurements to be 1.2×10^8 M^{-2}. Addition of more than 0.5 equivalents of pyrophosphate led to a decrease in excimer emission, which was attributed to the formation of a 1:1 complexes at higher concentrations of guest.

Rigid chiral bicyclic guanidinium moieties have been exploited successfully in phosphate anion recognition. For instance, Schmidtchen and co-workers reported the series of receptors **165** - **166**. Preliminary ^{1}H NMR spectroscopic titrations involving the addition of *p*-nitrophenylphosphate and CMP to the ditopic chiral hosts **165a** and **165b** provided support for the formation of 1:1 host:guest complexes in methanol.[253] Additional NMR spectroscopic analyses of host *R,R*-**166b** revealed the formation of 1:1 complexes with inorganic phosphate, 2′-AMP, 2′-deoxy-5′-adenosine monophosphate (dAMP) and 5′-AMP.[254] Interestingly, nearly identical binding constants were obtained for inorganic phosphate and 2′-AMP (log K= 4.26) in methanol, a finding that was consistent with little interaction between the receptor and the adenine base. The binding affinity of *R,R*-**166b** for 2′-AMP was reduced by approximately one third in DMSO as compared to that in methanol. It was also found that, as compared to 2′-AMP, dAMP was found to be bound more strongly. This latter finding could reflect the fact that the phosphate group is less sterically constrained in dAMP than 2′-AMP. The “addition” of a hydroxyl group (i.e. 5′-AMP) led to even higher affinities. While the phosphate interactions with receptor *R,R*-**166a** were not easily characterizable in methanol, when the analysis medium was unbuffered water, clean 1:1 complexes were formed with inorganic phosphates and a number of phosphate esters, including 5′-AMP, *p*-nitrophenylphosphate, *p*-nitrobenzylphosphate, guanylyl(3′-5′)adenosine (GpA), NAD, and ATP. However, the binding affinities in aqueous media were considerably reduced as compared to what was found in methanol (40-fold with 5′-AMP). In unbuffered water, the most stable complex was formed between receptor **166a** and inorganic phosphate (log K = 2.99). This reflects the formation of a complex that is stronger than that produced from the more charged species, ATP. In these studies, the organic moieties of the phosphate esters appeared to interfere with the phosphate interaction, in contrast to the contribution observed with polyammonium systems.

In an effort to avoid the influences of counteranions and the inherent lack of selectivity generally associated with strong electrostatic attractions, a *closo*-borane cluster was added to the bisguanidinium framework (**165c**).[255,256] The *closo*-borane cluster in receptor **165c** carries a double negative charge in a delocalized, hydrophobic environment. The presence of this cluster was expected to allow for neutralization of the two guanidinium groups without formation of a strong contact ion pair. However, ^{1}H NMR spectroscopic analyses in DMSO-

d_6 revealed evidence of dimerization, with an association constant of 250 M^{-1}. On the other hand, the use of UV-Vis titrations (and a lower concentration) allowed binding analyses to be carried out without having to account for aggregation effects. This method permitted a log *K* value of nearly 4 to be determined for the interaction between *p*-nitrophenylphosphate (TBA salt) and **165c** in DMSO. Using ITC, thermodynamic parameters were derived for the binding of *p*-nitrophenylphosphate in acetonitrile for **165c**, as well as those for control compounds **165d** and **165e**. While the three receptors displayed similar association constants (log *K* = 5), the negative entropy values found with receptors **165d** and **165c**, compared to the slightly positive value with **165e**, served to underscore the importance of the substituents on the central phenyl ring. Receptor **165c** was later used to prepare what was hoped to be a phosphate selective electrode.[257] While micromolar sensitivity for phosphate in chloride solutions was observed, and the stability was adequate, the electrode was not particularly selective against other anions.

The Schmidtchen group also investigated the phosphate anion extraction properties of receptors *S,S*-**166b, 165f**, and **165g**.[258] Each receptor was tested by following its ability to effect the extraction of inorganic phosphate, AMP, ADP, ATP, and other anions from aqueous solution (pH 7.4-7.8) into chloroform. Receptor *S,S*-**166b** displayed a selectivity for sulfate over inorganic phosphate. This receptor was also found to extract nucleotides AMP, ADP, and ATP more efficiently than inorganic phosphate. Under defined conditions, ATP was found to be 94% extracted, while ADP and AMP were extracted to the extent of 54% and 6%, respectively. Bromide and iodide anions were also efficiently extracted (82% and 96%, respectively). The more flexible isophthalic acid host **165g** was found to effect significantly less nucleotide extraction than *S,S* **166b**. On the other hand, in the case of **165f**, the increased rigidity and the additional hydrogen bonding sites provided by the amide moieties was thought to lead to significant extraction of all oxoanions studied, with selectivity even over iodide being observed.

Comparisons between **167a** and **167b** were used to determine the thermodynamic contribution of the four amide groups present in **167a** for the binding of inorganic phosphate.[259] A strong enthalpic contribution was expected in the case of **167a** from the addition of four hydrogen bonding sites. Upon the addition of TBA dihydrogenphosphate to **167a** in acetonitrile, ITC studies revealed an initial exothermic 1:1 complexation event followed by an endothermic 1:2 host:guest complexation process. Evidence for higher ordered complexes at high phosphate concentrations was also obtained. For receptor **167b**, 1:1 complexes were observed. While the binding constant was slightly higher for the amido receptor **167a**, the enthalpic contribution was actually smaller for this receptor. The enhanced affinity was thus ascribed to a large increase in the entropic component of binding with **167a** over that associated with **167b**. Based on a comparison of the binding of these receptors to other anionic guests, the cause of this effect was proposed to involve the weakened structural definition of the **167a:**phosphate complex compared to that formed with **167b**, as opposed to, e.g., differences in solvation. This conclusion was supported by the observation of higher order complexes in the case of **167a** but not in the case of **167b**. Taken in concert, these results help illustrate the importance of individual thermodynamic parameters in regulating the affinities of host-guest systems.

The complexation of bicyclic chiral guanidinium receptors (**168-169**) with AMP derivatives was studied by the de Mendoza group. The ability of these receptors to extract anions from aqueous media into chloroform was tested with *S,S*- and *R,R*-**168a** and *S,S*-**168b**.[260] No chiral recognition was observed based on interactions with 5′-AMP, 3′-AMP, 3′,5′-cAMP, or 2′,3′-cAMP. The cyclic AMP derivatives were extracted by **168a** less efficiently than the linear derivatives, presumably due to steric effects that precluded π–π donor-acceptor interactions involving the naphthalene ring. On the other hand, in the case of **168b**, the more

lipophilic cAMP derivatives were found to be extracted more effectively than the more hydrophilic linear derivatives. Overall, **168a** extracted this set of nucleotides more efficiently than **168b**. Finally, receptor *S,S*-**168c** was designed to interact with nucleotides through both π–π donor-acceptor and base pairing effects. Such interactions were clearly observed through ^{1}H NMR spectroscopy in DMSO-d_6 in the case of 3′-AMP but not 3′,5′-cAMP. Anion interactions with **168c** were not quantified.

Macrocyclic receptor **169** combines the bicyclic guanidinium unit with two urea moieties as additional hydrogen bonding sites and with amino acids to impart additional chirality.[261] The xanthene unit was included to add rigidity and aid with preorganization. Molecular mechanics calculations led to the prediction that a diphenylphosphate ion would be located inside the cavity, stabilized by hydrogen bonds involving all of the phosphate oxygen atoms. The two phenyl rings would thus protrude to each side. Proton NMR spectroscopic studies of the isolated **169**:diphenylphosphate salt in $CDCl_3$ were consistent with a complex of C_2 symmetry. However, the splitting of the signals seen at 213 K led to the suggestion that the ion was most likely residing outside the cavity and rapidly exchanging between both sides of the macrocycle at room temperature. Bulkier phosphates displayed similar ^{1}H NMR spectral features, providing support for the conclusion that the phosphate esters were not bound within the cavity as predicted. No association constants were reported in this study.

The Rebek group also studied a series of guanidinium receptors that incorporate additional functionalities. For example, receptor **170** was designed to combine the phosphate recognition ability of guanidinium subunits, the π-electron donor capabilities of aromatic carbazoles, and the hydrogen bonding ability of amides in order to create a receptor selective for dinucleotide phosphates.[262] In preliminary studies, receptor **170** was found capable of extracting a full equivalent of a dinucleotide (2′- deoxyadenylyl(3′,5′)-2′-deoxyadenosine, d(AA)) from aqueous solution into dichloromethane. Two-dimensional NMR spectroscopic experiments led to the suggestion that, while most of the binding was electrostatic in nature, significant hydrogen bonding existed between the base pairs of the dinucleotide and the receptor. The energetic contribution of the electrostatic interaction was examined by comparing the affinity of several adenine derivatives for receptor **171a** and control system **171b** in aqueous media at pH 6.[263] In these studies, adenosine and 9-ethyladenine were found to bind equally well to both **171a** and **171b**. This finding was considered consistent with the conclusion that the guanidinium moiety of **171a** did not interact with the nucleotide base and that the sugar moiety did not influence the binding process significantly. Furthermore, all four adenine derivatives (adenosine, 9-ethyladenine, 2′,3′-cAMP, and 3′,5′-cAMP) were found to bind to receptor **171b** with similar affinity; again, this is as would be expected were the adenine moiety interacting only with the carbazole portion of this receptor and its more elaborate analogue **171a**. In the case of **171a**, relatively higher binding affinities were observed for the cAMP derivatives, although this effect was somewhat mitigated at higher ionic strengths. Little dependence on ionic strength was observed for complexes lacking an electrostatic interaction. The free energy of each system was then calculated from ^{1}H NMR spectroscopic titration experiments with the goal of sorting out the energetic contribution of the electrostatic interactions. On this basis, the average phosphate-guanidinium interaction energy in this system was calculated to be 0.6 kcal/mol at an ionic strength of 51 mM (NaCl) and 0.3 kcal/mol at an ionic strength of 501 mM (NaCl).

Transport studies made using simple U-tube apparatus (two aqueous phases separated by a dichloroethane “membrane”) with receptors **172** revealed a preference for adenine over other nucleotide bases.[264-266] Transport was observed for 2′,3′-cAMP, 3′5′-cAMP, and 3′-AMP but not for 2′,3′-cGMP and 3′5′-cGMP. Interestingly, transport was also not observed in the case of 5′-AMP. The more effective transport seen for adenosine nucleotides relative to guanosine nucleotides was thought to reflect the loss of one hydrogen bonding interaction

in the corresponding complexes. Receptors **172a** and **172b** were found to transport dinucleotides containing adenine, although not those lacking an adenine moiety. Receptor **172d** did not transport any of the nucleotides studied. For the adenine nucleotides, receptor **172c** displayed the best transport properties among the set of receptors **172**. This was ascribed to the favorable combination of interactions provided by the hydrophobic naphthalene moiety and the carbazole moiety acting as a hydrogen bond donor.

Mandolini, de Mendoza, and co-workers combined a bicyclic guanidinium moiety with a calixarene (**173**) with the goal of facilitating the binding of dioctanoyl-L-α-phosphatidylcholine (DOPC), a transition state analogue that acts as an inhibitor for the enzyme-mediated hydrolysis of acetylcholine.[267-269] In the case of **173**, the guanidinium moiety was expected to bind the anionic phosphate group while the calixarene moiety was expected to bind the ammonium group through cation-π interactions with the calixarene in its cone conformation. Proton NMR spectroscopic studies and molecular modeling supported these assumptions, although an analysis of the data led to the conclusion that the phosphate group was involved in hydrogen bonding with the amide and proximal guanidinium protons instead of both guanidinium protons as originally expected. In the absence of a guest, receptor **173b** was found to have a stronger preference for the cone conformation, leading to a more preorganized receptor. Proton NMR binding studies carried out in $CDCl_3$ allowed log *K* values of 4.9 for DOPC:**173a** and 5.0 for DOPC:**173b** to be derived. These quantitative analyses revealed no significant difference in binding affinity based on the presence or absence of the cyclohexane substituent, even though the presence of this latter subunit was expected to increase the extent of preorganization. The addition of one percent CD_3OD to the $CDCl_3$ solvent served to reduce the binding constants by an order of magnitude.

The monocyclic and acyclic guanidinium receptors **174** and **175** were studied by the Hamilton group.[270] These systems contain neighboring acyl groups, a feature that was expected to enhance the rigidity of the receptors through intramolecular hydrogen bonding interactions. Acylation was also expected to decrease the basicity of the receptors. Despite this expected lowering in the pK_a values, both receptors were found to remain protonated under all conditions explored in these studies. Proton NMR spectroscopic studies of the receptors in CD_3CN provided support for the proposed rigid structure as evidenced by the lack of spectral splitting or line broadening. Spectral changes seen upon the addition of TBA diphenylphosphate were considered consistent with the formation of four hydrogen bonds to the anionic guest, which, in turn, was proposed to reside inside the cleft in the case of both receptors. Compound **175** displayed a first association constant of 5×10^4 M^{-1}, a binding event that was considered to be followed by the binding of two additional phosphate ions as inferred from ^1H NMR spectroscopic analyses. Only 1:1 binding was observed with compound **174**, a difference that was rationalized in terms of the absence of an additional hydrogen bond donor as compared to **175**.

Additional basic moieties were incorporated to produce receptors such as **176**. These latter systems were prepared in pursuit of catalysts that would facilitate transesterification reaction of 2-hydroxypropyl-*para*-nitrophenylphosphate.[271] The interactions between receptors **176b** and **177** with diethylphosphate in CD_3CN were studied using ^{31}P NMR spectroscopy. Receptor **176b** was found to form strong 1:2 host:guest complexes, while **177** formed weaker 2:1 host:guest complexes.

Several guanidinium receptors have been investigated by the Anslyn group. The first of these were the cleft-like monocyclic guanidinium receptors **178** and **179**.[272,273] Within this pair, it was considered likely that the binding affinity and selectivity could be tuned through variations in the cavity size and the overall conformational flexibility, brought about by

linking guanidinium moieties to cyclopenteno and cyclohexeno rings in the case of **178** and **179**, respectively. Both the *meso*- and *d,l*-diastereomers were isolated and tested in the form of various counter anion salts. Binding constants for the interaction with dibenzylphosphate were determined through ^{31}P NMR spectroscopic titrations carried out in various mixtures of water and DMSO. Low concentrations of water led to a mixture of 1:1 and 1:2 complexes, while concentrations near or above 20% led to the formation of only 1:1 complexes. This was true for both receptors. In pure DMSO and with the receptors studied in the form of their tetraphenylborate salts, stronger binding was found in the *meso*-forms of both receptors. This finding was attributed to enhanced cooperativity between the two guanidinium groups constrained on the same side of the cleft-like receptor. The putative increased flexibility present in receptor **179** was thought to underlie the slightly higher binding affinities observed for the *meso*-form of **179** as compared to *meso*-**178**. In addition, increased binding constants were observed in the presence of LiCl, NaCl, and KCl. However, decreased binding affinities were observed in the presence of $NaClO_4$ and NaSCN. These results were tentatively attributed to a "salting out" effect rather than an ionic strength effect. With dibenzylphosphate, the strongest measured binding constant was recorded when the chloride salt of *meso*-**178** was studied in pure DMSO. In this case, values of $K_1 = 4.0 \times 10^3\ M^{-1}$ and $K_2 = 1.0 \times 10^2\ M^{-1}$ were recorded.

The guanidinium-mediated complexation of several phosphate anions with preorganized C_{3v} symmetric receptors was also studied by the Anslyn group. These receptor systems, embodied by **180** and **181b**, were created in order to achieve geometric complementary in addition to allowing for hydrogen bonding, electrostatic interactions, and metal coordination. While highly selective for citrate, receptor **180** was also found to interact with ATP, displaying a binding constant of $1.2 \times 10^3\ M^{-1}$ as inferred from ^1H NMR spectroscopic analyses (D_2O, pH 7.4).[274] Receptor **181b**, in which six guanidiniums are sterically geared to converge and create a cavity, was used to effect the selective detection of inositol triphosphate (IP_3).[275] Using an indicator displacement assay, **181b** was shown to bind IP_3 more strongly than a variety of other phosphorylated molecules including ATP and fructose-1,6-diphosphate in an aqueous medium (HEPES, pH 7.4). Of the compounds tested, only benzene-1,3,5-triphosphate and phytic acid were found to bind with similar or greater affinity. The protonated amine receptor **181a** was tested for comparison. Although slightly higher binding constants were observed with **181a** compared to **181b**, evidence for non-specific interactions was also obtained. Binding constants between **181b** and IP_3 were found to be $4.7 \times 10^5\ M^{-1}$ in water and $1.0 \times 10^8\ M^{-1}$ in methanol. In neither case was a significant ionic strength dependence observed. Using fluorescence spectroscopy, IP_3 could be detected at 1 μM concentrations in water and 2 nM in methanol. These results led to the suggestion that significant binding can be achieved through geometric complementarity. In further studies, improved binding to IP_3 was observed when this assay was performed in the presence of 2% Triton X detergent at pH 4.0 (formate buffer).[276] This increased stability was attributed to the inclusion of the neutral host:guest complex within a micelle core.

A small library of guanidinium chemosensors (**182a**,**b**) was also developed by the Anslyn group and based on the preorganized "pinwheel" scaffold. This library was screened for selective binding to ATP.[277,278] Control receptor **183** was found to bind ATP with an association constant of $3.5 \times 10^2\ M^{-1}$ in water. However, it was not found to be selective among nucleotides.[277] The ability of the more elaborate receptors **182** to effect differentiation was expected to derive from the peptide arms (prepared in a combinatorial manner). Library **182a** was first screened for its ability to bind to a fluorescently labeled ATP analogue. Several receptors were observed to interact strongly with the fluorescent guest, and the peptide arms of a selection of these high affinity receptors were sequenced. A small subset of these receptors in the form of **182b** was then synthesized based on the sequences obtained. Fluorophores were then used in conjunction with **182b** so as to allow

for direct fluorescent sensing of ATP. Among the sequences used to create the **182b** library, Ser-Tyr-Ser exhibited strong binding (3.4×10^3 M^{-1}) and high selectivity for ATP over AMP and GTP. The lack of response to AMP and GTP provided support for the notion that specificity was dependent on both the length of the phosphate chain and the nucleobase. The specific subunits involved, namely tyrosine and serine, led to the consideration that binding modes involving interactions of the π-surfaces and hydrogen bonding were important. The library of peptides represented by structure **182a** was later used to create an array in which ATP, AMP, and GTP could be differentiated via pattern recognition analysis. Here, the relevant studies were carried out by noting the response of each library member to the analyte in an indicator displacement assay.[278] In this way, one array could be used to identify selectively these three different phosphorylated analytes.

Schmuck and Schwegmann used a variation on the guanidinium pinwheel to bind phosphorylated sugars through both ion pairing interactions and hydrogen bonds.[279] Receptor **184** was found through ^{1}H NMR spectroscopic titration to bind mono-substituted anionic sugars. With this receptor, a two-fold preference for phosphorylated sugars over carboxylated sugars and a three-fold preference for phosphorylated sugars over methylphosphate was reported. The highest binding affinities near neutral pH were found between **184** and mannose-1-phosphate, with an association constant of 1.25×10^3 M^{-1} being recorded in aqueous DMSO (30% water). Two-dimensional NMR spectroscopic experiments and molecular modeling analyses led to the suggestion that the anionic groups interacted with the guanidiniums, while the sugars participated in hydrogen bonds with the pyrrole and amide units. Receptor **185** was also found to bind sugar phosphates more strongly than sugar carboxylates.[280] In addition, this receptor was found to interact with AMP and 3′,5′-cAMP. While no obvious trends were observed among phosphorylated compounds, UV-Vis titrations carried out in aqueous DMSO (20% water) at pH 6 revealed that receptor **185** bound methylphosphate with an association constant of $K = 3.8 \times 10^3$ M^{-1}.

The Kunitake group developed guanidinium-functionalized monolayers for the binding of nucleotides at the air-water interface.[281,282] The guanidinium amphiphiles **186** were found to form a monolayer on pure water that was then observed to bind AMP and ATP.[281] Langmuir isotherm analysis of X-ray photoelectron spectroscopy (XPS) data yielded binding constants of 1.7×10^7 M^{-1} and 3.2×10^6 M^{-1} for ATP and AMP, respectively. Interestingly, ATP was found to effect saturation at a concentration of 10^{-5} M at a 1:3 ratio of ATP:**186a**, whereas with AMP saturation was seen at a 1:1 ratio. Such findings are consistent with each phosphate interacting with one guanidinium moiety. In addition, changes in the IR spectrum were observed that supported the notion that hydrogen bonding plays an important role in the recognition process. Amphiphiles **186b** and **186c** were mixed with equimolar ratios of **186a** in order to aid in discrimination between nucleotides.[282] Mixtures with adenine-substituted **186b** were found to have a slight preference for UMP over AMP, while the opposite trend was observed with the thymine-containing receptor **186c**.

A three-component monolayer consisting of the diaminotriazine derivative **187**, the guanidinium derivative **188**, and the orotate derivative **189** was used in an effort to bind the isoalloxazine, the phosphate, and the adenine subunits of flavin adenine dinucleotide (FAD).[283] Binding of FAD by the monolayer (1:2:1 **187:188:189**) was nearly stoichiometric at concentrations as low as 10^{-6} M. Significantly lower binding was observed with combinations of only two components or with other guest molecules such as ADP, AMP, and flavin mononucleotide (FMN). However, each of these multi-component systems suffered from competing hydrogen bonding interactions between the amphiphilic components. Binary mixtures were thus explored later on in an effort to bind FMN.[284] In the context of this work, it was found that a 1:1 mixture of **188** and melamine derivative **190**

bound FMN in stoichiometric fashion with saturation being observed at ca. 5×10^{-6} M. FTIR analysis provided support for interactions between the guanidinium subunits and the phosphate moiety and between the melamine units and the isoalloxazine moiety. A monolayer mixture made up from **190** and **191** was found to behave similarly. These studies demonstrated that appropriate combinations of individual guanidinium species and additional recognition motifs can be used to effect the selective binding of appropriately chosen phosphorylated substrates.

Recently, Mellet and Fernández reported a small library of monosaccharide pseudo-amide oligomers containing guanidinium, urea, or thiourea recognition units (**192-193**). These elaborated cores were found to bind both dimethyl and phenylphosphate in pure D_2O.[285] The relative binding affinities were found to depend on the nature of the pseudo-amide receptor. Specifically, as the acidic character of the receptors increased, the binding affinity was found to improve. Guanidinium receptors **192c** and **193c** were found to interact most strongly with these two test anions, with binding constants in the range of 48-60 M^{-1} being calculated in the case of sodium dimethylphosphate, as inferred from ^1H and ^{13}C NMR spectroscopic analyses.

4.1.3. Other Charged Systems—In addition to the ammonium and guanidinium receptors previously discussed, a number of other charged binding moieties have been investigated in the context of phosphate recognition. For example, receptor **194** was created by Göbel and co-workers in 1992 using an amidinium group, a chemical entity bearing considerable resemblance to the guanidinium cation.[286,287] While the measured association constant was small with catechol cyclic phosphate (200 M^{-1} in DMF as inferred from ^1H NMR spectroscopic titrations), this system was found to catalyze the hydrolysis of the phosphate unit of the guest. Presumably, this reactivity was the result of the neighboring alcohol group.

Thiouronium units have also been utilized for phosphate recognition. In 1998, the Hong group explored the use of thiouronium groups in creating receptors **195** and **196**.[288] The interactions of receptors **195** and **196** with a variety of TBA:oxoanion salts were studied via ^1H NMR spectroscopy in DMSO. Binding constants were found to correlate well with basicity except for dihydrogenphosphate ($K = 1.1 \times 10^3$ M^{-1}), which bound receptor **195** nearly twice as strongly as benzoate despite having a significantly lower basicity. This difference was attributed to an increased number of oxygen atoms available to hydrogen bond to the host in the case of dihydrogenphosphate. When constrained in similar receptor geometries, the thiouronium groups were estimated to be more effective anion recognition motifs than thiourea groups. However, the thiouronium-based systems proved much less effective than guanidinium-derived receptors. The latter observation was attributed to the larger size (and hence lower charge density) of sulfur compared to nitrogen.

As with many of the previous examples, additional functionalities can be combined with thiouronium motifs to give receptors with improved selectivity. This has been nicely demonstrated by the Hong group. For example, these researchers attached a thymine subunit to thiouronium-based skeletons to obtain receptors **197-198**. This allowed for the selective transport of 5′-AMP, presumably in U-tube type experiments using a chloroform membrane.[289] In terms of design, the use of charged receptors was expected to lead to enhanced transport by creating charge neutrality in the host:guest complex, while the thymine was expected to increase selectivity for substrates containing the complementary nucleobase (i.e., adenine). As predicted, receptors **197a** and **198a** displayed significantly higher transport rates for 5′-AMP as compared to receptors **197b** and **198b**, at both pH 5.0 and pH 7.0. Bis(thiouronium) receptors **197** were also significantly more effective than receptors **198**, particularly at pH 7.0 where 5′AMP exists primarily as a dianionic species.

Lower transport rates were observed for the non-complementary nucleotide 5′-GMP. These results provide yet another demonstration of how substrate specificity may be achieved through an appropriate combination of multiple binding moieties.

A fluorescent thiouronium receptor **199** was developed by the Kubo group.[290,291] A dramatic increase in the fluorescence of compound **199** was observed upon the addition of both TBA acetate ($K > 10^6$ M^{-1}) and TBA dibutylphosphate ($K = 5.6 \times 10^4$ M^{-1}) in acetonitrile, with binding affinities corresponding well with the basicity of the anions in question. Based on control experiments, the authors suggested that the thiouronium moiety quenched the naphthalene emission through a PET process involving electron transfer from the fluorophore to the attached cationic center. This process is then precluded upon anion binding to the thiouronium moiety, which results in a higher fluorescence intensity. A more dramatic response was observed for receptor **200**. However, in this case precipitation at higher concentrations prevented determination of a binding constant for the dibutylphosphate anion. Similar results to those obtained with receptor **199** were reported with the 1,3-bis(isothiouronium)-derived naphthalene receptor **201**.[292] Here, addition of HPO_4^{2-} and acetate (as the corresponding $[K^+$-[18-crown-6] salts) led to the restoration of the fluorescence emission of receptor **201** in acetonitrile (6% water). Job plot analysis provided support for the proposed formation of a 2:1 (host:guest) complex in the case of HPO_4^{2-}.

Teramae and co-workers developed a system ostensibly similar to **199** but where the naphthalene fluorophore was attached to the thiouronium anion recognition moiety through the sulfur atom instead of the nitrogen atom (**202**).[293] An emission increase was again observed in the presence of select anions, which were studied as their K^+-[18-crown-6] salts in methanol. Strong binding was observed with HPO_4^{2-} ($K = 1.1 \times 10^4$ M^{-1}). On the other hand, acetate was found to bind approximately one order of magnitude less well. The addition of dihydrogenphosphate or chloride anions led to little or no change in the emission spectra of receptor **202**. A direct comparison between the Teramae and the Kubo systems (**199**) was not possible as different solvents (methanol *vs.* acetonitrile) were used by the two research groups.

A more elaborate thiouronium receptor system **203** was prepared by Ahn and co-workers using a tripodal core. [294] The interactions of these receptors with dianionic sulfate and trianionic phosphate were measured in methanol using ITC. Strong 1:1 complexes were observed with sulfate. However, titrations with phosphate revealed complex equilibria that could not be resolved, although evidence of binding was inferred.

Kubo, Nakhara, and co-workers examined thiouronium groups organized in monolayers at the air-water interface in efforts to sense dihydrogenphosphate.[295] In preliminary studies, dihydrogenphosphate was observed to expand monolayers of receptor **204** to a much greater degree than acetate or chloride anions. Interestingly, picrate also expanded the monolayers. In Langmuir-Blodgett (LB) films, picrate was used as an indicator and allowed the sensing of dihydrogenphosphate via absorption spectroscopy. Indeed, a large decrease in absorbed picrate was observed in the presence of the dihydrogenphosphate anion. On the other hand, little change was observed in the presence of acetate or chloride. This method allowed for the simple detection of dihydrogenphosphate in a film sensor system.

The Kubo group also examined isothiouronium groups assembled on gold nanoparticles (**205**). These studies were carried out in an effort to develop an optical sensing system for oxoanions.[296] While little color change was observed upon the addition of chloride, red-shifts in the absorption spectrum were observed in the presence of acetate, monohydrogenphosphate, and malonate in 10% water/methanol. The addition of

monohydrogenphosphate led to a 13 nm shift in the plasmon absorption band of the nanoparticle, presumably due to increased aggregation. Malonate, however, produced an even greater shift (as well as an observable color change), indicating a lack of specificity for monohydrogenphosphate in these receptors.

Imidazolium groups, which contain cationic hydrogen bond donor sites, have been studied extensively as anion binding motifs in recent years. Compound **206**, for example, was designed by K. S. Kim to investigate $(C\text{-}H)^+$ - X^- hydrogen bonds.[297] However, this system was also found to bind dihydrogenphosphate. The nitro groups were intended to decrease the electron density of the ring. By means of ^1H NMR spectroscopic titrations carried out in DMSO, a binding constant of 2.5×10^3 M^{-1} was measured for dihydrogenphosphate (TBA salt). A higher association was recorded for chloride. Receptor **207** was designed to have a larger cleft and provide a fluorescent response upon exposure to targeted anions.[298] In this case, a tweezer-like binding mode was inferred, while an association constant of 1.3×10^6 M^{-1} for the binding of TBA dihydrogenphosphate in acetonitrile was calculated from fluorescence titrations. Interestingly, receptor **207** displayed selectivity for dihydrogenphosphate over chloride. A more rigid receptor, the macrocyclic system **208**, was found to bind dihydrogenphosphate with an affinity that was similar to that of **207**. However, a much great selectivity for dihydrogenphosphate over chloride and fluoride was reported (all anions added as TBA salts).[299] A water-soluble version, receptor **209**, was then prepared. This derivative could selectively sense GTP in aqueous solutions at pH 7.4 through a chelation-enhanced quenching mechanism.[300] The greatest change in the fluorescence (a notable increase) was seen with GTP. From the spectral changes involved, a 1:1 binding stoichiometry and a binding constant of 8.7×10^4 M^{-1} was inferred. Addition of ATP and ADP also engendered an increase in the fluorescence intensity, while anions such as pyrophosphate, dihydrogenphosphate, fluoride and chloride produced no appreciable change in the fluorescence. Based on molecular modeling, the authors suggested that the selectivity for GTP over ATP was due to enhanced π-H interactions between anthracene and the respective nucleobases. Further fluorescence studies, carried out in acetonitrile, revealed that receptors **207, 208, 210**, and **211** displayed a strong preference for pyrophosphate (TBA salt).[301] The highest pyrophosphate interaction was found with receptor **208**, for which a binding constant of ca. 1.01×10^8 M^{-1} was inferred. An extensive ^1H NMR spectroscopic study provided support for the inference that the dominant interaction mode between receptor **208** and pyrophosphate involved ion pairing.

Recently, the imidazolium receptor **212**, prepared by J. Yoon and co-workers, was studied as a fluorescent chemosensor.[302] This receptor was found to have a high affinity for dihydrogenphosphate (TBA salt) in acetonitrile with a binding constant of 2.2×10^5 M^{-1} being reported. The quinoxaline-imidazolium receptors **213-216**, prepared by K. S. Kim, were found to act as fluorescence sensors, allowing for the detection of pyrophosphate as well as acetate in acetonitrile (TBA salts).[303] However, binding constants for pyrophosphate could not be determined due the insolubility of the resulting adducts. These researchers also investigated the anion binding properties of the acridine derivative **217**.[304] The effects of a number of TBA anion salts (pyrophosphate, dihydrogenphosphate, hydrogensulfate, acetate, iodide, bromide, chloride, and fluoride) on the emission spectrum of this receptor were studied in acetonitrile. Interestingly, addition of pyrophosphate led to significant fluorescence quenching, while the addition of dihydrogenphosphate led to strong emission enhancement. The other anions tested produced only small spectral changes. An association constant of 4.9×10^7 M^{-1} was determined for the binding of pyrophosphate to receptor **217** through fluorescence titrations. Job plot analysis confirmed a 1:1 binding stoichiometry, and ^1H NMR spectroscopic analyses in DMSO-d_6 proved consistent with the presence of strong imidazolium C-H hydrogen bonding interactions in this complex. The emission increase observed upon the addition of dihydrogenphosphate was attributed to the formation

of a hydrogen bond between the acridine nitrogen atom and one of the OH groups of the phosphate anion. Job plot analysis was consistent with a 1:2 host:guest binding stoichiometry, and the binding constant was estimated to be greater then 10^8 M^{-2}. In competition experiments, it was found that the emission response of receptor **217** to pyrophosphate was not effected by a 10-fold excess of dihydrogenphosphate, acetate, or fluoride. However, no response to anions was observed under aqueous conditions (9:1 CH_3CN:H_2O).

K. S. Kim, J. Yoon, and co-workers reported the anion sensing abilities of receptor **218**, containing two imidazolium recognition units and two pyrene reporter units, in 2007.[305] Quenching of both the monomer and excimer pyrene emission peaks was observed upon the addition of a variety of anions ($H_2PO_4^-$, HSO_4^-, AcO^-, I^-, Br^-, Cl^-, and F^- as TBA salts) in acetonitrile. A modest preference for dihydrogenphosphate was observed with receptor **218a**, while a high selectivity for dihydrogenphosphate was found with receptor **218b**. Similar dihydrogenphosphate binding affinities for both receptors were found, as determined through fluorescence titrations ($K \approx 2 \times 10^5$ M^{-1}).

These same researchers recently reported the tetraimidazolium receptor **219**, a system put forward as a selective fluorescent sensor for ATP.[306] In neutral aqueous solution (20 mM HEPES, pH 7.4), unique ratiometric changes in the monomer and excimer peaks of the pyrene units of receptor **219** were observed upon the addition of ATP. From these spectral changes, a binding constant of 1.03×10^4 M^{-1} was calculated for the ATP:**219** interaction. Little to no change in the emission peak ratio was reported upon the addition of dihydrogenphosphate, pyrophosphate, CTP, UTP, TTP, GTP, AMP, and ADP. Calibration curves developed for ATP worked well even in the presence of 40 equivalents of other nucleotide triphosphates. One- and two-dimensional ^1H NMR spectroscopic studies carried out in DMSO-d_6 provided support for the suggestion that the adenine moiety of ATP was inserted between the pyrene units upon complex formation. On the other hand, it was inferred that the guanine nucleobase of GTP was bound to one pyrene unit on the outside of the cleft. The significant changes in the emission spectra of receptor **219** with ATP were attributed to this difference in nucleobase-pyrene interactions. In the case of both ATP and GTP, the triphosphate unit was found to be bound to the positively charged imidazolium moieties, while the ribose subunit was considered not to interact with this receptor (**219**), as inferred from NMR spectroscopic analyses. These binding modes were further supported by density functional theory calculations of the host:guest complexes. Finally, preliminary studies in cell cultures supported the use of receptor **219** as a real-time monitoring system for ATP levels.

In 2003, Sato and co-workers reported the anion binding ability of the imidazoliophane receptor **220**.[307] Proton NMR spectroscopic titration studies carried out in DMSO-d_6 revealed a strong size and shape selectivity when anions were added as their TBA salts. Binding affinities with receptor **220** decreased in the order hydrogensulfate > bromide > dihydrogenphosphate > chloride > iodide > perchlorate. A binding constant of 1350 M^{-1} was reported for dihydrogenphosphate.

A series of imidazolium receptors with varying symmetry was reported by Schatz and co-workers (**221** - **223**).[308] All receptors were found to have a similar binding affinity for dihydrogenphosphate ($K \approx 2000$ M^{-1}) in DMSO-d_6 as inferred from ^1H NMR spectroscopic studies. This observation led these researchers to propose that only two imidazolium units of each receptor are involved in phosphate anion recognition. The highest selectivity for dihydrogenphosphate against other anions (chloride, bromide, and hydrogensulfate) was observed for receptor **223b**. All anions were studied as the TBA salts.

Multifunctional imidazolium-binol receptors **224** - **225** were reported by Yu and co-workers in 2009.[309] Receptor **224a** displayed red-shifted emission intensity peaks upon addition of acetate and fluoride and a quenching of emission intensity upon addition of dihydrogenphosphate, chloride, bromide, and hydrogensulfate (TBA salts) in acetonitrile. The spectral shifts observed for fluoride and acetate anions were attributed to deprotonation and charge-transfer effects, respectively. Binding constants were found to be on the order of acetate ≈ dihydrogenphosphate ≫ hydrogensulfate ≈ chloride > bromide. The association constant for receptor **224a** and dihydrogenphosphate was determined to be 2.46×10^5 M^{-1} under these conditions. Anion-induced quenching was also observed upon the addition of these anions to receptors **224b** and **225**; however, no binding constants were reported.

In work also involving an imidazolium subunit, the Chang group screened a small library of chloro-substituted benzimidazolium receptors of general structure **226** for their ability to bind GTP.[310] Of this set, two compounds, **226a** and **226b**, were found to display an enhanced fluorescence in the presence of micromolar GTP at pH 7.4 (HEPES buffer, 1% DMSO). These turn-on sensors demonstrated little to no response to all other nucleotides and nucleosides included in the test set. However, compound **226b** displayed significant photobleaching and was not subject to follow-up study. On the other hand, more detailed studies involving **226a** revealed that it binds GTP more strongly than 2′-deoxy-5′-guanosine triphosphate (dGTP), a finding that provided support for the sugar moiety being involved in GTP binding. Due to its visible green emission, compound **226a** was dubbed “GTP green” by this group of researchers.

Ghosh and co-workers recently developed a series of benzimidazolium-based cleft receptors (**227** - **228**).[311] Preliminary studies with mono-anthracene receptors **227** and **228** revealed quenching of the emission spectra by several anions (acetate, dihydrogenphosphate, propanoate, benzoate, mandelate, pyruvate, and fluoride) when added as the TBA salts in DMSO. The strongest signal changes were observed for acetate and dihydrogenphosphate with the urea-based receptor **228**. Spectral changes were also observed through UV-Vis spectroscopy, which was used to determine 1:1 binding constants for the host:guest complexes. Receptors **227** and **228** were found to bind dihydrogenphosphate with binding constants of 1.73×10^3 M^{-1} and 1.12×10^4 M^{-1}, respectively. While dihydrogenphosphate displayed one of the highest signal changes, carboxylate anions were generally found to have the strongest binding affinities. Proton NMR spectroscopic experiments carried out in DMSO-d_6 led to the conclusion that all N-H groups and the benzimidazolium C-H groups in these receptors were involved in anion-binding interactions.

The bis-anthracene receptor **229** was reported later.[312] In this case, fluorescence titrations revealed a strong preference for TBA dihydrogenphosphate over acetate, propanoate, benzoate, chloride, bromide, iodide, and hydrogensulfate in acetonitrile (K ($H_2PO_4^-$) = 5.41×10^3 M^{-1}). Job plot analysis revealed a 1:2 host:guest binding stoichiometry for dihydrogenphosphate. Fluoride also produced a strong decrease in emission intensity, presumably due to deprotonation. However, the sensing ability of receptor **229** was significantly diminished in aqueous methanol solution (4:1 CH_3OH:H_2O). Support for the proposed N-H and C-H hydrogen bonding interactions with dihydrogenphosphate came from standard ^{1}H NMR spectroscopic studies carried out in DMSO-d_6.

Triazolium recognition units were used by Pandey to produce deoxycholic acid-containing scaffolds **230-231**.[313] Here, click chemistry was used, and this allowed for a facile synthesis of these receptors. The binding properties were studied via ^{1}H NMR spectroscopic titrations carried out in chloroform using various TBA anion salts. While *meta*-substituted receptor **230a** displayed no appreciable binding for dihydrogenphosphate, receptors **230b** and **231** displayed binding constants of 1.10×10^3 M^{-1} and 1.92×10^3 M^{-1}, respectively. These

receptors also displayed a significant selectivity for dihydrogenphosphate over the halide anions (F^-, Cl^-, Br^-, and I^-) as well as over acetate. This binding specificity was found not to correlate with basicity, which is noteworthy. Rather, the observed selectivity trend was attributed to the slightly larger cavity of receptor **230b** as compared to compound **230a**, as well as to the greater flexibility of receptor **231**. The ease of preparation and the observed selectivity for dihydrogenphosphate makes it safe to predict that triazolium receptors have a future in the area of phosphate recognition.

A relatively novel anion recognition functionality, aminothiazolinium, was investigated in the context of the previously described "pinwheel" scaffold by T. H. Kim in 2009.[314] On the basis of ITC studies carried out in methanol, receptors **232a** and **232b** were observed to interact with AcO^-, SO_4^{2-}, and PO_4^{3-}. Despite the higher charge of the trianionic phosphate guest, the lowest binding affinity for these two receptors was reported for this analyte, with apparent binding constants of 1.34×10^4 M^{-1} and 1.81×10^4 M^{-1}, respectively. A complex binding stoichiometry was inferred for both receptors based on ITC titration data and ESI mass spectrometric analyses.

Another example of charged receptor units comes from the group of Fabbrizzi. These researchers reported the pyridinium receptors **233** and **234**, which were expected to bind with anions via a combination of electrostatic and hydrogen bonding interactions.[315] In fact, spectrophotometric studies in DMSO led to the conclusion that receptor **233** undergoes deprotonation in the presence of basic anions such as acetate, fluoride, and dihydrogenphosphate (TBA salts). The less acidic receptor **234** was only deprotonated by the fluoride and acetate anions. In contrast, 1:1 adducts were produced with dihydrogenphosphate (log $K = 3.11$).

Beginning in 1996, Powell and co-workers began a series of studies involving the fluorescent pyridinium receptor **235**, a system characterized by an excited state with charge-transfer character.[316,317] An increase in fluorescence upon anion binding was observed in phosphate buffered solution at pH 7.2. This increase was interpreted in terms of the formation of twisted intramolecular charge-transfer (TICT) complexes when receptor **235** was treated with purine nucleotides; however, no interaction was observed for pyrimidine nucleotides. Receptor **235** permitted 3′,5′-cAMP and 3′,5′-cyclic guanosine monophosphate (3′,5′-cGMP) detection in aqueous solution but did not discriminate between the two purine guests (i.e., $K = 212$ M^{-1} for 3′,5′-cAMP and 285 M^{-1} for 3′,5′-cGMP).

In 2006, Steed and co-workers reported the binding of pyridinium receptor **236** to ATP.[318] Significant shifts in the ^1H NMR spectral peaks corresponding to the pyridyl aromatic protons were observed upon the addition of Na_2ATP to receptor **236** in 1:1 acetonitrile:water (deuterated). A binding constants of 70 M^{-1} was determined using this method. No ^1H NMR spectral changes were observed upon the addition of chloride, bromide, iodide, hydrogensulfate, sulfate, dihydrogenphosphate, acetate, or nitrate in deuterated acetonitrile. These results led these researchers to suggest that receptor **236** binds ATP through a combination of electrostatic interactions and π-surface interactions.

In the same year, the Steed group examined the ability of a preorganized urea-pyridinium hybrid (**237**) to bind simple inorganic anions.[319] Binding constants were determined through ^1H NMR spectroscopic titrations carried out in DMSO-d_6 with all anions added as the corresponding TBA salts. A preference for $H_2PO_4^-$ over Cl^-, Br^-, I^-, NO_3^-, AcO^-, HSO_4^-, $CF_3SO_3^-$, and ReO_4^- was observed. Log binding constants for the 1:1 and 1:2 host:guest complexes with $H_2PO_4^-$ were found to be 3.70 and 3.68, respectively.

A pyridinium-amide receptor (**238**) bearing anthracene substituents has recently been reported by Ghosh.[320] UV-Vis and fluorescent studies revealed that **238** bound strongly

basic anions such as acetate, dihydrogenphosphate, and fluoride, with an affinity constant on the order of 10^4 M^{-1} in the case of dihydrogenphosphate in acetonitrile. These researchers further reported selective dihydrogenphosphate sensing with the biphenyl-based pyridinium receptor **239**.[321] A strong increase in the emission intensity was seen upon the addition of dihydrogenphosphate (TBA salt) to a solution of receptor **239** in $CHCl_3$ containing 2% CH_3CN. Significantly smaller changes were observed upon the addition of acetate, hydrogensulfate, and a variety of dicarboxylates as the TBA salts. The observed increase in emission was attributed to a conformational change of the receptor upon anion binding. It was proposed that dihydrogenphosphate interacts with both pyridinium moieties, thus bringing the two anthracene arms closer together and increasing excimer emission. A binding constant of 1.22×10^4 M^{-1} was measured under these conditions. At higher concentrations of dihydrogenphosphate (greater than 4 equivalents relative to host **239**), the emission intensity was seen to decrease. This decrease was attributed to the formation of 1:2 host:guest complexes at higher concentrations. Significant changes were also observed in the UV-Vis and ^1H NMR spectra upon the addition of dihydrogenphosphate to receptor **239**. However, no excimer formation was observed upon the addition of anions in mixtures of water and acetonitrile, a more competitive environment.

Another charged recognition functionality that is commonly employed for phosphate anion recognition is viologen. In 2005, Ramaiah and co-workers reported the selective binding of ATP by viologen - anthracene receptor **240**.[322] This receptor displayed reduced absorption intensity upon the addition of ATP in 10 mM phosphate buffer. UV-Vis titrations were used to measure a 1:1 binding constant of 4.04×10^3 M^{-1} for the **240**:ATP complex. No significant changes in the absorption features were seen upon the addition of adenosine, AMP, or ADP. These results provided support for the suggestion that strong electrostatic interactions contribute to the binding process. Proton NMR spectroscopic experiments were consistent with strong π-surface interactions between the adenine base of ATP and the viologen moieties of receptor **240**. In addition, complex formation was inferred from the observed shift in the reduction potential of this receptor in the presence of ATP as measured using differential pulse voltammetry.

Receptor **240** exhibited negligible fluorescence, presumably due to a PET process from the anthracene units to the viologen units. In efforts to develop a fluorescent nucleotide sensor, an indicator displacement assay was developed using receptor **240** and 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) as the indicator.[323] In this system, the fluorescence of HPTS was quenched by complexation with receptor **240**. Addition of GTP, ATP, and inosine triphosphate (ITP) led to a recovery of the HPTS fluorescence, which was attributed to displacement of the indicator. A significantly stronger response to GTP (150-fold emission increase) was observed as compared to ATP (45-fold) and ITP (50-fold). The addition of other guests (adenosine, AMP, ADP, CTP, UTP) did not result in changes to the HPTS emission. The selectivity exhibited by this system allowed for the discrimination of GTP in the presence of other nucleotides and in biological fluids.

Schiller and Singaram reported that viologen receptors **241** and **242** were able to sense glucose-6-phosphate via fluorescent IDA in 2009.[324] Binding constants were measured using HPTS as the indicator in a phosphate buffered aqueous solution at pH 7. Receptor **241** bound glucose-6-phosphate ($K = 7.5 \times 10^2$ M^{-1}) more strongly than fructose ($K = 5.6 \times 10^2$ M^{-1}), presumably due to a charge-charge interaction between the pyridinium and phosphate groups. This additional interaction thus overcomes the inherent fructose > glucose selectivity of boronic acids. However, this selectivity was reversed in the case of receptor **242**, leading to the suggestion that the charge-charge interaction was not as strong in this case. Interestingly, no binding interactions were observed for either receptor with glucose-1-phosphate or sucrose. Receptor **241** and HPTS were then employed in a bioanalytical assay

for the activity of phosphoglucomutase (PGM), which converts glucose-1-phosphate to glucose-6-phosphate.

The use of larger aromatic moieties was explored through the use of *N,N*-dialkyl-diazapyrenium units, which represent known DNA intercalators. For example, nucleotide binding was studied with receptors **243** and **244**.[325] Addition of nucleotides to the more complex system **243** in neutral aqueous solution produced significant fluorescence quenching, allowing for the derivation of binding constants. Among the set of potential guests, adenine, AMP, ADP, and ATP, the binding constants were found to increase as the number of phosphate groups increased (e.g., log $K = 2.5$ and 3.1 for adenine and ATP, respectively). Among nucleotide monophosphates AMP, GMP, CMP, and TMP, the observed binding affinity was found to increase with decreasing oxidation potential. These trends led the authors to suggest that both electrostatic and charge-transfer interactions influence the overall binding affinity.

As discussed elsewhere in this review (e.g., Section 4.1.1), hydrogen bonding between complementary nucleobases has been used in several instances to create selective receptors. Receptors **245** and **246** combine this strategy with one that allows for charge neutralization by appending a phosphonium center to a clip-like framework. These systems were found to extract and transport GMP and AMP effectively through a chloroform membrane.[326] Concentration-dependent extraction studies provided support for the conclusion that 1:1 host:guest complexes were found at pH 5.0 and 2:1 host:guest complexes at pH 7.0. In accord with design expectations, better transport rates were observed with these combined receptors than was observed with either the phosphonium or nucleobase component alone or when the individual components were both contained separately within the organic phase.

Sapphyrins, a class of expanded porphyrin derivatives, generally bind phosphates in their protonated forms and charged receptors based on these motifs are known. They are treated in Section 4.4, which includes a discussion of several other macrocyclic receptor systems.

4.2. Hydrogen Bonding

Hydrogen bonding is a stabilizing interaction that exists between an electronegative atom and a hydrogen atom bound to another electronegative atom. This type of bonding always involves hydrogen atom(s) and is generally directional. The strength of a hydrogen bond is largely determined by electrostatic attraction (e.g., dipole-dipole interactions and charge-dipole interactions), although significant contributions are also observed from charge-transfer, dispersive, and covalent forces. Therefore, the typical hydrogen bond is stronger than van der Waals forces but weaker than covalent or ionic bonds.

The use of multipoint hydrogen bonding is commonly observed in the molecular recognition of biological compounds. In supramolecular chemistry, a variety of scaffolds have been used to preorganize the hydrogen bonding components with the goal of enhancing interactions between the receptor and the functional groups of the target compound.[327,328] Highly selective systems have been developed based on these interactions, although for the most part the systems in question have been proven effective in less-competitive organic solvents. In the specific case of phosphate binding, amide and urea-based binding moieties have been the most commonly employed, reflecting their known ability to act as strong hydrogen-bond donors.[329,330] However, other subunits have been employed, as have systems containing several different hydrogen bonding functionalities. In this section, we present a discussion of neutral receptors that have been used for phosphate anion recognition. They are organized to the best of our ability according to the specific functional group involved.

Prior to commencing a discussion of individual receptor systems, it is important to point out that many neutral hydrogen bond-based phosphate receptors are plagued with low solubility. As a general rule, these receptors also display low binding affinities in competitive media (as the result of what are effectively weaker receptor-anion hydrogen bonds as compared to those involving solvent). As a result, a variety of solvents and solvent mixtures have been used in the analysis of neutral hydrogen bond-based phosphate anion receptors. Such solvent differences, however, generally prevent direct comparison of the anion binding properties of different receptor systems. It is to be further noted that, as a general rule, studies with neutral receptors have relied on the use of tetraalkylammonium salts. In most cases, these salts allow for solubility in organic media. As such, it has long been assumed that tetraalkylammonium cations are innocent and do not interfere in the recognition process. However, the validity of this assumption is starting to be called into question.[331,332] Nevertheless, unless otherwise stated, all studies in this section were carried out using tetraalkylammonium salts.

4.2.1. Amide Systems—The amide group has a time-honored place in anion recognition chemistry. This presumably reflects its ease of preparation, as well as its effective hydrogen bond donating ability. On the other hand, amide groups can also serve as hydrogen bond acceptors, and this can lead to complications in terms of receptor design.[333] Nevertheless, to date amide subunits have been incorporated into a wide variety of both cleft-like and macrocyclic receptors. Amides have also been studied on their own. For instance, the study of a simple system, *N*-methylacetamide, revealed an association constant of 26 M^{-1} for TBA dihydrogenphosphate in $CDCl_3$ as inferred from ^1H NMR spectroscopic analyses.[334] This modest affinity for a single amide unit provides a baseline against which the success of other more elaborate receptors may be judged.

In 1995, phosphate receptors **247** and **248** incorporating amide moieties were reported by Raposo and co-workers.[335] These triangular receptors were designed to be complementary to tetrahedral phosphate anions. The binding properties were studied using ^1H NMR spectroscopic methods, with the actual analyses being carried out in $CDCl_3$, CD_3OD and DMSO-d_6 as dictated by solubility considerations. In chloroform, the solubility of phenylphosphonic acid was found to be enhanced in the presence of receptor **247**, leading to the suggestion of a host:guest interaction. However, the specifics of the putative binding process were not fully characterized. No interaction was observed between **247** and a variety of phosphates in mixtures of chloroform and methanol. On the other hand, evidence for a weak interaction was found upon addition of tris(tetramethylammonium) phosphate in DMSO. In the case of receptor **248**, measurable complex stabilities were recorded in chloroform/methanol mixtures, as well as in DMSO and pure methanol. Presumably, this reflects the presence of the additional hydrogen-bond donors present in **248** as compared to **247**. Phenylphosphonic acid, propylphosphonic acid, bis(tetrabutylammonium) phenylphosphonate and tris(tetramethylammonium) phosphate were all tested as possible substrates for receptor **248**. In DMSO, the strongest interactions were observed with the most negatively charged targets, dianionic phenylphosphonate and trianionic phosphate (K = 1.5×10^4 M^{-1}, $K > 10^5$ M^{-1}, respectively). In methanol, trianionic phosphate was also found to be complexed well by **248**, with an association constant of 1.2×10^4 M^{-1} being calculated for this interaction.

In 1992, the Hamilton group examined the phosphate interactions of a set of related cleft and macrocyclic receptors containing pyridine bis-amide cores.[336] Specifically, the pyridine bis-amide clefts **249** and **250** and the corresponding macrocycle (**251**) were studied through UV-Vis and NMR spectroscopy in chloroform. While the addition of phosphotriesters resulted in no significant change in the absorption spectrum, the addition of a phosphodiester, bis-(4-nitrophenyl)phosphoric acid, led to spectroscopic changes similar to those caused by simple

pyridine protonation. In contrast to what is true for pyridines, however, the spectroscopic changes could be reversed via the addition of methanol. This then led to the conclusion that a hydrogen-bonded complex was formed following protonation. Proton NMR spectroscopic and a preliminary single crystal X-ray diffraction analysis provided support for a structure wherein the amide NH and protonated pyridine protons act to stabilize a bidentate phosphate complex similar to those observed with guanidinium receptors (discussed in detail in section 4.1.2). Shifts in the NMR spectrum were observed for the amides protons of **250b** and **251** that are closest to the phenyl spacer. Presumably, this is due to inclusion of the guest. This latter conclusion was supported by NOE studies. In addition, a Job plot constructed for the interaction of **250a** and **251** with the phosphodiester provided support for the proposed 2:1 guest:host binding stoichiometries. In analogy to what was true for the diesters, the phosphomonoester dodecylphosphoric acid was found to bind to **249** with a binding constant of 2.8×10^4 M^{-1} in chloroform. However, addition of the monoester to **250a** led to minor changes in the absorption spectrum. Further, NMR spectroscopic studies provided support for a more symmetrical complex wherein both amide protons and both pyridine nitrogen atoms participated in hydrogen bonding interactions (as hydrogen-bond donors and acceptors, respectively). However, little evidence of interactions with the proximal phenyl spacer was found. A binding constant of 1.0×10^5 M^{-1} in chloroform and a stoichiometry of 1:1 were then inferred. These results provided support for the conclusion that the four hydrogen bonds present in the complex stabilized by the cleft system of **250a** led to a stronger interaction than would be obtained using the simpler system **249**.

A series of convergent macrocyclic receptors containing amide moieties, specifically systems **252, 253**, and **254**, were also analyzed by these researchers.[97,337,338] These rigid structures provided convergent C_3 symmetric hydrogen bonding groups in the interior of the macrocyclic scaffold, a design feature that was thought to enable the macrocycles to bind tetrahedral anions with size and shape selectivity. Tetrahedral anions, such as *p*-TsO^-, HSO_4^-, and $H_2PO_4^-$, were observed to bind strongly, as inferred from ^1H NMR spectroscopic analyses, even in more competitive hydrogen bonding solvents, such as DMSO. At the same time, the binding of halides and nitrate anions proved dramatically less effective in this latter solvent. All anions were studied as the TBA salts.

Receptor **252b** displayed a 1.5×10^4 M^{-1} binding affinity toward dihydrogenphosphate in DMSO, while the linear analogue **253** displayed an affinity of only 500 M^{-1}.[337,338] The equilibrium between dihydrogenphosphate and receptor **252a**, however, proved too slow on the NMR time scale to yield an accurate binding constant. Nevertheless, these results provided strong support for the intuitively appealing notion that increasing the preorganization of a macrocyclic receptor (relative to the corresponding open-chain system) serves to increase the binding affinity substantially.

A coumarin fluorophore was attached to the basic scaffold in order to generate a selective fluorescent sensor (**252c**).[97] While system **252c** displayed only a small change in the emission spectrum in the presence of tetrahedral anions, binding affinities of 5.9×10^5 M^{-1} and 2.7×10^6 M^{-1} could nevertheless be determined for the binding of phenylphosphonate and dihydrogenphosphate (TBA salts) in a solvent mixture consisting of a 1:1 DMSO:1,4-dioxane. The fluorophore was later covalently incorporated into the macrocycle structure, producing **254**, in an effort to achieve greater spectral changes. As expected, receptor **254** displayed more dramatic spectral changes, including ones that were anion dependent. On the other hand, the actual binding affinities proved similar to those recorded in the case of **252c**. Control studies led to the suggestion that the spectral changes observed for receptor **254** were due to proton transfer from the fluorophore excited state to the anion (ESPT). A sensitive fluorescent sensor for phosphate anions was thus achieved.

Simpler but related systems were later analyzed by Gale and Paver.[339] While the simple bis-amide **255**, a compound earlier developed by Crabtree as a halide anion receptor,[340,341] was not found to bind dihydrogenphosphate, the coordination of two metal carbonyl fragments to the phenyl groups (**256**) led to a weak interaction with dihydrogenphosphate. A binding constant of 119 M^{-1} was determined from ^1H NMR spectroscopic titrations carried out in CD_3CN. The increased binding was attributed to the electron withdrawing nature of the coordinated metals, which rendered the amides better hydrogen bond donors. However, the highest interactions were observed with halide anions.

In further studies, Gale and co-workers examined the effect of incorporating an anthraquinone moiety at the central phenyl ring giving receptor **257**.[342] The interactions of this latter receptor and the control compound **258** with TBA anion salts were then studied via ^1H NMR spectroscopic titrations carried out in DMSO-d_6-0.5% water. Under these conditions, receptors **257** and **258** displayed significantly higher binding constants for dihydrogenphosphate than the chloride, benzoate, hydrogensulfate, or bromide anions. The anthraquinone derivatives generally bound dihydrogenphosphate more strongly than the phenyl derivative. It was proposed that receptors **257** adopted a twisted conformation as a result of steric clashes with the anthraquinone oxygen atoms and that this conformation was appropriate for hydrogen bonding interactions with dihydrogenphosphate. Particularly strong binding was observed upon the incorporation of electron withdrawing substituents in receptor **257c**, which displayed a 1:2 host:guest stoichiometry (K_1 = 1520 M^{-1}, K_2 = 65 M^{-1}).

Lees and co-workers studied the behavior of the amide receptors **259** - **261**, which incorporated bis(4,4′-di-*tert*-butylbipyridine) rhenium complexes.[343,344] Receptor **259a**, a luminescent cleft-like receptor, was found to act as fluorescent anion sensor, displaying a high preference for halides, cyanide, and acetate anions as inferred from fluorescence quenching studies carried out in dichloromethane. On the other hand, the interaction with dihydrogenphosphate was found to be weak, characterized by a binding constant of 147 M^{-1} in this same solvent. Interestingly, dihydrogenphosphate complex formation was slightly weaker with the 2-substituted bipyridyl receptor system **259b** (K = 110 M^{-1}) as compared to **259a**. On the other hand, much stronger binding was observed with the phenyl-linked receptor **259c** ($K = 1.8 \times 10^5$ M^{-1}), linear receptor **260** ($K = 5.5 \times 10^4$ M^{-1}), and monotopic receptor **261** ($K = 1.6 \times 10^4$ M^{-1}). This trend was not observed with any of the other anions tested.

The multifunctional amidopyridine receptor **262** and several other two-arm receptors (e.g., **263** and **264**) were studied by the Fang group.[345,346] It was proposed that the central pyridine moiety serves to restrain the rotation of the appended amide bonds through hydrogen bonding, leading to the formation of the cleft-like structure shown explicitly in the case of **262**. This conformation was confirmed through a single crystal X-ray diffraction analysis of **262a**, a study that revealed a pseudo-tetrahedral geometry characterized by multiple hydrogen bonding sites. Proton NMR spectroscopic studies with TBA anion salts revealed that both **262a** and **262b** bound $H_2PO_4^-$ in DMSO-d_6, with association constants of 549 M^{-1} and 1374 M^{-1} being recorded in the case of these two receptors, respectively. The corresponding association constants for acetate were found to be 3 to 6-fold lower than those of $H_2PO_4^-$. Other studied anions (NO_3^-, Br^-, I^-, SCN^-, ClO_4^-, $H_2SO_4^-$) displayed little response.

The pyrene-bearing receptor **262b** was found to display both monomer and excimer emission in THF in the absence of anion. Upon addition of $H_2PO_4^-$ or PO_4^{3-} in THF, a reduction in the excimer emission and an increase in the monomer emission was observed. Based on control studies, the authors suggested that this change was due to an increase in the

distance between the two arms that occurred upon phosphate anion complexation. Association constants for complexes of **262b** with $H_2PO_4^-$ and PO_4^{3-} were calculated to be 1.8×10^5 M^{-1} and 3.3×10^5 M^{-1}, respectively. While weaker binding was generally observed in DMSO, it was found that hydrogenpyrophosphate displayed significant binding ($K = 1.1 \times 10^4$ M^{-1}) in this highly competitive solvent. The fluorescence response proved selective for phosphate anions over all other anions tested.

More recent studies examined the ability of receptor **262d** to bind the metabolite geranyl pyrophosphate (GPP) in DMSO.[347] Among the series of receptors **262b-e**, receptor **262d** displayed the strongest binding affinity for GPP (3300 M^{-1}) and di(hexadecyl)phosphate (DHP, 1830 M^{-1}) (TBA salts), as inferred from ^1H NMR spectroscopic experiments. The increased affinity of receptor **262d** for these analytes relative to **262c** (a system containing four aliphatic substituents) was attributed to preorganization of the receptor cavity by the pyrene moieties of **262d**. Receptor **262b** displayed the strongest binding affinity for dihydrogenphosphate among this series. In emission studies, no change in the fluorescence of receptor **262d** was observed upon the addition of GPP. A FRET-based displacement assay was then developed in which coumarin phosphate was first complexed with receptor **262d** to form a strong FRET pair. Upon displacement of coumarin phosphate from the receptor by GPP, a decrease in FRET efficiency was observed. This change was used to determine a binding constant of 2.3×10^4 M^{-1} in DMSO, a significantly higher value than that observed through ^1H NMR spectroscopic methods. This sensing ensemble was selective for the binding of GPP over simple anions (fluoride, chloride, bromide, thiocyanate, acetate, nitrate, and perchlorate) as well as carboxylate ions of fatty acids. The increased signal for GPP over other anions was attributed to hydrophobic interactions between the host and guest aliphatic tails and multi-point hydrogen bonding interactions between the phosphate unit and the amide units of receptor **262d**.

In separate work, Fang and co-workers synthesized receptor **263**, a system that contains both electrochemical and fluorescent sensing units.[348] A control receptor bearing a single arm (**264**), was also prepared and analyzed. Proton NMR titrations of receptor **264** with a variety of TBA anions in DMSO provided evidence for 1:1 binding interactions. The associated affinity constants were found to range from $1.5\text{-}2.5 \times 10^2$ M^{-1}, with a preference for dihydrogenphosphate being seen over acetate, iodide, nitrate, perchlorate, and hydrogensulfate. In contrast to what was seen for the control system **264**, the two-armed receptors (**263**) displayed a 1:2 host:anion stoichiometry. Based on ITC measurements carried out in THF, the authors suggested that the binding behavior of both types of receptors was favorable in both enthalpic and entropic terms, with the association constants of **263b** ($K_1 = 1.97 \times 10^5$ M^{-1}, $K_2 = 8.30 \times 10^3$ M^{-1}) being much higher than those of **264b** ($K = 9.10 \times 10^3$ M^{-1}). In the end, both receptors proved to be effective sensors with a fluorescence response to dihydrogenphosphate being observed for receptor **263c** and an electrochemical response to dihydrogenphosphate being observed in the case of **264a**.

In 2004 Kovalchuk and co-workers reported a different pyridine bis-amide cleft-type receptor. The system in question, **265**, was designed to serve as a charge-transfer type fluorescent sensor.[349] As designed, the central amidopyridine unit was expected to act as the anion receptor while the two styryl chromophores would participate in donor-acceptor interactions. Of the anions tested, only $H_2PO_4^-$ and acetate (TBA salts) led to an enhancement in the receptor fluorescence, at least when tested in DMSO:water (95:5) mixtures. This enhancement allowed binding constants of 55 M^{-1} and 74 M^{-1} to be determined for acetate and dihydrogenphosphate, respectively. Since other potentially complexing anionic species, including halide, sulfate, nitrate, cyanide and carboxylate anions, did not produce such an enhancement, it was proposed that, in spite of the low

binding constants, the relatively specific, on-off fluorescence response displayed by **265** would make it a potentially useful anion sensor.

A slightly different combination of amides and pyridine was used to create receptors **266** and **267**, reported by Kondo and co-workers in 2005.[350] Within this set, the strongest interaction with dihydrogenphosphate (added as the TBA salt) was observed in the case of **267c**. This finding provided support for the notion that the pyridyl groups participated in the binding event. It was further postulated that the four amide protons bound the carbonyl-type and anionic oxygen of the bound phosphate while the pyridyl hydrogen atoms interacted with the protonated oxygen groups of the $H_2PO_4^-$ guest. The corresponding binding affinity was determined via standard UV-Vis titrations, yielding a binding constant of 1.20×10^5 M^{-1} in 10% DMSO-CH_3CN. Receptor **267c** also displayed high selectivity, binding dihydrogenphosphate 85-fold more strongly than either acetate or sulfate.

The inclusion of a carbazole moiety within a cleft-like arrangement was used by Jurczak and co-workers to generate receptors **268a** and **268b**. Here, the goal was to create a receptor system containing an additional hydrogen bond donor compared to the earlier systems, while at the same time retaining a high level of rigidity.[351] The chlorine substituents present in **268** were meant to increase the hydrogen bond-donating ability by withdrawing electron density. The binding behavior of **268a** and **268b** was studied using standard ^1H NMR spectroscopic methods and various TBA anion salts. In DMSO-d_6 containing 0.5% H_2O, both receptors **268a** and **268b** were found to be selective for dihydrogenphosphate, displaying relative affinities of $H_2PO_4^- > PhCOO^- \gg Cl^-$. The selectivity over chloride was attributed to the cleft being too large for the smaller anion, as judged from a single crystal X-ray diffraction analysis. Interestingly, the aliphatic amide system (**268b**) was found to bind anions more strongly than its aromatic analogue (**268a**). The reason for this difference was not, however, discussed in detail.

Sessler and co-workers have reported the diamidopyrazole receptor **269**.[352] However, quantitative ^1H NMR spectroscopic analyses could not be carried out due to the formation of aggregates at the concentrations required for study. On the other hand, ITC titrations carried out in DMSO provided evidence that receptor **269** bound dihydrogenphosphate ($K = 5.76 \times 10^5$ M^{-1}) in preference over benzoate, perchlorate, cyanide and bromide; however, receptor **269** was also found to interact strongly with hydrogensulfate. All anions were studied as the TBA salts.

Early on, more flexible receptors, based on the use of diethylenetriamine and tren (**78**) scaffolds, were reported by Reinhoudt and co-workers.[353] The systems in question, receptors **270-272**, were described in 1993 and were found to display a selectivity for dihydrogenphosphate over chloride and hydrogensulfate anions, as inferred from ^1H NMR spectroscopic analyses carried out in chloroform (all anions studied as the corresponding TBA salts). Host:anion 1:2 adducts were established with dihydrogenphosphate, and this stoichiometry was attributed to the formation of an intermolecular hydrogen bond between both dihydrogenphosphate guests. Receptor **272b** displayed the highest affinity of the series ($K(H_2PO_4^-) = 1.4 \times 10^4$ M^{-1}). This finding was rationalized in terms of the relatively increased preorganization of its binding pocket as the result of: 1) interactions involving the π-surfaces of the naphthyl units and 2) the more acidic character of the sulfonamide NH protons compared to the corresponding amide analogues.

Hiratani and co-workers incorporated a hydroxynaphthamide moiety in receptors **273** in order to provide additional hydrogen bonding sites as well as a signaling moeity.[354] Upon addition of tetraethylammonium fluoride, TBA dihydrogenphosphate, and TBA acetate to **273a** in acetonitrile, a strong increase in emission at 486 nm was observed. A red-shift in the

emission maximum as well as an increase in intensity was also observed when these anions were added to a solution of **273b**. However, in the case of dihydrogenphosphate the spectral changes could not be fit to a simple binding profile, precluding calculations of the association constants.

Phenylborate and anthracene moieties were included in macrocyclic **274**, a system also prepared by Hiratani.[355] Fluorescence titrations carried out in acetonitrile:chloroform (9:1) revealed that receptor **274** interacted with dihydrogenphosphate more strongly than with acetate, sulfate, chloride, bromide and iodide (all anions studied as their TBA salts). In this solvent mixture, the association constant was found to be on the order of 10^4 M^{-1} for dihydrogenphosphate. Proton NMR spectroscopic studies led to the conclusion that the hydroxy, amide, and ether groups all participated in hydrogen bonding interactions with the bound dihydrogenphosphate guest. On this basis, the increase in the fluorescence intensity was attributed to a Lewis acid interaction with the phenylborate that, in turn, served to overcome the otherwise inherent PET quenching of the anthracene fluorescence.

Cleft receptors based on 2,2-biimidazoles (**275**) with various amide groups at the 4- and 4′-positions were studied by the Allen group.[356] The intrinsic fluorescence of the biimidazole unit was quenched, without an emission shift, upon addition of dihydrogenphosphate or chloride (TBA salts) in CH_2Cl_2. In this study, 1:1 complexes were formed with association constants on the order of 10^4 M^{-1} for both $H_2PO_4^-$ and Cl^-. Receptor **275b** proved to be the strongest binding receptor, while receptor **275f** was found to be the weakest. The receptor-based differences in affinities were found to be small for dihydrogenphosphate (ca. 3-fold) but higher for chloride (ca. 100-fold). Bromide and nitrate anions produced a much smaller change. An effort was made to probe whether the syn or anti conformation of the amide groups gave rise to higher affinities. However, solution state ^1H NMR spectroscopic studies proved uninformative. On the other hand, a single crystal X-ray diffraction analysis revealed that the anti conformation was the preferred conformer, at least in the solid phase.

The use of diphenylglycouril as a scaffold for the formation of concave phosphate binding clefts, such as receptors **276** and **277**, was reported by Kang.[357,358] While the strongest binding interactions were observed with acetate and benzoate (K values on the order of 10^4 M^{-1} in CH_3CN), a binding constant of 1.6×10^3 M^{-1}, corresponding to the interaction between receptor **276** and dihydrogenphosphate was measured by means of UV-Vis titrations carried out in acetonitrile. All anions were studied as the TBA salts. In the case of receptor **276**, the binding affinities were found to correlate well with anion basicity. Receptor **277** contains a built-in fluorophore. It thus allowed anion binding to be monitored by following the substrate-induced emission quenching in acetonitrile. Using this receptor and this solvent, a variety of anions were found to be bound, although only bromide was found to bind more strongly than dihydrogenphosphate (K ($H_2PO_4^-$) = 7.5×10^4 M^{-1}). The relatively good selectivity for dihydrogenphosphate displayed by **277** was attributed largely to the size of this anion relative to others studied.

The fipronil-based receptor **278** was recently prepared by Qian and Li.[359] This system was found to interact with highly basic TBA anion salts, such as acetate, fluoride and dihydrogenphosphate, through deprotonation. UV-Vis titrations allowed for the determination of what can be considered pseudo-association constants in acetonitrile, with a value of 2.27×10^5 M^{-1} being recorded for dihydrogenphosphate. However, the highest interaction was found with acetate.

Several macrocyclic amide systems have also been analyzed for their anion binding properties, in particular by Bowman-James and co-workers. Strong binding interactions with receptor **279a** were observed for both dihydrogenphosphate and hydrogensulfate as

determined by ^{1}H NMR spectroscopic titrations carried out in $CDCl_3$ (log K = 4.50 and 4.66, respectively), while other anions were found to bind two or three orders of magnitude less well (all anions added as the TBA salts).[360] While 1:1 complexes were observed in solution for these two anions, single crystal X-ray diffraction analysis revealed 2:1 macrocycle:anion sandwich complexes. The binding affinities were attributed to efficient geometric complementarity between the amide hydrogen atoms and the anion oxygen atoms, as inferred from the solid-state structural studies.

A follow-up report detailed the anion binding ability of the pyridine and thioamide derivatives **279b-d** as well as bicyclic derivatives **280**.[361] Proton NMR spectroscopic studies in DMSO-d_6 revealed the formation of 1:1 complexes with a number of anions studied as the TBA salts. In general, the strongest binding interactions were observed for dihydrogenphosphate, hydrogensulfate, and fluoride with chloride, bromide, iodide, nitrate, and perchlorate being bound significantly less well if at all. Thioamide monocycles **279c-d** were found to bind anions more strongly than the respective amide derivatives **279a-b**. However, the opposite trend was observed for bicyclic receptors **280**. Here, weaker binding affinities were observed for the thioamide derivative **280b** relative to the amide derivative **280a**. Such differences were attributed to increased intramolecular hydrogen bonding interactions between the thioamide groups on different arms of the receptor, thus leading to a more compact cavity. In support of this latter conclusion was the finding that fluoride was bound by receptors **280** with high selectivity over other anions.[362] The highest measured phosphate affinity in these studies was seen for **279c** and dihydrogenphosphate (log K = 4.97). Macrocycle **279c** was also found to display significant selectivity for dihydrogenphosphate over hydrogensulfate (log K (HSO_4^-) = 3.15).

Separate analysis revealed that the corresponding quaternary ammonium receptors **281a** and **281b** displayed, in general, higher anion affinities than receptors **279**. This finding was ascribed to the combination of hydrogen bond and electrostatic interactions available with the former macrocycles.[363] Indeed, ^{1}H NMR spectroscopic titrations performed in DMSO-d_6 revealed that receptors **281a** and **281b** interacted well with dihydrogenphosphate (as the TBA salt), with log K = 4.06 and 5.32 for these two systems, respectively.

Optimization of cryptand-based binding was pursued through expansion of the core of receptor **280a** producing receptor **282** and quaternization of the ammonium centers giving receptor **283**.[364] Single crystal X-ray diffraction analyses revealed a capsule shape for receptor **282** but a bowl conformation for receptor **283**. Despite its expanded core, receptor **282** was found to be selective for fluoride, in analogy to what was seen for receptor **280a**, as inferred through ^{1}H NMR spectroscopic titrations carried out in DMSO-d_6 using the TBA anion salts. Receptor **283**, on the other hand, was found to be highly selective for dihydrogenphosphate under these conditions ($K = 1.2 \times 10^4$ M^{-1}). This latter receptor was found to bind dihydrogenphosphate with a four-fold selectivity relative to chloride and a 35-fold selectivity relative to hydrogensulfate. Expanded monocyclic structures (**284**) were also explored under these conditions.[365] These analyses revealed that receptor **284b** bound dihydrogenphosphate with an affinity constant of 4.4×10^3 M^{-1}; however, this value was lower than the association constant obtained with the analogous receptor **279b**. Macrocycle **284b** was found to be selective for hydrogensulfate and displayed weak binding for fluoride, chloride, bromide, and acetate. Single crystal X-ray diffraction analysis of the complex of **284b** and dihydrogenphosphate revealed the binding of two guest molecules in a dimeric fashion within the receptor cavity (Figure 6). In this case, each dihydrogenphosphate molecule participated in two hydrogen bonds with neighboring amide substituents as well as two hydrogen bonds with the other guest molecule. On the other hand, relatively small anion affinities were observed for *N*-substituted receptors **284a** and **284c**.

Nearly contemporaneously with the studies of Bowman-James, the anion binding behavior of the amido-macrocyclic receptors represented by structure **285** was being studied by Jurczak and co-workers.[366-368] This latter set of macrocycles allowed the effect of size on selectivity to be probed. It was found to be quite pronounced despite the relatively high conformational flexibility of the receptors in question. The 20-membered macrocycle (**285b**) was found to bind the tested anions (TBA salts of Cl^-, Br^-, CN^-, $PhCOO^-$, AcO^-, $H_2PO_4^-$, *p*-NO_2-Ph-O^-, and HSO_4^-), more strongly than **285a** or **285d** in DMSO-d_6 as determined by ^{1}H NMR spectroscopic analysis (K (**285b**:$H_2PO_4^-$) = 7.41×10^3 M^{-1}). It was proposed that receptor **285b** embodied the best balance of flexibility and preorganization among the series. Receptor **285c** was not sufficiently soluble in DMSO to allow for the determination of binding constants. Furthermore, dihydrogenphosphate selectivity was reported for receptors **285b** and **285d** while receptor **285a** exhibited selectivity for acetate. These trends were attributed to differences in the geometric complementarity of the host:guest systems. The binding behavior of receptor **285b** and the linear analogue **286** with chloride, acetate, and dihydrogenphosphate (TBA salts) was also compared. Similar selectivity but significantly lower binding affinities (ca. 20 - 40 fold) were observed. Proton NMR spectroscopy and single crystal X-ray diffraction analysis led to the suggestion that linear receptor **286** exists largely as a folded structure with significant intramolecular hydrogen bonding interactions in the absence of an anionic guest. The lower binding affinities displayed by **286** were attributed in part to the structural reorganization required for guest binding. The influence of the pyridine ring present in receptor **285b** on anion binding was studied through the analysis of phenyl derivatives **287** and **288**.[369,370] On this basis it was determined that the bis-phenyl receptor **287** was a less efficient host. The reduced binding ability was attributed to intramolecular hydrogen bonds between the distal amide units in the free receptor. These interactions were thought to lead to a high energetic cost of reorganization upon anion binding. Hybrid receptor **288** was observed to binding anionic guests more strongly than **287** and **285b**, presumably due to a more favorable conformation of the free host. Similar selectivity was reported for all three receptors ($H_2PO_4^-$ > $PhCOO^-$ > Cl^- > Br^- > HSO_4^-). On the basis of ^{1}H NMR spectroscopic titrations, the binding constant corresponding to the interaction between receptor **288** and dihydrogenphosphate was estimated to be greater than 8.0×10^3 M^{-1} in DMSO-d_6 and 2.6×10^3 M^{-1} in DMSO-d_6 / 5% water.

The amide-based macrocyclic sensor **289** was also studied by the Jurczak group. This system, which contains a built-in chromophore, allowed for the colorimetric detection of $H_2PO_4^-$, as well as F^- and AcO^-, in both DMSO and CH_3CN solutions.[371] The response was found to be both anion and solvent dependent for the aforementioned anions. In DMSO, the binding affinities followed the order of the anion basicities, such that $F^- \gg AcO^-$ > $H_2PO_4^-$. However, dihydrogenphosphate was found to bind more strongly than acetate (K = 4.2×10^3 M^{-1} *vs.* 5.03×10^2 M^{-1}, respectively) in acetonitrile. This effect was attributed to the additional hydrogen bond interaction available between the OH group of the phosphate and an ether oxygen of receptor **289**. This hydrogen bond would be disrupted by the solvent to a much greater extent in DMSO than in acetonitrile. Interestingly, the three test anions, namely F^-, $H_2PO_4^-$, and A_2O^-, produced three distinct colors when added to colorless solutions of receptor **289** in both DMSO and acetonitrile. On the other hand, solutions of **289** remained clear when treated with Cl^-, Br^- and HSO_4^-. All anions were studied as the TBA salts.

In 2004, Costero and co-workers studied the anion binding of amide-containing macrocycles **290** and **291**.[372] While all of these receptors were found to bind fluoride anion strongly, only macrocycle **290** was found to interact appreciably with larger anions such as chloride, acetate, and dihydrogenphosphate as inferred from ^{1}H NMR spectroscopic experiments carried out in CD_3CN (all anions studied as the TBA salts). The results obtained were found

to be consistent with the presence of hydrogen bonding interactions between the amide moieties near the pyridine unit and these larger anions.

He and co-workers studied the anion binding ability of hybrid amine-amide macrocycle **292** in the same year.[373] Interactions of fluoride, chloride, dihydrogenphosphate, acetate, bromide, and iodide (as the TBA salts) were studied by fluorescence and ^{1}H NMR spectroscopy in chloroform. Emission quenching was observed on the order fluoride > chloride > dihydrogenphosphate ≈ acetate while bromide and iodide did not affect the emission intensity. A similar trend was found in the binding constants determined through fluorescence titration. The dihydrogenphosphate binding affinity was measured to be 1.39×10^4 M^{-1}. Proton NMR spectroscopic experiments were consistent with the presence of hydrogen bonding interactions with both the amine units and the amide units of these host:guest complexes.

Several recent studies have examined more complicated amido macrocycles. For example, ditopic receptors **293** and **294** were prepared by Morey and co-workers and analyzed using an indicator displacement assay.[374] Naked eye anion detection was achieved via the addition of potassium picrate to a 1:1 DMSO:H_2O solution of these receptors, followed by the addition of the anion salts of interest. In addition to sodium phytate, TBA salts of $H_2PO_4^-$, GMP, and ADP could be visually detected in aqueous DMSO solution through a yellow-to-green color change. On the other hand, the TBA salts of Cl^-, Br^-, I^-, NO_3^-, and HSO_4^- engendered no change in the yellow color of the solution.

Another amide macrocycle, the dithioxamide receptor **295**, developed by Gupta, was used to produce a phosphate-selective electrode.[375] This system, which involves incorporation of the receptor into a PVC membrane, was found to have a working detection range of 1.7×10^{-6} to 1.0×10^{-2} M at pH 8. Semi-empirical calculations supported the assumption that all four amide hydrogen atoms participate in phosphate binding.

A larger polyamide macrocycle, receptor **296**, was synthesized by Cheng and co-workers. This system is noteworthy in that the closed ring contains a disulfide bridge derived from oxidize cystine groups.[376] UV-Vis studies in acetonitrile provided support for the inference that this receptor binds dihydrogenphosphate, although a preference for acetate and fluoride anions was also observed (anions added as TBA salts). Due to interference from aggregation, the interactions with dihydrogenphosphate could not be assessed in a quantitative manner.

A different approach to anion detection was explored by the Watanabe group. In this work, a gold nanoparticle surface modified with amide ligands (**297**) was tested as a possible optical sensor for a number of different anions, added as the TBA salts.[377] An increase in the plasmon absorbance band at 520 nm was observed with the addition of up to 0.5 equivalents of dihydrogenphosphate in dichloromethane. This effect was attributed to anion-induced aggregation of particle **297** as the result of hydrogen-bond interactions between the anion and interparticle amide ligands. Further addition of anions to the solution caused a decrease in absorbance, reflecting the deaggregation of the supranananoparticles composed of compound **297** and anions. Compound **297** was capable of acting as an optical sensor and detecting changes in the anion concentration at the 10^{-6} M level. This constitutes a three order of magnitude increase in the detection sensitivity relative to what can be obtained using the constituent, neutral amide ligands. A preference for HSO_4^{2-} and $H_2PO_4^{2-}$ over other anions was observed, a finding that was interpreted in terms of hydrogen-bonding interactions mediating the receptor-anion binding events and the nanoparticle aggregation phenomena.

4.2.2. Urea and Thiourea Systems—Urea and thiourea groups are widely used in the design of artificial anion receptors. These moieties provide two hydrogen bond donors within a single functionality, with the consequence that these motifs often provide for effective anion recognition. As a general rule, thiourea-based systems give rise to stronger $H_2PO_4^-$ binding than do those containing ureas. This can be rationalized by examining the pK_a of the thiourea group. It is roughly 6-9 pK_a units lower than the pK_a of the urea group. This difference makes thioureas much stronger hydrogen bond donors than ureas but also makes the corresponding receptors more susceptible to deprotonation. The use of thiourea groups offers additional advantages, including generally enhanced solubility in lower polarity solvents and a lower propensity to undergo self-association. Presumably, these features reflect in part the lower hydrogen bond acceptor ability of sulfur versus oxygen.[378]

In 1992, the Wilcox group reported phosphate binding studies of the urea-based cleft receptor **298**.[379] Proton NMR spectroscopic studies supported the predicted anion-receptor hydrogen bonding interactions, while UV-Vis titrations carried out in chloroform were used to measure binding affinities. Binding constants were found to be on the order of $10^3 - 10^4$ M^{-1}, with diphenylphosphate binding less strongly than benzoate but more strongly than tosylate. Each anion was tested in the form of its TBA salt.

In 1994, Kelly and M. H. Kim reported the mono and bisurea receptors **299** and **300**. These rather rigid acyclic systems were screened for their ability to bind a variety of TBA phosphate salts under different solvent conditions by means of ^{1}H NMR spectroscopy.[380] As expected, the binding constants recorded in DMSO-d_6 proved significantly lower than those obtained in $CDCl_3$. It was found that the selectivity correlated well with basicity, such that, in the case of **299**, phenylphosphonate and phenylphosphate bound more strongly than phenylsulfonate but less well than benzoate. Similar results were observed with ditopic anions, such as phenyldiphosphonate and receptor **300**. Here, however, much higher association constants were observed than would be expected given the increased charge of the anionic guests. For instance, receptor **300** was found to bind phenyldiphosphonate with an association constant of 3.4×10^5 M^{-1} in DMSO-d_6 *vs.* 140 M^{-1} for phenylphosphonate.

The Alcázar group examined both tris(2-aminoethyl)amine and 1,3,5-tris(aminomethyl)cyclohexane as spacers for the construction of tripodal urea receptors. Using these building blocks, they produced systems represented by structures **301** and **302**.[381] An association constant of 1.1×10^4 M^{-1} was measured for tris(tetramethylammonium) phosphate with the more flexible receptor **301a** as determined by ^{1}H NMR spectroscopic titrations carried out in DMSO-d_6. Studies with system **302**, a receptor containing a more rigid spacer, resulted in a decreased association constant. This result was attributed to the cavity size being too large for this particular anion. Interestingly, the thiourea analogue of **301a** (receptor **301b**) also displayed a lower association constant for trianionic phosphate than did **301a**, with the difference being roughly one order of magnitude. Finally, an association constant of 1.0×10^2 M^{-1} was measured for the water-soluble analogue **301c** under conditions where trianionic phosphate was added to water. However, no pH or buffer conditions were reported for these latter experiments. Additional binding functionality was incorporated through the use of chromane groups. The resulting receptor, **303**, was found to bind tris(tetramethylammonium) phosphate roughly five-fold more effectively than receptor **301a**. It is important to note the use of trianionic phosphate as a guest in these studies, which is expected to give rise to binding affinities that are substantially enhanced relative to what would be expected for dihydrogenphosphate based on simple electrostatic considerations.

The pentafluorophenyl substituted receptor **301d**, synthesized by Ghosh and co-workers,[382,383] provided support for the intuitively reasonable supposition that the presence

of the electron withdrawing groups on the phenyl rings would lead to higher anion binding affinities. In fact, receptor **301d** was found to display a preference for dihydrogenphosphate (studied as the TBA salt), binding this substrate with a log $K = 5.52$ in DMSO-d_6 as inferred from ^{1}H NMR spectroscopic studies. A single crystal X-ray diffraction analysis revealed that dihydrogenphosphate was encapsulated within the C_{3v} symmetrical cavity of receptor **301d**, being held in place by a combination of seven hydrogen bonds and what were taken to be two additional intermolecular anion-π interactions.

Ganguly and Das recently reported receptor **301e** that contains nitrobenzene as a built-in chromophoric subunit.[384] In an acetonitrile/water mixture (95/5, v/v) this receptor was found to bind the dianionic sulfate anion in preference over other anions; however, strong interactions were observed with other tetrahedral anions including dihydrogenphosphate (log $K = 4.26$), as inferred from UV-Vis spectroscopic titrations. All anions were studied as the TBA salts, added as commercially available aqueous solutions.

Another tris (2-aminoethyl)amine-derived compound, the fluorescent tripodal urea receptor **304**, was designed and tested by the Wu group.[385] In this case, a significant enhancement in the fluorescence intensity was observed upon the addition of TBA anion salts in DMF, with the derived association constant being 1.1×10^4 M^{-1} for dihydrogenphosphate. Hydrogensulfate was found to bind significantly more weakly, and bromide and iodide anions displayed no spectral change. Based on control studies, the authors suggested that the spectral response and the strong binding affinity resulted from protonation of the central tertiary amine, an event that would lead to a reduction in the PET quenching and a strong charge-charge association within the resultant complex (**304**-$H_2PO_4^{2-}$).

A more rigid, tribenzyltrindane C_{3V} -symmetric scaffold (**305**) was later reported by the Choi group.[386] This scaffold allowed for the preorganization of all three urea arms on the same side of the receptor. The binding properties were analyzed via ^{1}H NMR spectroscopy in DMSO-d_6 using various TBA anion salts. On this basis, it was determined that receptor **305** binds dihydrogenphosphate with an association constant of 5.21×10^2 M^{-1}. Other anions (nitrate, hydrogensulfate, chloride, bromide and carbonate) were found to bind with much lower affinities.

In 1997, the Umezawa group studied the anion binding ability of fluorescent urea receptor **306** through its incorporation into a PVC membrane that was immobilized on a glass slide.[387] Presumably as the result of the appended cytosine moiety, this receptor was found to display a preference for guanosine derivatives over other nucleotides. Further, this system was found to function as an efficient fluorescent sensor for 5′-GTP, displaying specificity over 5′-ATP in an aqueous buffer (10 mM tris/ HCl at pH= 7.4). Receptor **306** was also incorporated into a monolayer for channel mimetic sensing.[388] In this case, selective binding of 5′-GMP over 5′-AMP was manifested in terms of a reduced permeability of the monolayer to $[Fe(CN)_6]^{4-}$ when it was analyzed by CV in the presence and absence of the anions. Interestingly, systems based on receptors containing a urea moiety were found to display an increased selectivity when compared to monolayers based on cytosine derivatives. Glassy electrodes based on receptor **306** were found to be unstable.

Reinhoudt and co-workers developed a series of multidentate urea receptors (**307** – **308**), which allowed them to probe the effect of substituents as well as the effect of ring closure on the anion affinities.[389] All anion interactions were studied in DMSO-d_6 by means of ^{1}H NMR spectroscopic titrations using the corresponding TBA anion salts. Each receptor displayed significant spectral shifts with $H_2PO_4^-$ but little to no change in the presence of Cl^-, Br^-, NO_3^-, or HSO_4^-. While association constants could not be determined with receptors **307a** or **307c** due to what were considered to be indeterminate binding

stoichiometries, 2:1 guest:host binding was observed for **307b** and **307d** with association constants near 10^7 M^{-2}. In this case, the thiourea moiety of **307d** did not improve binding for dihydrogenphosphate relative to **307b**. On the other hand, its presence did lead to a modest affinity for the chloride anion. In contrast, systems **308**, macroyclic analogues of **307**, were found to bind $H_2PO_4^-$ with a 1:1 binding stoichiometry and with affinities near 10^3 M^{-1}. This latter system displayed a low binding affinity for chloride. In fact, receptor **308b** displayed a greater than 100-fold selectivity for dihydrogenphosphate over chloride. On the basis of NMR spectroscopic experiments, the relatively low affinity for chloride displayed by these receptors was rationalized as being due to the fact that only the outside urea moieties can participate in chloride binding, whereas all four urea moieties can participate in dihydrogenphosphate binding. Certainly, it appears safe to conclude that the number and spatial organization of the hydrogen bonding units within receptors **307** and **308** play a critical role in determining their selectivity.

Gale and co-workers later explored the anion binding ability of the internal bisphenylurea binding unit of receptors **307** and **308** (**309a**) as well as the related cleft receptors **310** and **311**.[390] The anion binding ability of these receptors was explored using ^{1}H NMR spectroscopy in DMSO-d_6 / 0.5% water with all anions added as the TBA salts (Cl^-, Br^-, AcO^-, $PhCOO^-$, $H_2PO_4^-$, and HSO_4^-). Receptor **309a** displayed a significantly lower binding affinity for dihydrogenphosphate ($K = 7.32 \times 10^2$ M^{-1}) than the larger systems **307** and **308**, although the use of slightly different solvent systems must also be considered. This receptor further displayed significant selectivity for acetate and benzoate over dihydrogenphosphate. Receptor **310**, containing only two hydrogen bond donor sites, produced the lowest binding affinities, while experiments with pyrrole-based receptor **311** led to intermediate values. The decreased binding of receptor **311** relative to receptor **309a** was attributed to the formation of a smaller more closed binding cleft in the former receptor. Receptor **311** was also found to bind dihydrogenphosphate in DMSO-d_6 as inferred from ^{1}H NMR spectroscopic studies carried out by Cheng and co-workers.[391] Further studies by Gale and co-workers focused on probing the effect of electron withdrawing substituents in receptors **309b-e**.[392] Within this series, receptor **309b** displayed the highest binding affinities, with the largest increase being observed for the binding of dihydrogenphosphate ($K = 4.72 \times 10^3$ M^{-1}). The strong binding of receptor **309b** relative to the other receptors in the series was attributed to increased intramolecular hydrogen bonding interactions between the carbonyl oxygen atoms and the nearby hydrogen atoms of the central phenyl ring that served to preorganize the binding cleft. It was also proposed that the *ortho*-nitro substituents of receptors **309d** and **309e** led to reduced binding through steric interactions within the binding site. The low binding of thiourea derivative **309f** was also attributed to unfavorable steric interactions involving the relatively large sulfur atom and the central phenyl ring. This interaction was expected to distort the binding cleft. The incorporation of an additional hydrogen bond donor unit (as in receptors **312** and **313**) also served to increase the anion binding ability relative to receptor **309a**, particularly the *ortho*-substituted receptor **313**. Receptor **313** displayed a 1:2 host:guest binding stoichiometry with dihydrogenphosphate and a high overall binding constant (1.85×10^5 M^{-2}), presumably due to the interactions of this anion with the additional hydrogen bonding sites. These researchers also incorporated an *ortho*-phenylenediamine unit into a macrocyclic system (**314**).[393] However, this receptor displayed a low binding affinity for dihydrogenphosphate ($K = 1.42 \times 10^2$ M^{-1}). Chemical shift analysis led to the conclusion that this anion only interacted with the amide hydrogen atoms closest to the pyridine ring. On the other hand, this macrocycle was found to bind the acetate anion well.

Schneider and co-workers examined the mono- and tri-dentate urea receptors **315-317**. These systems incorporate aromatic units and were designed to permit the binding of nucleotide monophosphates.[334] The relevant interactions were studied using ^{1}H NMR

spectroscopy (DMSO-d_6) and fluorescence emission spectroscopy (H_2O, unbuffered). System **315a**, with a direct link between the urea and aromatic moiety, proved to be the strongest binder. In this case, 1:1 association constants on the order of 10^4 M^{-1} were recorded in DMSO-d_6 for AMP, CMP, GMP and TMP (add as sodium salts). The corresponding association constants for receptor **315b** could not be determined. Receptors **316** and **317** were designed to act as improved receptors. These compounds contain additional aromatic units, which were expected to improve the π-surface interactions. While receptor **317** displayed weak interactions, the monodentate receptors **316a** and **316b** were indeed found to bind various nucleotides well. The resulting association constants were approximately 10^3 M^{-1} in H_2O, with lower affinities being noted in DMSO-d_6. Interestingly, the free energies of association recorded in the case of unsubstituted pyrene were found to be only 2 – 5 kJ lower than the free energies of association with receptors **316a** and **316b**. This led to the suggestion that the contribution from the urea unit was small, possibly due to steric restraints that precluded the full benefit of concurrent full chelation and π-surface interactions.

A number of chromophores have been appended to urea receptors in order to facilitate optical signaling. For example, Jeon, Nam, and co-workers appended urea units to an anthraquinone unit in receptor **318**.[394] All three receptors displayed a chromogenic shift in the absorption spectrum upon the addition of TBA dihydrogenphosphate in DMSO. The strong color shift (ca. 100 nm) observed upon the addition of dihydrogenphosphate and acetate to receptor **318a** was determined to reflect abstraction of the phenolic hydrogen atoms. However, the UV-Vis spectrum of receptors **318b** and **318c** was found to undergo a 30 nm shift upon the addition of dihydrogenphosphate but not acetate, chloride, or hydrogensulfate. The selectivity for dihydrogenphosphate was attributed to the formation of four hydrogen bonding interactions with the urea NH units. Binding constants were measured via ^1H NMR spectroscopic titrations carried out in DMSO-d_6. All three receptors were found to display similar affinities for dihydrogenphosphate ($K \approx 1 \times 10^4$ M^{-1}), and lower affinities for the other tested anions. Significant changes in the chemical shifts of these receptors upon the addition of dihydrogenphosphate were only observed for the NH units closest to the anthraquinone unit, leading to the conclusion that these hydrogen bond donors were the most critical for binding. Less dramatic shifts were reported for the addition of acetate.

Receptor **319**, recently developed by Fabbrizzi and co-workers,[395] contains 4-nitrophenyl substituents. This design feature allowed its use as a colorimetric anion sensor. The electron withdrawing nitro groups also serve to enhance the acidity of the NH groups. UV-Vis titrations, carried out using a variety of TBA anion salts in acetonitrile, demonstrated that receptor **319** exhibits a preference for oxoanions, with a log *K* for dihydrogenphosphate of 5.37. The binding affinity was found to reflect the basicity of the anions in question (i.e., log *K* falling in the order $AcO^- > C_6H_5COO^- > H_2PO_4- > NO_2^- > HSO_4^- > NO_3^-$). The urea linker was also functionalized via the attachment of two naphthalimide groups. In this case, the resulting receptor, **320**, proved effective for the detection of carboxylic acids, as determined through colorimetric and fluorescent experiments. Receptor **320** was also found to form a supramolecular adduct with $H_2PO_4^-$ at low concentrations. However, the addition of excess anion resulted in deprotonation. Deprotonation was also observed for fluoride and acetate anions. Fabbrizzi and co-workers later developed the chiral diurea receptor **321** based on a bridging (*R,R*)-cyclohexane-1,2-diamine subunit.[396] Receptor **321** was studied via UV-Vis titrations carried out in DMSO. These analyses revealed a 1:2 (host:guest) binding stoichiometry. The formation of this 1:2 adduct was attributed to the presence of an extra hydrogen bond between both anions and an unexpected cooperative effect. The first and second log *K* values for dihydrogenphosphate were determined to be 2.96 and 3.46, respectively. The binding interaction with pyrophosphate was characterized by a log *K* of

4.63. Incorporation of a nitrobenzofurazan moiety (**322**) led to the production of more dramatic changes in the absorption spectrum upon anion addition in acetonitrile.[397] In analogy to what was seen for other systems, a hydrogen bonding complex was observed at less than one equivalent of anion, and further additions led to deprotonation of the receptor. A log *K* value of 5.40 was calculated for the complex with dihydrogenphosphate on the basis of UV-Vis titration studies. Binding selectivities correlated with anion basicity in this case.

Gunnlaugsson and co-workers have also combined urea and naphthalimide moieties for anion recognition (producing, e.g., receptors **323**).[398] The interactions of acetate, dihydrogenphosphate, and fluoride with receptors **323** were followed by ^{1}H NMR, UV-Vis, and fluorescence spectroscopy in DMSO. These spectral changes were attributed to a combination of hydrogen bonding interactions and deprotonation. No changes to the spectra of **323** were observed upon the addition of chloride or bromide. The UV-Vis titration data with acetate and dihydrogenphosphate could generally be fit to a 1:1 binding equation. A log *K* of ca. 3 was reported for the complexation of dihydrogenphosphate by receptors **323a** and **323c**; however, receptor **323b** displayed more complex binding behavior with dihydrogenphosphate and could not be fit to a 1:1 model. All studies with fluoride were consistent with full deprotonation of the receptor. Importantly, a strong green-to-purple color change was observed upon the addition of acetate and dihydrogenphosphate to these receptors. All anions were studied as their TBA salts.

These researchers were also able to achieve anion sensing by combining urea binding subunits with anthracene fluorophores (giving receptors **324**).[399] Significant fluorescence quenching was observed upon the addition of acetate, dihydrogenphosphate, and fluoride anions as their TBA salts in DMSO. The reduced emission intensity was attributed to an increase in PET quenching of the anthracene unit by the urea nitrogen atom upon anion complexation. Similar binding constants were measured for interactions of all receptors with dihydrogenphosphate and acetate, with log *K* values being ca. 2; however, acetate exhibited more significant quenching. Fluoride was found to effect the largest quenching and to display the highest binding constants. The addition of chloride and bromide anions produced little to no effect on the fluorescence spectra of these receptors.

J. Yoon, J. S. Kim, and co-workers studied the anion binding of phenylurea-anthracene receptors **325** - **326**.[400] These receptors were found to be highly selective for fluoride and hydrogenpyrophosphate over dihydrogenphosphate, chloride, bromide, iodide, hydrogensulfate, and acetate in DMSO. The interactions of TBA anion salts with receptors **325a** and **326a** were followed through UV-Vis spectroscopy while the binding behavior of receptors **325b** and **326b** were followed through fluorescence spectroscopy. Using the latter method, binding constants of 6.0×10^3 M^{-1} and 2.6×10^3 M^{-1} were determined for the interaction of hydrogenpyrophosphate with receptors **325b** and **326b**, respectively. Interestingly, ^{1}H NMR spectroscopic studies of receptors **325a** and **325b** in DMSO-d_6 led to the conclusion that, in addition to the urea units, the 9-H atom of anthracene played a role in mediating the anion binding process.

A hybrid acridone - urea fluorescent sensing system (**327**) was reported by Gale and co-workers in 2007.[401] Dramatic quenching of the receptor emission was observed upon the addition dihydrogenphosphate (TBA salt) in an acetonitrile:DMSO solution (94:6). Addition of chloride led to a much smaller decrease in emission intensity, and the addition of acetate and benzoate produced a slight increase in intensity. The emission enhancement observed with the latter anions was attributed to deprotonation of the receptor. The binding of dihydrogenphosphate was probed by ^{1}H NMR spectroscopy in DMSO-d_6 / 0.5% water.

Interestingly, a combination of supramolecular complexation, deprotonation, and receptor tautomerization was inferred from these studies.

The use of a small series of urea-functionalized carbazole receptors (**328**) allowed for the differential recognition of several anions.[402] These systems, reported by K. S. Kim and co-workers, combined the hydrogen bonding moieties of the carbazole and the urea units within one receptor. These receptors allowed the optical properties of a carbazole motif to be complemented by those of various substituents. In the case of receptor **328a**, this combination produced an effective colorimetric receptor that displayed visible color changes in the presence of several TBA anion salts in 9:1 CH_3CN:DMSO. The actual color change was found to depend on the identity of the anion. For example, dihydrogenphosphate caused a color shift from yellow to orange, while hydrogenpyrophosphate caused a color change from yellow to red. More dramatic changes were observed with the hydroxide and fluoride anions. The anion-based differences could also be monitored using UV-Vis and fluorescence spectroscopy. While binding constants were not reported, two-dimensional plots of the absorbance wavelength change *vs.* fluorescence intensity change allowed for the differentiation of most anions tested, including dihydrogenphosphate and pyrophosphate.

Gale and co-workers recently reported the anion binding properties of the monourea carbazole receptors **329** - **331**.[403] These receptors display a strong selectivity for acetate and bicarbonate. However, they also display a moderate affinity for dihydrogenphosphate as inferred through ^{1}H NMR spectroscopy (DMSO-d_6 / 0.5% water). All anions were studied as the TBA salts. Titrations of dihydrogenphosphate with receptors **329** and **330** did not allow for the determination of binding constants; however, a 1:1 binding constant was determined for receptor **331** ($K = 6.14 \times 10^3$ M^{-1}). Chloride was found to be weakly bound by these receptors.

Urea moieties have also been combined with a benzoxazole reporter unit, as embodied in receptor **332**.[404] It was proposed that in the case of this latter receptor, anion binding would disrupt the internal hydrogen bonding between the oxazole nitrogen atom and the proximal urea N-H. Fluorescence experiments carried out in DMSO revealed that while acetate followed the proposed model, fluoride led to deprotonation and the less basic dihydrogenphosphate participated in hydrogen bonding interactions without disrupting the internal hydrogen bonding of the receptor. All anions were studied as their TBA salts. Due to the presence of three different mechanisms, the additions of fluoride, acetate, and dihydrogenphosphate produced distinct signals and could thus be differentiated by receptor **332**. No spectral changes were observed upon the addition of chloride, bromide, iodide, and hydrogensulfate. A more acidic derivative, sulfonamide receptor **333**, was found to be deprotonated by fluoride, acetate, and dihydrogenphosphate in DMSO.

The liquid-liquid extraction ability of urea functionalized dendrimers (**334**) was reported by Stephan, Johannsen, Vögtle, and co-workers in 1999.[405] Both second generation (G2) and third generation (G3) dendrimers functionalized with hexyl, octyl, or dodecyl alkyl chains were studied. Extraction of AMP, ADP, and ATP from aqueous solutions at pH 5.4 (2-(N-morpholino)ethanesulfonic acid (MES) buffer) and 7.4 (HEPES buffer) into chloroform was observed with second generation dendrimers of type **334a** and **334c** as well as third generation dendrimers of type **334b** and **334c**. Extraction efficiency was found to correlate with the charge of the guest (ATP > ADP > AMP) and the generation of the dendrimer (G3 > G2). In addition, dendrimers with shorter alkyl chains (hexyl and octyl) at the periphery extracted anions more effectively than dodecyl-functionalized dendrimers. As a result, the highest degree of extraction was observed for dendrimer **334b-G3**. Mass spectrometry studies supported the formation of a 1:5 **334b-G3**:ATP complex.

Thioureas have also played an important role in anion recognition. In 1995, the interaction of TBA dihydrogenphosphate with a series of bis-urea and thiourea receptors (**335** and **336**) was studied by Umezawa. This study was motivated by a desire to compare the two functionalities, as well as the effect of the size of the appended hydrocarbon group. With such goals in mind, the binding behavior of **335a** and **335b** were first compared.[378] Both 1:1 and 2:2 host:guest complexes were observed at high concentrations of dihydrogenphosphate in DMSO-d_6 by ^{1}H NMR spectroscopy. Significantly stronger binding was observed for the thiourea congener **335b** (K = 820 M^{-1} versus 110 M^{-1} for **335a**). These dihydrogenphosphate binding constants were nearly two-fold higher than those observed for acetate. NMR spectroscopic studies supported the conclusion that all four N-H groups in the receptor participate in hydrogen bonding interactions involving the bound phosphate. Anions Cl^-, HSO_4^-, NO_3^- and ClO_4^- did not show significant binding interactions. Several other trends were analyzed through comparisons of **335b-d** and the **336** series.[406] All association constants were measured in DMSO-d_6 via ^{1}H NMR spectroscopy.

A first comparison can be made between **335b** and **335c**, wherein replacement of the butyl substituent with a phenyl substituent serves to increase the association constant by more than five-fold ($K = 8.20 \times 10^2$ M^{-1} and 4.60×10^3 M^{-1}, respectively). This effect was explained in terms of the electron-withdrawing nature of the phenyl groups, which increases the acidity of the thiourea. The naphthyl group in host **335d** was not nearly as beneficial, likely due to steric interactions with the phosphate guest. Interestingly, the use of a xanthene spacer in **336a** and **336b** greatly increased the binding affinity toward dihydrogenphosphate (5.5×10^4 M^{-1} and 1.95×10^5 M^{-1}, respectively). This increase in binding affinity was attributed to the enhanced rigidity of the spacer. High selectivities for dihydrogenphosphate were also observed in this series, with binding constants for dihydrogenphosphate being two-fold higher than those for acetate in most cases. Ion channel-mimetic sensors based on receptor **336a** were then constructed. These displayed the largest relative changes for dibasic phosphate as compared to other common anions, while ion-selective electrodes based on the same receptor gave responses in line with the Hofmeister series.[407]

Teramae and co-workers later illustrated the use of receptor **336a** in an amperometric ion selective electrode in which several anions, including dihydrogenphosphate, could be distinguished.[408] Receptor **335e**, containing nitrophenyl substituents to enhance acidity, was found to transport dihydrogenphosphate in a 1:2 host:guest stoichiometry across a nitrobenzene/water interface. The increased binding stoichiometry provided greater overall transport efficiency than that observed with receptor **336a**, although the rates of transport were similar. Receptor **335e** also displayed a high selectivity for dihydrogenphosphate over chloride. Ion transfer polarography studies of receptor **335e** confirmed the transportation of dihydrogenphosphate across the nitrobenzene-water interface in the form of a 2:1 adduct.[409]

Macrocyclic derivatives of receptors **335** were investigated by Hong and co-workers in 2000.[410] Receptor **337a** was reported to be selective for dihydrogenphosphate over acetate and chloride (TBA salts) as inferred from ^{1}H NMR spectroscopic experiments carried out in DMSO-d_6. This selectivity was in agreement with that observed for acyclic derivatives **335**. In contrast, receptor **337b** was found to bind anions in order of basicity (i.e. acetate > dihydrogenphosphate > chloride). Binding constants determined in the case of receptor **337b** were two-fold higher for dihydrogenphosphate and 15-fold higher for acetate then those reported with receptor **337a**. This increase in binding affinity was attributed to increased preorganization of the macrocyclic core in the case of the ethyl substituents.

Thiourea based receptors with alkyl, pyrene, and crown ether moieties (**338** - **341**) were developed by the Teramae group. In preliminary studies, **338** (n = 1) was shown to bind dihydrogenphosphate and acetate with log K values of 4.3 and 5.7, respectively, in

acetonitrile as determined via UV-Vis titrations with the corresponding TBA salts.[411] The electron-withdrawing *p*-nitrophenyl group attached to the thiourea moiety was thought to provide an effective intramolecular charge transfer conjugate upon anion binding, a feature that was expected to result in an anion-induced color change. In a subsequent study, receptor **338** (n = 1-8) was mixed with a detergent to create vesicles.[412] While association constants were not determined, the anion selectivities of this series of receptors were found to be highly dependent upon the length of the alkyl chains when studied under these conditions. However, none of these vesicle systems displayed a preference for dihydrogenphosphate.

Fluorescent pyrene receptors were also found to be effective. For example, the directly conjugated receptor **339** displayed a significant emission increase upon the addition of TBA acetate and TBA dihydrogenphosphate in acetonitrile. A binding constant of 8.70×10^2 M^{-1} was determined for the dihydrogenphosphate complex, but a strong preference for acetate was also reported.[413] Receptor **340** was designed to contain a spacer between the recognition and the reporter subunits. This sensor displayed a quenching of the pyrene monomer emission followed by intramolecular exciplex formation between the thiourea and the pyrene in the presence of anions.[95] The quenching was attributed to the increased electron donating ability of the thiourea when complexed to an anion. Based on the magnitude of this effect, it was concluded that receptor **340** binds acetate more effectively than either $H_2PO_4^-$ or Cl^-. A binding constant of 5.2×10^3 M^{-1} was reported for the dihydrogenphosphate complex in acetone. However, the use of different solvents in these studies precludes a direct comparison between the receptors.

Studies of the putative ditopic receptor **341** revealed a decreased binding affinity for TBA dihydrogenphosphate in acetonitrile relative to phenyl thiourea. Nevertheless, the affinity for dihydrogenphosphate proved higher than that of the other anions tested.[414] However, attempts to add sodium cation in the form of ($NaBPh_4$) in an effort to increase the anion affinity through concurrent cation and anion complexation were stymied due to precipitation. Further studies compared the anion binding ability of receptors **336a, 338**, and **341** at a liquid-liquid dichloroethane-water interface through the use of interfacial tension measurements.[415] While receptors **336a** and **338** did not show any activity, crown ether conjugate **341** displayed selectivity for sodium dihydrogenphosphate under these conditions. No changes in the tension were observed in the presence of CH_3COO^-, Cl^-, Br^-, or ClO_4^-. It is to be noted that the interaction was limited to the liquid-liquid interface as receptor **341** did not extract the $Na^+{\cdot}H_2PO_4^-$ ion pair from aqueous solution into the organic phase.

The synthetic addition of a thiouronium group and the removal of the crown ether moiety (leading to receptor **342**) served to improve the binding of dihydrogenphosphate at the DCE-water interface, without leading to a reduction in the phosphate selectivity observed in the case of receptor **341**.[416] Based on control experiments, the authors suggested that both the thiourea and thiouronium arms contributed to the strong binding observed in the case of **342**.

The C_3-symmetric optically-active receptor **343** was prepared by the Wu group.[417] A UV spectral response was observed for both TBA dihydrogenphosphate and TBA sulfate, whereas no change was observed with bromide, iodide, acetate or trifluoroacetic acid (all as the TBA salts). Among the two tetrahedral anions studied, receptor **343** displayed a 1:1 binding affinity for dihydrogenphosphate that was two orders of magnitude higher than that for sulfate in DMF ($K = 1.1 \times 10^5$ M^{-1}). Proton NMR spectroscopic analyses led the authors to conclude that the dihydrogenphosphate guest was tightly bound within the receptor cavity via hydrogen bonds. The observed binding affinity was attributed to the availability of six hydrogen bonding sites as well as the three-dimensional and flexible nature of the cavity.

Tobe and co-workers studied the influence of macrocyclic preorganization in a series of metacyclophane-based thiourea receptors, namely **344-347**.[418,419] Proton NMR spectroscopic titrations carried out in DMSO-d_6 using various TBA anion salts revealed that these receptors bound $H_2PO_4^-$ in preference over other anionic species with the selectivity order being $H_2PO_4^- > CH_3COO^- > Cl^- > HSO_4^- > Br^-$. In all cases, the binding stoichiometry was 1:1. These cyclic receptors displayed higher stability constants than the analogous acyclic compound **335b** in this solvent system. For example, cycle **346** was found to bind dihydrogenphosphate with an association constant of 1.2×10^4 M^{-1}, while receptor **335b** displayed an association constant of 5.2×10^2 M^{-1}. The strongest interactions were observed for the lariat-type receptor **347**, for which the association constant proved too high to determine using standard ^1H NMR spectroscopic methods.

Herges, König, and co-workers later reported the anion binding properties of a thiourea macrocycle containing ether linkages (**348**).[420] Proton NMR spectroscopic experiments carried out in DMSO-d_6 led to the suggestion that this macrocycle bound a number of anions (added as the TBA salts) with varying stoichiometries. Complexes of 1:1 host:guest stoichiometry were indicated for nitrate, acetate, cyanide, bromide, iodide, and hydrogensulfate, while 2:3, 2:1, and 1:2 complexes were found for sulfate, dihydrogenphosphate, and chloride, respectively. The overall binding constant for binding to dihydrogenphosphate was determined to be 5.3×10^4 M^{-2}.

Tripodal thiourea fluorescence sensors **349** were reported by the Suzuki group in 2001.[421] The pyrene substituted receptor **349a** was found to display a fluorescence emission band that was shifted to longer wavelength upon the addition of anions in acetonitrile. The anthrylmethyl substituted receptor **349b**, however, displayed a fluorescence signal whose intensity was quenched upon the addition of anions. The binding affinities for receptor **349a** were generally greater than those of **349b**. Presumably, this reflects the increased acidity of the thiourea groups that results from direct conjugation to the fluorophore. Both receptors displayed a slight selectivity for dihydrogenphosphate over acetate in acetonitrile, with binding constants of 3.7×10^5 M^{-1} and 1.9×10^4 M^{-1} being obtained for receptors **349a** and **349b**, respectively. In both cases, the relative change in emission intensity was found to be $H_2PO_4^- > CH_3COO^- > Cl^- \approx ClO_4^-$. In control studies involving the constituent monomeric units, a selectivity for acetate over dihydrogenphosphate was observed. This latter result provided confirmation that geometry matching between the host and guest confers anion binding selectivity and enhances sensitivity in the case of receptors **349**. All anions were studied as the TBA salts.

A number of thiourea receptors were studied by the Gunnlaugsson group beginning in 2001. In preliminary studies, the monothiourea receptors **350** were studied by monitoring the change in fluorescence seen upon the addition of TBA anion salts in DMSO.[422,423] The addition of dihydrogenphosphate, acetate, and fluoride resulted in quenching of the anthracene-derived fluorescence intensity. The corresponding log K values were 3.35, 2.55, and 2.05 for the three anions, respectively, in the case of receptor **350a**. No change was observed upon the addition of chloride or bromide to either **350** or **351** under these same conditions. The association constants were higher for **350a** than those observed for receptor **350b**, presumably due to an increase in acidity of the thiourea groups in the former due to the electron-withdrawing trifluoromethyl group. Consistent with this same trend, receptor **350c** displayed the lowest overall binding affinities. Interestingly, however, this latter system displayed a reversed selectivity for dihydrogenphosphate over acetate. Photochemical studies of the urea version of **350b** were carried out by the Arai group.[424] The addition of phosphate to this latter receptor resulted in fluorescence quenching and a significant decrease in the fluorescent lifetime of the receptor. No binding constants were reported.

Changes in the fluorescence spectra of the bis-thiourea receptors **351a** and **351b** upon addition of monoanion TBA salts in DMSO were also analyzed by the Gunnlaugsson group.[425,426] Dihydrogenphosphate, acetate, and fluoride all led to quenching of the fluorescence intensity. However, no signal modulation was observed upon the addition of chloride, bromide, or perchlorate. Both fluorescence and NMR spectroscopic data supported the formation of 1:2 receptor:anion complexes in the case of these monodentate anions. Even greater changes were observed upon the addition of bis-anions such as pyrophosphate, which bound to receptors **351a, 351b**, and **351c** with log *K* values of 3.40, 3.07, and 2.72, respectively (producing 1:1 complexes in all cases). Similar association constants were observed for bis-carboxylates. The differences in pyrophosphate binding affinity reflected the expected acidities of the hydrogen bonding donor units, as seen in other receptor systems and noted earlier in this review. Proton NMR spectroscopic studies and the absence of excimer/exciplex emission provided support for the formation of 1:1 bridging complexes in the case of pyrophosphate. The reduction in emission intensity seen in the case of all of these systems was attributed to an enforcement of PET quenching of the anthracene fluorescence by the thiourea/urea groups induced by anion binding.

Later, Gunnlaugsson, Pfeffer, and co-workers developed the series of naphthalimide-containing receptors **352** as fluorescent thiourea sensors for anion recognition.[427] The naphthalimide moiety in these systems emits in the green spectral region and does so with a high quantum yield. However, the intensity of this signal was quenched in the presence of anions. This effect was attributed to an increase in electron transfer from the thiourea unit to the fluorophore as the result of anion binding. While chloride and bromide did not produce a change in the emission features, fluoride, acetate and dihydrogenphosphate were all found to quench the fluorophore. Dihydrogenphosphate displayed the lowest association constants among these three anions, with log *K* values of 2.9 and 3.7 being reported for **352a** and **352b**, respectively, in DMSO when the anions were added as the TBA salts. The higher association constant for receptor **352b** was attributed to an increase in the acidity of the thiourea brought about as the result of the electron-withdrawing nature of the trifluoromethyl substituent. Further analysis of receptors **352b** and **352c** revealed strong colorimetric changes upon the addition of TBA anions in both DMSO and protic media.[428] Naked-eye detection of dihydrogenphosphate in protic media was observed as a yellow-to-purple color change.

These same researchers later investigated receptor **353** with the expectation that the N-H unit of the naphthalimide moiety would be able to participate in hydrogen bonding as a result of the increased flexibility of the aliphatic unit compared to the phenyl spacer of **352a**.[429] Proton NMR spectroscopic analyses proved consistent with the participation of this third N-H proton in the binding of dihydrogenphosphate (TBA salt) in DMSO-d_6. The presumed additional interaction was also supported by molecular modeling studies and the measurement of an increased binding constant (log K (**353**:$H_2PO_4^-$) = 3.4) relative to that of the analogous receptor **352a**. In studies of other anions, chloride and bromide anions did not significantly change the ^{1}H NMR spectrum. Acetate displayed a similar association constant but interacted with only the thiourea protons (i.e., not the naphthalimide N-H), whereas the addition of fluoride resulted in deprotonation. However, no changes in the emission spectrum were observed upon the addition of anions to receptor **353** in DMSO, presumably due to the fact that the increased flexibility disrupted the fluorescence signaling mechanism observed in receptors **352**.

In a subsequent design, an *ortho*-substituted phenyl linker was incorporated into the framework in order to organize better the anion binding site. This led to receptor **354**.[430] Proton NMR spectroscopic analysis in DMSO-d_6 led to the conclusion that with this new system dihydrogenphosphate (TBA salt) was again bound to all three N-H atoms.

Significantly stronger binding was observed for dihydrogenphosphate (log $K \approx 4$) as compared to receptor **353**, and a selectivity for dihydrogenphosphate over acetate was achieved. Acetate was observed to bind to only the thiourea protons, in analogy to what was found for receptor **353**. The addition of both anions led to a decrease in the emission intensity of receptor **354**, although a larger quenching effect was observed for acetate.

In 2008, Gunnlaugsson and co-workers reported the colorimetric anion sensing ability of amidothiourea receptor **355**.[431] This receptor was found to respond to acetate, fluoride, dihydrogenphosphate, hydrogenpyrophosphate, AMP, and ADP as inferred from UV-Vis experiments conducted in both DMSO and DMSO:H_2O (4:1) solutions, whether the anions were present as their TBA or sodium salts (in the latter solvent system). These binding events were also reflected in a transition from colorless to yellow colored solutions. No alteration in the absorption spectrum was observed upon the addition of chloride, bromide, or ATP. UV-Vis titration data was fit to multiple binding models and led to the conclusion that dihydrogenphosphate (TBA salt) was bound in a 1:2 host:guest ratio with a log K value of 4.06. Pyrophosphate was found to form a 1:1 complex with a log K value of 6.53. Proton NMR spectroscopic titrations were consistent with a binding mode that involved both hydrogen bonding and deprotonation of the receptor.

The anion recognition properties of thiourea receptors **356** were investigated by Kruger and Gunnlaugsson in 2009.[432] All three stereoisomers of **356** were investigated for their ability to bind acetate, dihydrogenphosphate, and fluoride (as the TBA salts) through a number of spectroscopic techniques. Binding constants were measured through ^{1}H NMR spectroscopy in wet (ca. 2% H_2O) DMSO-d_6, with enantiomers **356a** and **356b** displaying similar association constants for acetate and dihydrogenphosphate (log K value ca. 2 for both receptors with both anions). Receptor **356c** displayed a slight selectivity for acetate over dihydrogenphosphate (log K values of 2.86 and 1.83, respectively). This preference in anion binding was attributed to a tighter binding pocket in **356c** relative to **356a** and **356b** that precluded binding of the bulkier dihydrogenphosphate anion. The presence of such a binding pocket was supported by both ^{1}H NMR spectroscopic studies and single crystal X-ray diffraction analysis. Binding constants for fluoride could not be determined due to competing deprotonation of the thiourea protons. Interestingly, neither the absorbance nor emission spectra of these compounds was observed to change significantly upon the addition of the anions in question in acetonitrile. For the chiral receptors **356a** and **356b**, changes in the CD spectra were observed in the presence of acetate and dihydrogenphosphate in DMSO. No receptor-anion interactions were observed for chloride using either ^{1}H NMR or CD spectroscopic techniques.

Hennrich, Resh-Genger, and co-workers reported the iminoylthiourea fluorescent sensors **357** and **358**, along with their oxidized 1,2,4-thiadiazole derivatives **359** and **360** in 2001.[433] Increases in the naphthalene emission were observed upon the addition of sodium salts of HCO_3^-, CO_3^{2-} and HPO_4^{2-} in methanol. This finding was attributed to a rigidifying effect brought about by anion binding. Little or no increase in emission intensity was seen in the presence of other anions, including $H_2PO_4^-$. Significantly larger changes were generally seen with the reduced forms **357** and **358** as compared to the oxidized forms. This observation is consistent with the presence of a greater number of hydrogen bond donors in the reduced forms. No binding constants, however, were reported. It should be noted that a much greater fluorescent enhancement was observed for the bicarbonate and carbonate anions than for monohydrogenphosphate.

Also in 2001, the Hong group reported a class of chromogenic azophenol-thiourea based anion sensors (**361** and **362**) that permitted the naked-eye detection of anions. Receptor **361a** is representative of this class. It was found to undergo a large red-shift in its absorbance

maximum in the presence of $H_2PO_4^-$, F^-, and AcO^- anions (TBA salts) in chloroform solution.[434] A visible color change from light yellow to deep red was observed at micromolar concentrations of receptor in the presence of three molar equivalents of these anions. While other anions did not induce such a color change, no discrimination could be made among the $H_2PO_4^-$, F^-, and AcO^- anions. While the color change was attributed to the participation of the phenolic hydrogen in hydrogen-bonding, no color change was observed in control systems in which the thiourea groups were absent. Receptors **361b** and **361c** were analyzed in an effort to tune the selectivity of the system.[435] It was found that the presence of the phenyl substituents in **361b** did not lead to a change in the observed selectivity. On the other hand, replacing these phenyl substituents with nitrophenyl groups, as in **361c**, led to a red-shift in the absorbance band upon nitrophenyl anion complexation. In the case of **361c**, addition of dihydrogenphosphate (as the TBA salt) produced the largest wavelength shift, allowing for the selective detection of this species among anions of similar basicity. This effect was attributed to the additional hydrogen bonding interactions that the phosphate anion can support in comparison to the fluoride and acetate anions. In any event, this serves to underscore how thiourea moieties can be used to tune the selectivity of an anion receptor.

The indoaniline receptor **362** was produced by the Hong group in 2002 with the goal of obtaining a receptor whose selectivity would differ from that predicted solely on considerations of anion basicity.[436] In contrast to the azophenol receptors, a blue-shift of absorption was observed with receptor **362** in the presence of anions. This shift was attributed to a breakdown in the intramolecular hydrogen bonding interactions between the thiourea NH protons and the carbonyl of the receptor upon the addition of anions. This loss of internal hydrogen bonding serves to free the carbonyl group, leading to the observed blue absorbance shift. A preference was observed for tetrahedral anions, such as $H_2PO_4^-$ and HSO_4^-, over spherical or planar anions, likely due to a geometric complementarity between the receptor and these tetrahedral guests. Quantitative UV-Vis spectroscopic analyses revealed an order of magnitude difference in binding affinity for $H_2PO_4^-$ and HSO_4^- relative to other anions (10^4 M^{-1} *vs.* 10^3 M^{-1}) in chloroform when the anions were studied as the TBA salts. A similar trend was observed in acetonitrile, although the absolute value of the respective binding constants proved to be considerably lower than in chloroform.

From 2002-2009, Jiang and co-workers reported a number of hybrid amidothiourea anion receptors. Preliminary studies examined the anion binding ability of receptor **363a**.[437] This host contained a combination of a thiourea recognition moiety and a *p*-dimethylaminobenzamide dual fluorescent reporter that were linked by a hydrazine spacer. Modeling studies (AM1) and ^1H NMR spectroscopic studies in DMSO-d_6 led to the conclusion that the receptor was held in a rigid conformation through the hydrogen bond network shown below. Addition of anions (as their TBA salts) to a solution of this receptor in acetonitrile served to perturb the emission spectrum significantly. Specifically, the charge-transfer state band was quenched while the locally excited state band was enhanced. In general, anions were observed to interact with this receptor in the order of increasing basicity, and a large preference for acetate was observed. Dihydrogenphosphate was found to bind the receptor with an association constant of 4.7×10^4 M^{-1}.

The ability of this system to function as a chemosensor was further probed by varying the substituents on the benzene ring of the recognition motif (**363b-h**).[438] Interestingly, the ^1H NMR, absorption, and fluorescence spectra of these complexes were consistent with a lack of coupling between the thiourea recognition moiety and the reporter unit. This decoupling effect was presumably due to a twisted conformation of the hydrazine single bond in these receptors prior to anion binding. At the same time, strong changes in the absorption and fluorescence spectra were observed upon the addition of anions in acetonitrile. These changes were attributed to an anion-induced conformational change that facilitated

electronic communication between the recognition and reporter units. The measured binding constants were found to correlate with the electronic-withdrawing nature of the benzene substituent as well as the basicity of the anionic guest. The highest binding constant for dihydrogenphosphate was reported for receptor **363h** ($K = 1.3 \times 10^5$ M^{-1}), as determined via absorbance spectroscopy in acetonitrile. A 1:1 host:guest stoichiometry was inferred on the basis of Job plot analyses for all systems.

Pyrene was also studied as a fluorescent reporter in these systems (**364**).[439] An enhancement in the PET quenching of the pyrene unit by the thiourea moiety was observed upon the addition of a number of anions as their TBA salts in acetonitrile. The degree of quenching was found to follow the order $F^- > AcO^- > H_2PO_4^- \gg HSO_4^- > Cl^- > Br^- > I^- > NO_3^- > ClO_4^-$. Binding constants for dihydrogenphosphate were determined as 4.29×10^5 M^{-1}, 1.63×10^6 M^{-1}, and 8.86×10^6 M^{-1} for receptors **364a, b**, and **c**, respectively. A 1:1 binding stoichiometry was confirmed through Job plot analysis. The binding affinity of acetate and fluoride was too high to be determined. These neutral sensors also responded to anions in mixtures containing up to 8% water in acetonitrile.

Further iterations varied the substitution of the benzamide reporter unit (**365a** - **h**).[440] These receptors displayed a change in the absorption spectrum upon anion addition in acetonitrile. These experiments also supported an anion-induced coupling mechanism (*vide supra*). The binding constants generally correlated with the electron-withdrawing nature of the substituent and the basicity of the anion, although a few exceptions were observed. For example, the *p*-Br substituted receptor **365f** displayed stronger binding to dihydrogenphosphate ($K = 5.09 \times 10^5$ M^{-1}) than the *p*-NO_2 substituted receptor **365h** ($K = 1.38 \times 10^5$ M^{-1}) despite the increased electron-withdrawing ability of the latter substituent. Receptor **365f** was also observed to bind dihydrogenphosphate more strongly than fluoride. The studied benzamide receptors (**365a-h**) displayed higher binding affinities toward anions than simpler thio-derived counterparts (**366a-h**). Based on these findings, it was concluded that the contribution of the hydrogen bonding ability of the amide unit in receptors **365a-h** was more significant than the increased acidity of the NH protons in receptors **366a-h**. The effects of further expansion were investigated by preparing and studying the symmetrical receptors **367i-l**.[441] In general, these *N,N′*-bis(benzamido)thiourea receptors displayed increased anion binding affinities relative to the related *N*-benzamidothiourea receptors **365i-l**. Within this class, the chloro-substituted receptors **367k** and **367l** were found to bind dihydrogenphosphate the most strongly ($K \approx 10^5$ M^{-1}).

Phosphate binding constants on the order of 10^6 M^{-1} have been achieved with *N*-benzamido-*N′*-benzoylthiourea receptors of general structure **368**, reported by Liu and Jiang in 2008.[442] Significant UV-Vis spectral changes were observed upon the addition of dihydrogenphosphate, acetate, and fluoride (as TBA salts) to receptors **368** in acetonitrile. Little to no changes were observed upon the addition of other monoanions (HSO_4^-, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-). A 1:1 host:guest stoichiometry was confirmed through Job plot analysis. As with previous systems, the signal mechanism was proposed to arise from the breaking of strong intramolecular hydrogen bonds by the binding of the anionic guest to the thiourea moiety. Interestingly, the binding affinity was found to show little dependence on the aryl substituents in the case of these receptors.

These researchers also prepared a series of *N*-(isonicotinamido)-*N′*-(substituted-phenyl)thiourea receptors **369**.[443] These acyclic receptors were not only found to bind carboxylate anions well but also to interact with dihydrogenphosphate with a binding constant on the order of 10^5-10^6 M^{-1} as inferred from UV-Vis titrations carried out in acetonitrile (anions studied as their TBA salts). It was suggested that the hydrophobic environment created around the thiourea group facilitated hydrogen bonding interactions

with the bound anions. Spectral changes were also observed in water - acetonitrile mixtures, although no binding constant was reported for dihydrogenphosphate.

Jiang and co-workers further examined *N*-(acetamido)thiourea based anion receptors **370** and **371**.[444] Variations at the aceto-position were examined through studies with receptors **370a-d**, and variations at the benzene unit were examined through studies with receptors **371a-f**. Binding constants on the order of 10^4 - 10^7 M^{-1} in acetonitrile were reported for acetate, fluoride, and dihydrogenphosphate anions (studied as the TBA salts) in the case of receptors **370a-d** and **371a-e**. These values were measured by monitoring changes in the absorption spectra of the receptors upon anion addition. The large wavelength shift seen in the case of receptor **371f** led to the conclusion that deprotonation of this receptor was occurring in the presence of anions. This result was ascribed to the increased acidity of this system relative to the other derivatives. Little to no changes in the absorption spectra were observed upon addition of hydrogensulfate, chloride, bromide, iodide, nitrate, and perchlorate. In these studies, a preference for acetate and fluoride over dihydrogenphosphate was observed.

In 2004, He and co-workers reported the colorimetric sensors **372**, which also incorporated amidourea hydrogen bonding sites.[445] The addition of anions (TBA salts) to receptors **372** in DMSO was followed through UV-Vis spectroscopy. Significant color changes were observed with fluoride, acetate, and dihydrogenphosphate. On the other hand, chloride, bromide, and iodide did not affect the absorbance spectrum. Interestingly, the presence of the pyridine ring in receptor **372b** did not influence the binding affinity toward dihydrogenphosphate, and binding constants on the order of 10^4 M^{-1} were reported for receptors **372a** and **b**. However, these two systems displayed a preference for acetate and fluoride over dihydrogenphosphate.

These researchers later studied receptor **373** containing *p*-nitrophenylthiourea groups and a spacer between the amide and thiourea units.[446] Spectrophotometric titrations carried out in DMSO established that this mixed amide-thiourea system interacted similarly with acetate and dihydrogenphosphate anions ($K(H_2PO_4^-) = 1.41 \times 10^4$ M^{-1}). However, the highest affinity was found for fluoride, in which case naked-eye detectable color changes were observed. This same group also reported systems **374**.[447,448] Preliminary studies with receptors **374a** and **374b** were performed using UV-Vis and fluorescence titrations, respectively, using various TBA anion salts. Binding affinities for the interactions with dihydrogenphosphate were determined in DMSO. Receptors **374a** and **374b** were found to bind dihydrogenphosphate with association constants of 1.85×10^4 M^{-1} and 1.61×10^3 M^{-1}; however, binding was not selective against acetate, *p*-nitrophenolate, or *p*-nitrophenylphosphate. The incorporation of a pyridine unit led to the development of receptors **374c** and **374d**. Both acetate and dihydrogenphosphate were found to participate in hydrogen bonding interactions with all the NH units present in these receptors as inferred from ^{1}H NMR spectroscopic measurements carried out in DMSO-d_6. Anion-dependent changes in the UV-Vis spectrum of receptor **374c** (DMSO) were also seen. The same proved true for the emission spectrum of receptor **374d** ($CHCl_3$). The use of different solvents for the two receptors, however, prevented comparison of the binding ability. Both receptor **374c** and **374d** displayed a high affinity for dihydrogenphosphate ($K = 1.6 \times 10^4$ M^{-1} (DMSO) and 1.0×10^6 M^{-1} ($CHCl_3$), respectively), but a preference for acetate. Other anions (bromide, chloride, iodide, *p*-nitrophenolate, *p*-nitrophenylphosphate) were found to bind more weakly.

Martínez-Máñez, Rurack, and co-workers reported the colorimetric detection of dihydrogenphosphate and other anions using monourea and thiourea receptors bearing an appended chromophore unit (**375a**,**b**).[449] These receptors were investigated as part of a

larger study of the **375** - **376** receptor series. Analysis of the binding behavior of these receptors was conducted through UV-Vis spectroscopy in acetonitrile using the TBA salts of all studied anions. While all receptors in this set were found to bind dihydrogenphosphate, only receptors **375a** and **375b** underwent a significant bathochromic shift (ca. 30 nm) upon the addition of this anion. These receptors also displayed the strongest binding constants with dihydrogenphosphate ($K = 3.9 \times 10^3$ M^{-1} and 7.4×10^3 M^{-1}, respectively). While lower binding affinities were reported for chloride, significantly higher binding constants were observed for the interaction of these receptors with acetate and benzoate as compared to dihydrogenphosphate. Little to no spectroscopic change was observed upon the addition of hydrogensulfate, thiocyanate, nitrate, bromide, and iodide anions. Deprotonation was often reported upon the addition of fluoride and cyanide.

In 2005, the Fabbrizzi group reported the results of an extensive study of the anion binding behavior of the urea and thiourea receptors **377**.[450] In the case of urea receptor **377b**, UV-Vis titrations in DMSO demonstrated that 1:1 adducts were formed with all anions when added as the TBA salt, excluding fluoride for which deprotonation was observed. The binding constant corresponding to the interactions between receptor **377b** and dihydrogenphosphate was found to be log $K = 4.47$. In analyzing the thiourea receptor **377a** deprotonation was observed for fluoride, acetate, benzoate, and dihydrogenphosphate, a finding consistent with the more acidic character of the thiourea receptor **377a**. This study provides a classic demonstration of the tenant that a balance must be maintained in receptor design if strong hydrogen bond donating systems are to be obtained that will not deprotonate under conditions of use. Since these latter can vary (anion, solvent, concentration, temperature, counter cation, etc) a caveat coming from this and other studies[451-453] is that great care must be exercised in the analysis of new hydrogen bond-based anion receptors.

The effect of urea and thiourea moieties on anion binding was also directly compared using the anthraquinone receptors **378** reported by Ganguly and Das in 2005.[454] Colorimetric responses were observed with both receptors in DMSO:CH_3CN (1:9), although the urea derivative **378a** required heating to 60°C before a color change was observed. This heating was thought to reflect a need to break strong intramolecular hydrogen bonds within the urea compound before binding the anion becomes competitive. UV-Vis titrations were consistent with 1:1 binding behavior when the anions were added as the TBA salts. Moderate selectivity for dihydrogenphosphate over acetate, benzoate and hydroxide was observed. Further, the binding affinities were higher in the case of the thiourea derivative than the urea derivative (K for $H_2PO_4^-$ = 1.0×10^6 M^{-1} and 1.3×10^4 M^{-1}, respectively). Computational studies led the authors to suggest that the receptor binds anions in a tweezer-like fashion, wherein the dihydrogenphosphate anion is held via all four hydrogen bond donors. In contrast, acetate was thought to be held by only three hydrogen bond donors.

At the same time, the diurea and dithiourea receptors **379-380** were reported by Yoon and Kim.[455] Proton NMR spectroscopic studies provided support for the conclusion that all members of this series stabilized 1:2 (host:guest) complexes when anions were added as the TBA salts. Across the board, these receptors displayed a strong affinity for adipate. An interaction with pyrophosphate was also observed. Only in the case of receptor **379a**, however, was the affinity toward pyrophosphate substantial. In this case a binding constant of ca. 1×10^5 M^{-1} was recorded in CD_3CN:DMSO-d_6 (9:1).

In 2005, Pfeffer, Gunnlaugsson and co-workers first reported a family of [3]polynorbornane frameworks. These receptors were designed in order to obtain a set of conformationally preorganized aromatic thiourea receptors (**381** and **382**).[456,457] While **381b** displayed a colorimetric response to TBA anion salts, the binding behavior for all receptors was studied most extensively via ^1H NMR spectroscopic titrations carried out in DMSO-d_6. These

receptors displayed an excellent affinity for the dihydrogenphosphate ($H_2PO_4^-$) and dihydrogenpyrophosphate ($H_2P_2O_7^{2-}$) anions, among others. Consistent with their respective charges, these anions were bound in 1:1 and 2:1 (host:guest) ratios, respectively. Based on this finding and other considerations, it was proposed that the cavity of the receptors was appropriately organized for the formation of four hydrogen bonds to one phosphate group, thus allowing for the formation of a sandwich-type complex in the case of pyrophosphate.

Receptors **381a** and **381b** exhibited similar binding affinities, with specific log *K* values of 3.9 and 7.9 being derived for the binding of TBA $H_2PO_4^-$ and TBA $H_2P_2O_7^2$, respectively, to receptor **381a**. The estimated binding constants for receptor **382** were slightly lower, a finding that was attributed to its additional flexibility. Receptors **381** and **382** were also found to bind acetate, although with a lower binding affinity and in a 1:2 host:guest ratio. Fluoride caused deprotonation of the receptors.

Functionalized norbornene scaffolds with smaller cavities (**383-385**) have also been prepared. As a general rule, these were found to interact with acetate and dihydrogenphosphate (TBA salts) to form 2:1 complexes as deduced from ^{1}H NMR spectroscopic titrations carried out in DMSO-d_6.[458,459] Receptor **383a**, for example, displayed a log K_1 = 3.66 and a log K_2 = 3.00 for the dihydrogenphosphate anion. Negligible binding was observed with bromide, chloride, and hydrogensulfate. Subtle differences in binding affinities were attributed to the varying steric constraints associated with each receptor.

Ramamurthy, Thirumalai, and co-workers developed a dual-fluorescent reporter system based on the acridinedione fluorophore (**386**).[460] The effects of a number of anions on the UV-Vis and fluorescence spectra of receptor **386** were studied in acetonitrile (F^-, Cl^-, Br^-, I^-, HSO_4^-, ClO_4^-, AcO^-, $H_2PO_4^-$, and BF_4^-, added as their TBA salts). Only the addition of dihydrogenphosphate led to changes in the absorbance spectrum of receptor **386a**; however, the addition of dihydrogenphosphate, acetate, and fluoride affected the fluorescence spectrum of receptor **386a**. Interestingly, the addition of acetate and fluoride was found to quench the fluorescence of receptor **386a**, while the addition of dihydrogenphosphate led to emission enhancement. It was proposed that acetate and fluoride participated in hydrogen bonding interactions with the thiourea moiety and thus increased the PET quenching of the fluorophore by the thiourea unit. On the other hand, dihydrogenphosphate was thought to participate additionally in a hydrogen bonding interaction with the nitrogen atom of the fluorophore in receptor **386a**. This additional interaction was expected to facilitate an ICT process and a corresponding enhancement in emission intensity. Support for this mechanism came from studies with receptor **386b**, which lacked the additional hydrogen bond donor. As expected, fluorescence quenching was observed upon the addition of dihydrogenphosphate. A 1:1 binding constant of 380 M^{-1} was determined for the complex **386a**:$H_2PO_4^-$.

In 2006, a receptor system containing a (benzylideneamino)thiourea unit (**387**) was reported by Fabbrizzi and co-workers.[461] This receptor displayed a strong response to acetate, fluoride, and dihydrogenphosphate anions (TBA salts) as inferred from UV-Vis spectroscopic studies carried out in acetonitrile. Proton NMR spectroscopy led to the conclusion that addition of fluoride led to deprotonation, while acetate and dihydrogenphosphate participated in strong hydrogen bonding interactions with receptor **387**. Similar log binding constants were observed for the 1:1 complexes of these anions (log $K(H_2PO_4^-)$ = 3.34, log $K(AcO^-)$ = 3.62).

A practical application for thiourea sensors was recently introduced by the Anzenbacher group. Specifically, these researchers demonstrated that an array of sensors (**388-389**) could be used to detect phosphate concentrations in blood serum.[462] All of the receptors in this

series formed 1:1 complexes with anions (TBA salts) according to ^{1}H NMR spectroscopic studies, with **388b-d** and **389b-d** displaying an increase in the observed fluorescence intensity upon anion binding. This emission increase was attributed to a reduction in the conformational flexibility of the fluorophores as a result of interactions between the bound anions and each arm with the guest. Affinities were similar for each anion ($K \approx 10^6$ M^{-1} in DMSO), but a general trend of $H_2PO_4^- > HP_2O_7^{3-} > AcO^- \gg Cl^- > Br^-$ was observed for each receptor. The low relative selectivities, coupled with the differential binding behavior observed for each receptor, allowed Anzenbacher and co-workers to exploit this series of receptors in a pattern recognition protocol. By immobilizing the receptors in a polyurethane matrix, and employing principle component analysis (PCA), AMP, ATP, phosphate, and pyrophosphate could be detected independently of one another in the complex medium of blood serum.

In 2008, the Jang group reported the hybrid receptor **390a**. This receptor combined thiourea moieties with benzimidazole groups.[463] Fluorescence studies of **390a** revealed a quenching of the emission intensity only after the addition of PO_4^{3-} in DMSO:water (8:2) with a binding constant of 1.0×10^4 M^{-1}. No quenching was observed upon addition of other common anions (F^-, Cl^-, Br^-, I^-, CN^-, ClO_4^-, $H_2PO_4^-$, HPO_4^{2-}, AcO^-, NO_2^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, and HSO_4^-). All anions were studied as the sodium salts. The selectivity for PO_4^{3-} was attributed to two factors, namely the geometrical complementary between receptor **390a** and PO_4^{3-} and the high negative charge of the anion. Receptor **390b**, in which the NH groups of the benzimidazole entities were substituted by sulfur units, interacted more weakly with PO_4^{3-}, resulting in a binding constant of 1.8×10^3 M^{-1} and a lack of selectivity. These results help illustrate the important role that additional hydrogen bond donor moieties can play in stabilizing anion-receptor complexes.

4.2.3. Pyrrole- and Indole-derived Receptors—Another hydrogen bonding motif that has been studied for the purpose of phosphate anion recognition is pyrrole. It and other nitrogen-containing heterocycles provide a source of NH donors that can be neutral or positively charged depending on the system in question. It should be noted that these heterocycles are only discussed here in the context of acyclic receptors. Polypyrrolic macrocycles, including calixpyrroles and expanded porphyrins, will be discussed in Section 4.4.

Some of the most interesting pyrrolic receptors developed for the purpose of anion recognition have been based on quinoxaline scaffolds. A variety of these receptors, which have been exploited for the colorimetric detection of anion binding, have now been designed and tested. The earliest contributions came from Sessler and co-workers, beginning in 1999. Nearly all of the quinoxaline receptors reported to date produce a strong colorimetric response to fluoride. However, some receptors were also found to bind dihydrogenphosphate. The first receptors synthesized, **391a-b**, displayed a low affinity for dihydrogenphosphate ($K < 100$ M^{-1}) as determined by fluorescence quenching measurements carried out in dichloromethane (using the TBA salt of this anion).[464] The incorporation of electron withdrawing fluorine substituents at the pyrrole beta positions (**391c**) increased the affinity to 1.7×10^4 M^{-1} and also enhanced the selectivity of the receptor for dihydrogenphosphate over chloride.[465] Receptor **391c** was also found to undergo a visible color change (yellow to orange) upon the addition of dihydrogenphosphate. Additional hydrogen binding sites were incorporated in receptors **392** and **393a**, which bound dihydrogenphosphate with binding constants of 4.3×10^3 M^{-1} and 5.0×10^5 M^{-1}, respectively, as inferred UV-Vis spectroscopic titrations carried out in dichloromethane.[466] Based on the observed increase in affinity relative to **391**, it was concluded that the additional pyrrole units contribute strongly to phosphate binding.

Interestingly, incorporation of electron withdrawing nitrile groups on the quinoxaline unit (**393b**) decreased the affinity for dihydrogenphosphate and reversed the selectivity.[467]

The Anzenbacher group sought to tune further this class of receptors through incorporation of aromatic substituents on the quinoxaline rings (**394-395**). While low binding was observed with dihydrogenphosphate, a strong selectivity for pyrophosphate was exhibited in preliminary studies with receptors **394a-c** and **395a**.[468] Receptor **394a** proved to have the strongest binding constant (9.4×10^4 M^{-1}) as determined through fluorescence quenching studies carried out in dichloromethane using the TBA salts of pyrophosphate. Remarkably, the quenching could be seen using a simple UVA lamp, such as is commonly used in the laboratory for the analysis of TLC plates. The general anion binding properties of receptors **394** - **395** were further examined.[469] These receptors were found to undergo both a visual colorimetric change as well as fluorescence quenching upon the addition of fluoride and pyrophosphate (TBA salts) in dichloromethane. An analysis of the binding constants revealed a slight preference for pyrophosphate over fluoride for these receptors. This stands in contrast to what is observed for the parent receptor **391a**. The strongest binding constant was reported for the complexation of pyrophosphate by receptor **394a** ($K = 9.37 \times 10^4$ M^{-1}). Incorporation of these receptors into a polyurethane matrix allowed for the colorimetric sensing of anions in aqueous solution near neutral pH.

Further studies by these researchers sought to improve the fluorescence response through the incorporation of pyrene fluorophores (**396-397**).[470] These receptors indeed displayed a much stronger fluorescence increase compared to the parent receptor **391a**. In the case of receptor **396**, this enhancement was attributed to a resonance energy transfer mechanism from the pyrene (donor) units to the dipyrrolylquinoxaline (acceptor) unit. With receptor **397**, it was proposed that the extended conjugation allowed for delocalization of the excited state, which was expected to increase the overall fluorescence intensity. In addition, in dichloromethane, enhanced anion binding affinities were observed for these receptors relative to receptor **391a**. For example, pyrophosphate (TBA salt) was found to bind receptor **397** with a binding constant of 2.95×10^5 M^{-1}.

Sessler and co-workers further investigated the anion-binding properties of a terpyrrolic analogue of dipyrrolylquinoxalines.[471] Receptor **398** was observed to undergo a visual color change upon addition of dihydrogenphosphate, fluoride, and chloride (TBA salts) in dichloromethane. Quenching of the emission intensity was also reported in the presence of these anions. A strong increase in binding affinity was found for these anions as compared to the unfunctionalized dipyrrolylquinoxaline (**391a**). For instance, the K for the formation of **398**:$H_2PO_4^-$ was determined to be 1.75×10^4 M^{-1}. The increased binding affinity was attributed to the electron-withdrawing nature of the ester substituents. High selectivity for dihydrogenphosphate over chloride was also observed (K (Cl^-) = 1.60×10^2 M^{-1}).

Simpler pyrrole receptors, such as **399**, in which the hydrogen bonding ability of the pyrrole moiety was combined with amide groups were designed and tested by Gale and co-workers. While both receptors **399a** and **399b** were produced as the result of this effort, these two systems displayed vastly different selectivities as determined from ^1H NMR spectroscopic titrations.[472,473] Specifically, the alkyl substituted receptor **399a** displayed selectivity for benzoate in acetonitrile, while **399b** displayed selectivity for dihydrogenphosphate in DMSO/H_2O 0.5% ($K = 1.45 \times 10^3$ M^{-1}) among the various anions studied. Because receptor solubilities demanded the use of different solvents, direct comparisons could not be made. The importance of the pyrrole N-H was further supported by the vastly decreased binding of furan-derivatives **400** with acetate and dihydrogenphosphate (TBA salts studied in DMSO/H_2O 0.5%).[474]

The putative ditopic receptor **399c** was also prepared and analyzed. However, no improvement in binding affinity was observed in the presence of a metal cation.[475] Thus, no direct benefit as the result of appending a crown ether could be inferred. Gale and co-workers also studied dipyrrolylmethane analogues of his basic receptor system (cf. structures **401-402**). Of these, receptor **401b** displayed a high selectivity for dihydrogenphosphate (studied as the TBA salt).[476] The associated binding constant proved too large to be measured reliably via ^{1}H NMR spectroscopic titrations in 5% water. However, analysis in 25% water in DMSO allowed a binding constant of 2.34×10^2 M^{-1} to be determined. The chemically more stable dimethyl substituted dipyrrolylmethane receptors **402** displayed similar selectivities but was characterized by reduced binding constants as compared to receptor **401**.[477] Proton NMR spectroscopy and computational studies of these receptors supported a linear binding cleft with hydrogen bonds between one phosphate oxygen atom and both the pyrrole and amide hydrogen atoms on each side of the receptor.

A small library of receptors derived from pyrrole-2,5-diacetic acid (**403**) were also reported by Gale.[478] Proton NMR titrations in CD_3CN revealed that receptor **403a** interacted most strongly with dihydrogenphosphate and hydrogensulfate. These and other anions were studied as the TBA salts. This result was attributed to a proton transfer process from the oxoanions to the amine groups of the receptor. In addition, receptors **403b-403d** were found to display the following selectivity order: Benzoate > dihydrogenphosphate > chloride, a sequence that correlates well with that obtained in the case of the parent systems **399**. However, the anion affinities of the receptors **403a-403d** were higher than those found with receptors **399**. The presence of a methylene group between the pyrrole and amide units was thus credited with creating an improved cavity for anion binding. Amidourea substituted pyrroles, such as **404** – **405**, were also analyzed in the hope that the additional hydrogen bonding functionality present in these systems would increase the binding affinity and selectivity.[479] While these receptors were able to bind dihydrogenphosphate in pure DMSO ($K = 10^3 - 10^5$ M^{-1}), little selectivity was observed among the anions tested. Additional pyrrolic units were also attached through synthetic means (giving systems **406**). These receptors were then compared to receptor **399b**.[480] Based on ^{1}H NMR spectroscopic studies carried out in DMSO, it was concluded that receptor **406** binds dihydrogenphosphate (as the TBA salt) more strongly than receptor **399b** ($K = 5.50 \times 10^3$ *vs.* 1.45×10^3 M^{-1}). However, it displays a reversed selectivity toward benzoate, binding the latter anion twice as strongly as dihydrogenphosphate. It is evident that by varying the nature of the substituents, selective pyrrolic cleft receptors can be developed for dihydrogenphosphate, although the specific design criteria needed to achieve this objective have yet to be fully elucidated.

Even simpler pyrrole receptors, of general structure **407**, were studied by the Jurczak group. These systems were prepared in the course of efforts designed to compare directly the binding ability of amide and thioamide recognition moieties.[481] It was concluded that the amide-containing receptors bound to dihydrogenphosphate more strongly than their thioamide counterparts. The opposite trend was observed for binding interactions involving the benzoate and chloride anions. Interestingly, the lack of phenyl substituents on the pyrrole led to decreased binding affinities, with the highest affinity found to be 2.03×10^2 M^{-1} for dihydrogenphosphate and receptor **407b** in DMSO/H_2O 0.5% (all anions studied as the TBA salts). Single crystal X-ray diffraction analysis revealed that, at least in the solid state, the pyrrole amide receptors preferred an *anti-anti* conformation in the absence of anions. However, rotation to give a *syn-syn* conformation (thus forming a binding cleft) occurs in the presence of anions. Based on computational experiments, the authors suggested that thioamides have a stronger preference for the *anti-anti* conformation, which may account for their decreased binding affinities relative to their amide analogues.

A series of dipyrrolyldiketone difluoroboronate receptors (**408**) were produced by Maeda and co-workers.[482] These receptors were found to bind various anionic guests via hydrogen bonding interactions that involve both the pyrrolic N-H and bridging C-H protons. While receptor **408a** displayed a preference for the fluoride anion, absorption-based spectroscopic titrations revealed that this receptor also interacted strongly with dihydrogenphosphate ($K(H_2PO_4^-) = 1.3 \times 10^4\ M^{-1}$) in dichloromethane. It appeared that the incorporation of difluoroboronate groups into these receptors served to enhance the interaction with the anions, especially in the case of dihydrogenphosphate. The substitution of the β pyrrole positions with fluoride units (giving receptor **408b**) led to an increase in the dihydrogenphosphate affinity ($K = 1.9 \times 10^5\ M^{-1}$ in dichloromethane).[483] However, the strongest binding interaction was observed with acetate ($K = 9.6 \times 10^5\ M^{-1}$). Furthermore, the N-"blocked" receptors **409a** and **b** and the C-modified receptor **410** displayed similar fluoride affinities as those displayed by receptor **408a**.[484] However, for the other anions subject to study, the binding constant was found to decrease as the result of such substitution ($K(H_2PO_4^-) = 1.4 \times 10^3\ M^{-1}$ for receptor **409a**, $3.2 \times 10^3\ M^{-1}$ for receptor **409b** and $250\ M^{-1}$ for receptor **410**). On the basis of these findings, it was concluded that in order to bind the small fluoride anion, only 1 or 2 binding sites are actually needed. However, with this type of receptor, more recognition interactions are needed to bind larger anions. Further studies involving the β-tetraethyl substituted dipyrrolylketones **408c** and **408d** were also conducted.[485] It was found that receptor **408c** displayed higher anion affinities (e.g., $K(H_2PO_4^-) = 9.1 \times 10^4\ M^{-1}$) than receptor **408a**, with the exception of fluoride. These results were attributed to the presence of a relatively stable inverted conformation in the case of **408c**. However, receptor **408d**, which bears ethoxycarbonyl groups in the α-pyrrole positions, was found to display a lower anion binding affinity, presumably as the result of increased electrostatic and steric repulsions. All anions were studied as the TBA salts.

The aryl-substituted receptors **411** were also prepared and studied.[486] They were found to display the following anion selectivities: $F^- > AcO^- > H_2PO_4^- > Cl^-$ (all anions as the TBA salts). Further, the anion affinities were found to increase in the order **411c** < **411b** < **411a**. This binding affinity trend was rationalized in terms of both the number of C-H units available as well as to differences in planarity. Receptors **412** and **408e**, characterized by substituted α and β pyrrole positions, displayed the highest affinities for acetate. However, these systems were also found to interact with dihydrogenphosphate, with binding constants of 2.2×10^3 and $3.6 \times 10^4\ M^{-1}$ being derived for receptors **408e** and **412**, respectively, in dichloromethane.[487]

Changes in the boron substituents were also explored.[488] Replacing the fluoride substituents of receptor **408c** with pinacol (**413a**) served to decrease the anion affinity ($K(H_2PO_4^-) = 21{,}000\ M^{-1}$) in dichloromethane. The binding affinity was partially restored upon substitution with a catechol group (**413b**, $K(H_2PO_4^-) = 67{,}000\ M^{-1}$) and found to be comparable to receptor **408c** in the case of nitrocatechol substitution (**413c**, $K(H_2PO_4^-) = 115{,}000\ M^{-1}$).

Significantly stronger binding was achieved by incorporation of addition pyrrole substituents (**414a**).[489] On the basis of UV-Vis titration experiments carried out in $CHCl_3$ containing 5% ethanol, binding constants in the range of $1.2 - 3.0 \times 10^6\ M^{-1}$ could be determined for chloride, acetate, and dihydrogenphosphate. These values were two to three orders of magnitude higher than the binding constants obtained for receptors **408c** and **408e** under the same conditions. The anion binding affinities of the pyrrolic receptor **414a** also proved larger than those seen for the furan (**414b**) and thiophene (**414c**) derivatives. This increased binding affinity was attributed to the additional hydrogen bonding interactions available in the case of receptor **414a**, as well as to the extended π-conjugation present in this system.

The effect of alkyl substituents at the α-position of the pyrrole units (**415b-k**) was also investigated.[490] In these studies, anion binding affinity was observed to decrease upon increasing the length of the alkyl substitution in dichloromethane. For example, unsubstituted receptor **415a** was found to bind dihydrogenphosphate rather strongly ($K = 2.7 \times 10^5$ M^{-1}), but the binding affinity of the methyl-substituted receptor **415b** fell to 1.5×10^5 M^{-1} and that of ethyl-substituted receptor **415c** to 7.6×10^4 M^{-1}. These reductions in affinity were attributed to the electron donating ability of the alkyl chains. Support for the critical role of these electronic contributions came from studies involving fluoroalkyl receptor **415k**. Here, a strong increase in the binding affinity relative to that of the unsubstituted receptor **415a** was observed ($K(H_2PO_4^-) = 5.6 \times 10^5$ M^{-1}), with this enhancement attributed to the electron withdrawing nature of the fluoro-substituent. Interestingly, the binding affinity of receptor **415k** for anions was found to about two-fold higher than the binding affinity of the β-fluoro-substituted receptor **408b**.

In 2009, Maeda and Eifuku reported the anion binding properties of various alkoxy-substituted receptors of general structure **416**.[491] No significant differences in binding affinity were observed among the series of receptors **416c-f**, and the affinities were found to be comparable to that of unsubstituted aryl receptor **411a**. No appreciable binding was observed with *ortho*-substituted receptors **416a-b**. Taking into account the wide range of dipyrrolyldiketone difluoroboronate receptors studied, it becomes apparent that seemingly small differences in the shape, size, and hydrogen bonding donor number can have significant effects on the anion binding affinities and selectivities within this series of receptors.

More recently, Cheng and co-workers examined the anion binding ability of the tripodal pyrrole receptor **417**.[492,493] This receptor was studied in DMSO-d_6 using ^{1}H NMR spectroscopy and various TBA anion salts. Of the anions tested, the highest binding affinity was observed for dihydrogenphosphate ($K = 2.40 \times 10^2$ M^{-1}), followed by fluoride, chloride, and hydrogensulfate. A single crystal X-ray diffraction analysis of the complex formed between receptor **417** and dihydrogenphosphate revealed the expected C_3-symmetry and confirmed that the anion is held within the receptor cavity through hydrogen bonds.

A different set of amide-pyrrolic receptors **418-419** was studied recently by Yin and Cheng.[494] In this case, proton NMR titrations performed in DMSO-d_6 using various TBA anion salts revealed that receptor **419** is a less effective anion receptor than **418**. Presumably, this reflects the presence of greater steric encumbrance in host **419** as the result of the bulky Boc substitution. Receptor **418a**, containing a pyridine group, displayed the strongest interactions with anions within the set of receptors ($K = 448$ M^{-1} for $H_2PO_4^{2-}$). This relatively higher binding affinity was rationalized in terms of intramolecular hydrogen bonding interactions between the two amide protons and the pyridinic nitrogen, which led to a rigid, preorganized receptor even in the absence of an anion. Quantitative measurements revealed that receptors **418b-419** display a preference for the dihydrogenphosphate anion with the associated binding constants being on the order of 10^2 M^{-1}. On the other hand, receptor **418a** was found to interact most strongly with the fluoride anion.

Indole moieties are yet another N-heterocyclic aromatic functionality that have been successfully incorporated into phosphate anion receptors. Some of the first indole receptors tested were the acyclic systems **420** – **423**.[495] In this case, the interactions with TBA anion salts were monitored using UV-Vis spectroscopy in acetone. It was found that the strongest binding affinities were observed for benzoate followed by dihydrogenphosphate. In general, receptor **423** displayed the highest binding affinity in this solvent (log $K = 5.3$ for dihydrogenphosphate), followed by receptor **421**. Addition of dihydrogenphosphate also led to significant fluorescence enhancement in the case of this latter receptor in acetone.

Receptor **424**, reported by Jeong in 2006, was designed to contain both indole hydrogen bond donor and pyridine acceptor functionalities within a rigid scaffold.[496] Changes in the absorption spectrum were observed in acetonitrile in the presence of TBA dihydrogenphosphate, from which an association constant of 1.1×10^5 M^{-1} could be derived. Based on experiments with control compounds, the authors suggested that each pyridine moiety increased the binding affinity by ca. 15-fold. Selectivity for dihydrogenphosphate over a variety of other anions was observed, with the closest competitor being acetate, a species that bound 5-fold more weakly. This study thus served to support the conclusion that good affinity and selectivity for phosphate species can be achieved via incorporation of an appropriate number and arrangement of hydrogen bond donors and acceptors. Later studies explored the ability of appended hydroxyl groups (**425**) to participate in hydrogen bonding interactions with anions.[497] UV-Vis titrations in acetonitrile / 1% water led to the determination of a strong binding affinity between receptor **425** and dihydrogenphosphate (TBA salt, $K = 2.9 \times 10^4$ M^{-1}). Proton NMR spectroscopic experiments were consistent with this anion being bound through hydrogen bonding interactions involving both the indole NH units and the hydroxyl groups. Significantly lower association constants were seen with control receptor **426**, a finding that was taken as evidence that the OH units play an important role in anion binding. Single X-ray diffraction studies revealed the formation of a 2:2 host:guest complex in the solid state. In this complex, the OH units participated in dihydrogenphosphate binding, acting as both a hydrogen bond donor and acceptor. Despite these strong interactions, receptor **425** was found to bind acetate and chloride more strongly than dihydrogenphosphate.

Biindole moieties were also incorporated into macrocycles by Jeong and co-workers.[498] While the binding interactions with anions (added as the corresponding TBA salts) could also be observed using ^{1}H NMR spectroscopy, association constants for receptors **427** and **428** were determined using UV-Vis spectroscopic titrations carried out in acetonitrile. Association constants for the binding of dihydrogenphosphate proved to be in the range of 10^6 M^{-1}; however, similar values were observed for chloride and several other anions.

Several other biindole receptors (**429-431**) were prepared by the same group and tested in an effort to gauge the effect of incorporating additional hydrogen bond motifs into a given acyclic framework. [499] The binding constants of these receptors with TBA anion salts were measured by carrying out UV-Vis titrations in DMSO containing 0.1 - 0.2% water. While receptor **429** displayed a low affinity ($K \approx 10^2$ M^{-1}) for most simple anions, significantly higher affinities were observed for the elaborated receptors **430** and **431**. Receptor **431** was found to bind most anions slightly more strongly than **430**, although the effect was not dramatic (K ($H_2PO_4^-$) $= 7.5 \times 10^5$ M^{-1} and 1.4×10^5 M^{-1} for **431** and **430**, respectively). Proton NMR spectroscopic studies carried out in DMSO-d_6 led to the suggestion that the terminal NH groups of the urea functionalities present in receptor **431** did not participate fully in the anion binding process, thus accounting for the modest increase in observed binding affinity. However, receptor **431** was found to bind hydrogenpyrophosphate an order of magnitude more strongly than **430**, providing support for the suggestion that all six hydrogen bond donors are involved in substrate recognition in this case. These receptors were found to bind acetate approximately two to three-fold less well than they bound dihydrogenphosphate. On the other hand, dicarboxylate anions were found to bind with affinities similar to those seen for dihydrogenphosphate.

To date, a number of symmetric indole-substituted clefts have been reported, and a number of groups have contributed to the development of this chemistry. For example, Sessler and co-workers combined indole moieties with quinoxaline scaffolds (*vide supra*) to obtain receptors **432a** and **432b**.[500] The association of receptors **432a** and **432b** with anions was analyzed by carrying out UV-Vis spectroscopic titrations in dichloromethane. Reasonable

selectivity was observed for dihydrogenphosphate over chloride, fluoride, benzoate, and hydrogensulfate (all studied as the TBA salts). The association constants for the binding of $H_2PO_4^-$ to receptors **432a** and **432b** were determined to be 6.8×10^3 M^{-1} and 2.0×10^4 M^{-1}, respectively. In the case of dihydrogenphosphate binding to receptor **432b** the corresponding constant was 5.0×10^2 M^{-1} in DMSO. The higher selectivities relative to those observed with dipyrrolylquinoxaline systems were attributed to the more open conformation created by attachment at the "beta" position of the pyrrolic portion of the indole subunit. As true for the nitro-substituted dipyrrolylquinoxalines (*vide supra*), receptor **432b** displayed a visible color change in the presence of fluoride and dihydrogenphosphate.

Indole clefts containing a variety of ancillary recognition motifs were reported by Gale and coworkers. Receptor systems **433** and **434**, for example, incorporate pyridine, amide, urea, and thiourea moeties.[501,502] The interaction of receptors **433** with anions (TBA salts) was studied by ^{1}H NMR spectroscopic methods in DMSO/water mixtures. The highest affinities were observed for fluoride. However, receptor **433b** displayed an association constant of 1.14×10^3 M^{-1} with dihydrogenphosphate in 0.5% water. This value dropped to 2.6×10^2 M^{-1} in 5% water. Interestingly, a much lower association constant was observed for the interaction between receptor **433a** and dihydrogenphosphate. The small library of compounds **434** was screened for binding to acetate, dihydrogenphosphate, benzoate, and chloride. The selectivity of receptors **434a-c** was found to be a function of anion basicity, with each receptor displaying similar binding constants for dihydrogenphosphate (3×10^2 – 4×10^2 M^{-1}) in DMSO/0.5% water as determined from ^{1}H NMR spectroscopic titrations. Receptors **434d-f** were all found to bind anions with essentially the same selectivity. However, as compared to the previous receptors, these compounds were found to be more effective binding agents. The actual binding affinities followed the trend **434d** < **434f** < **434e**. The most effective receptor, the urea compound **434e**, was found to bind phosphate with an association constant of 4.95×10^3 M^{-1}.

Diindolylureas **435** were later synthesized by this same group and analyzed as anion receptors.[503,504] In general, these urea-bridged receptors displayed a preference for dihydrogenphosphate as inferred from ^{1}H NMR titrations carried out in DMSO-d_6/0.5% water. The diindolylthioureas **435c** and **435d** were found to have only a moderate affinity for the $H_2PO_4^-$ anion (K = 3830 M^{-1} for receptor **435c** and 1630 M^{-1} for receptor **435d** (studied as the TBA salt)). On the other hand, the diindolylurea receptors **435a** and **435b** exhibited much stronger affinities for $H_2PO_4^-$, with $K > 10^4$ M^{-1}. The lower affinity of the thiourea compounds was attributed to the larger sulfur atom that served to destabilize the planar conformation in the case of receptors **435c** and **435d**.

Recently, amide receptors based on 1H-indole-7-amine (**436-439**) were prepared by Jurczak and co-workers; these systems were found to interact with dihydrogenphosphate as judged from ^{1}H NMR spectroscopic studies carried out in DMSO-d_6 / 0.5% water with TBA anion salts.[505] Receptor **437** displayed the highest affinity for dihydrogenphosphate, with an association constant 2×10^3 M^{-1} being calculated for this solvent system.

In 2009, these researchers investigated diamido-diindolylmethane receptors **440**.[506] Stability constants were determined in DMSO-d_6 / 5% H_2O for dihydrogenphosphate, benzoate, chloride, and bromide (TBA salts). A very strong preference was observed for dihydrogenphosphate, particularly with receptor **440a**. The dihydrogenphosphate:**440a** binding affinity proved too high to be accurately measured by this method in 5% water solutions. In 10% water DMSO-d_6 solutions, however, a binding constant of 6.0×10^3 M^{-1} was measured for this complex; this value represented a 10-fold increase in affinity as compared to what was seen for benzoate using this same receptor (**440a**). Bromide and chloride displayed little interaction with any of the receptors, even in 0.5% water solutions.

Receptors **440b** and **440c** displayed much weaker interactions with dihydrogenphosphate, a result that can be rationalized in terms of unfavorable steric interactions. The use of similar solvent conditions allowed direct comparison to the dipyrrolylmethane-based receptors **401**, which were expected to share a similar hydrogen bond donor arrangement. Interestingly, receptor **440a** bound dihydrogenphosphate with a significantly higher binding constant than any of the these latter systems.

Pfeffer and co-workers examined the effects of combining monoindoles with other H-bond donors. Towards this end, the Pfeffer group prepared receptors **441**- **443**.[507] The interactions with $H_2PO_4^-$ (TBA salt) were then studied using ^{1}H NMR spectroscopic titrations. This provided support for the suggestion that all available hydrogen bond donors interact with dihydrogenphosphate under these conditions. Binding constants for dihydrogenphosphate were similar (within error) for all three receptors (log $K \approx 3.5$) and reasonably similar to those found for acetate. While the authors of this review are not certain, these titrations appear to have been performed in DMSO.

Later studies by Lin and co-workers demonstrated that an indole receptor bearing a phenylhydrazone moiety (**444**), binds dihydrogenphosphate well in DMSO ($K = 2.25 \times 10^4$ M^{-1}), as inferred from UV-Vis spectroscopic titrations carried out using the TBA salt.[508] However, the highest affinity was seen for acetate, perhaps reflecting the geometrical constraints of this particular receptor.

Caltagirone and co-workers recently reported the anion binding ability of several bis-indole systems containing a bipyridine scaffold (e.g., receptors **445** - **446**).[509] It was expected that the coordination of platinum by the scaffold **445** would pre-organize receptor **446** so as to enhance anion binding. All anions were tested as their TBA salts. In the absence of platinum(II), receptor **445** displayed a slight selectivity for dihydrogenphosphate over fluoride, acetate, benzoate, and chloride as inferred from ^{1}H NMR spectroscopic measurements carried out in DMSO-d_6 / 0.5% water. A binding affinity of 90 M^{-1} for dihydrogenphosphate was reported under these conditions, and a 1:1 anion binding stoichiometry was proposed on the basis of a Job plot analysis. In the presence of platinum(II), a significant increase in binding affinity was observed ($K(H_2PO_4^-) = 3600$ M^{-1} for **446**). Phosphorous and ^{195}Pt NMR spectroscopic studies led to the conclusion that the phosphate anion did not interact with the platinum center. As a result, it was proposed that coordination of platinum by the bipyridine moiety prevented free rotation around the pyridine-pyridine bond, which led to pre-organization of the hydrogen bonding indole cleft.

Bis-amidoindole systems **447** - **448** were recently reported by Gale and co-workers.[510] Proton NMR spectroscopy titrations in DMSO-d_6-0.5% water allowed for the calculation of binding constants between these receptors and a number of monoanions (TBA salts). On this basis, it was concluded that receptors **447a, 448a**, and **448b** bind dihydrogenphosphate over fluoride, acetate, benzoate, and chloride. Receptor **447b** on the other hand displayed a preference for acetate over dihydrogenphosphate. This change in binding behavior was attributed to steric interactions between the dihydrogenphosphate anion and the nitro groups within the binding cleft of receptor **447b**. This conclusion was further supported by the formation of 1:2 host:guest complexes with nitro-substituted receptor **448b** while all other complexes displayed 1:1 stoichiometries. The strongest single binding constant was reported between receptor **447a** and dihydrogenphosphate ($K = 260$ M^{-1}). Interestingly, receptor **447a** was found to bind a dihydrogenphosphate dimer in the solid phase as deduced from a single crystal X-ray diffraction analysis.

In 2009, Jurczak and co-workers reported the anion binding properties of a series of novel isoindole-based receptors (**449**).[511] Intense color changes were produced upon the addition

of basic anions (acetate, benzoate, dihydrogenphosphate, and fluoride) as the TBA salts in DMSO solution. No significant changes were observed for the less basic chloride anion. Titrations with the series of anions was performed by both UV-Vis spectroscopic and ^{1}H NMR spectroscopy in DMSO / 0.5% water. Analysis by both methods led to the conclusion that the more basic anions serve to deprotonate the receptors under these conditions. This phenomenon prevented the determination of accurate binding constants.

4.2.4. Other Hydrogen Bonding Systems—Several other types of hydrogen bonding motifs have been used for phosphate recognition. Included in these are imidazolidone-based receptors **450-451** prepared by Kang and co-workers.[512] Proton NMR and UV-Vis spectroscopic titrations carried out in DMSO:CH_3CN (1:9) provided evidence that the urea-based receptor **450** binds anionic guests more strongly than does the corresponding amide receptor **451** (anions studied as the TBA salts). Such a finding is consistent with what was seen for other receptor systems (see previous discussions). Interestingly, receptor **450** was found to interact more strongly with acetate, whereas receptor **451** was found to display a preference for dihydrogenphosphate (K ($H_2PO_4^-$) = 6.8×10^2 M^{-1}).

Peng, Han, and co-workers incorporated a benzimidazole unit into a fluorophore (**452**) and used it to effect colorimetric and fluorescent anion sensing.[513] UV-Vis and fluorescence spectral changes were observed upon the addition of fluoride, acetate, and dihydrogenphosphate (TBA salts) in acetonitrile. The degree of spectral change was found to correlate with basicity, such that fluoride > acetate > dihydrogenphosphate. No changes were observed upon the addition hydrogensulfate, chloride, and bromide. Proton NMR spectral studies carried out in CD_3CN led to the conclusion that these anions formed hydrogen bonds with receptor **452** when up to one equivalent of anion was added. However, further anion addition was found to lead to deprotonation of the benzimidazole hydrogen atom.

A series of benzimidazole receptors were analyzed by the Jang group. Their first system, receptor **453**, was found to undergo a quenching of fluorescence when exposed to dihydrogenphosphate and a variety of other anions added as the TBA salts.[514] An association constant of 2.01×10^5 M^{-1} was determined for receptor **453** and dihydrogenphosphate in acetonitrile. This relatively high affinity was attributed to the formation of four hydrogen bonds between the receptor and the anion. Receptors **454**, also produced by Jang and co-workers, were found to bind dihydrogenphosphate in addition to acetate and fluoride in a 9:1 CH_3CN:DMSO solution.[515] The interaction between $H_2PO_4^-$ and receptor **454a** was quantified by UV-Vis spectroscopy, giving rise to a K of 3.3×10^3 M^{-1}. On the other hand, receptor **454b** displayed a significantly larger association constant of 3.1×10^5 M^{-1} for this same substrate. The stronger binding interaction with receptor **454b** was unexpected. This is because the nitro groups on compound **454a** were expected to increase the acidity of the hydrogen bonding functionalities. Proton NMR spectroscopic titrations led to the suggestion that DMSO may occupy half of the binding sites in receptor **454a**, leaving fewer hydrogen bonds available for anion interactions.

In 2009, these researchers developed the benzothiazole-based cleft system **455**.[516] This receptor displayed strong fluorescence quenching upon the addition of dihydrogenphosphate in a solvent mixture of 98:1:1 CH_3CN:DMSO:water containing a HEPES buffer. An association constant of 7.9×10^3 M^{-1} was determined using this method. Little change in emission intensity was observed upon the addition of fluoride, acetate, hydrogensulfate, hydrogenphosphate, trianionic phosphate, iodide, bromide, chloride, or nitrate. This high selectivity was attributed to the specific combination of both hydrogen bond donating and accepting moieties within the cleft. Strong binding within the cleft of receptor **455** was further supported by the absence of significant anion binding interactions with receptor **456**

under these conditions. Molecular modeling studies and ^{1}H NMR titration experiments also supported the proposed binding mode.

Concomitantly, T.H. Kim and co-workers reported colorimetric anion recognition using the benzothiazole-based receptor **457**.[517] A solution of this receptor in acetonitrile underwent a light to dark yellow color change upon the addition of acetate, fluoride, and dihydrogenphosphate (studied as their TBA salts). No color change was observed upon the addition of chloride, bromide, iodide, or hydrogensulfate. The intensity of the color change was observed to correlate with the basicity of the anion. Proton NMR spectroscopic studies in DMSO-d_6 led to the suggestion that the resultant color change was due to deprotonation of the NH proton of receptor **457**. ITC studies further supported this conclusion.

Another subunit that has been explored in dihydrogenphosphate anion recognition is oxadiazole. This moiety was used by Wang and co-workers to prepare the hydroxyphenyl-oxadiazole receptors **458**.[518] The free receptors displayed both short and long wavelength emission bands, with the long wavelength bands being attributed to an excited state intramolecular proton transfer (ESIPT) process wherein a phenol proton is transferred to an oxadiazole nitrogen atom. This long wavelength band becomes quenched upon the addition of TBA dihydrogenphosphate, while the short wavelength band increases in intensity. The intensity changes were attributed to a decrease in proton transfer due to hydrogen bonding between the anion and the phenolic proton. Higher binding constants and increased selectivity were seen for receptor **458b**. In this case an association constant of 1.8×10^6 M^{-1} was recorded for the binding of dihydrogenphosphate (TBA salt) in DMF. Lower association constants of 4.1×10^4 M^{-1} and 5.0×10^2 M^{-1} were reported for the fluoride and chloride anions, respectively. Visual changes from colorless to yellow were observed for both receptors in the presence of dihydrogenphosphate and fluoride.

A series of azole containing peptide macrocycles (**459-460**) were studied by Haberhauer and co-workers in 2009.[519] Proton NMR spectroscopy supported the presence of hydrogen bonding between the amide NH group and azole nitrogen atom, which was expected to preorganize the binding cavity in the conformation shown. The anion binding ability of these macrocycles was evaluated by means of ^{1}H NMR spectroscopic titrations carried out in DMSO-d_6 / 5% $CDCl_3$ using the TBA anion salts. On the basis of Job plot analyses, all anions were found to bind as 1:1 host:guest complexes. Anion binding affinities of receptors **459** were shown to correlate with the acidity of the azole nitrogen atom such that the thiazole receptor **459c** bound anions more strongly than oxazole receptor **459b**, followed by the imidazole receptor **459a**. The lowest binding affinities were generally observed with receptor **460**, a finding that was attributed to the increased size of the macrocycle interior. In general, affinities for different anions were found to correlate with the size and basicity of the guest. As a result, the strongest binding constants were reported for dihydrogenphosphate, acetate, and fluoride over hydrogensulfate, toluene sulfonate, methyl sulfonate, chloride, nitrate, bromide, iodide, and perchlorate. The preference for dihydrogenphosphate over all other anions, however, did not fit this trend. The observed selectivity was attributed to the formation of addition hydrogen bonds between the phosphate hydrogen atoms and the azole nitrogen atoms. As a result, the highest dihydrogenphosphate:acetate selectivity (10-fold) was observed for the more basic imidazole receptor **459a**. The strongest binding constant was recorded for the **459c**:dihydrogenphosphate complex ($K = 3.0 \times 10^4$ M^{-1}).

As seen in previous sections, pyridine subunits have been extensively exploited in the design of phosphate anion sensors. Their utility reflects both acid-base and hydrogen bonding properties. The ability of pyridine (and related quinolines) to form salts was supplemented in early work by creating receptors, such as **461, 462**, and **463**, that provide for the

preorganized placement of hydrogen bond donor groups. Preliminary studies provided a confirmation that receptor **461a** facilitated the precipitation of FMN from a biphasic aqueous:chloroform mixture.[520] Salt formation between **461a** and FMN was inferred from the fluorescence quenching of FMN seen in the presence of receptor **461a** in apolar media. Such quenching was not observed with compound **461b**. Later, ^{1}H NMR spectroscopic studies of receptor **462a** and dodecylphosphoric acid in methanol led to the suggestion that salt formation involving the pyridine subunit, as well as hydrogen bonding interactions with the hydroxyl groups, contributed to the binding behavior. An association constant of 1.2×10^3 M^{-1} was calculated under these conditions.[521] Titrations with receptor **462b** produced similar results. In a separate study, analogous interactions were observed between receptor **463** and methyl phenylphosphonic acid.[522] In this case, an association constant of 7.1×10^2 M^{-1} was calculated from ^{1}H NMR spectroscopic titrations carried out in chloroform. However, direct comparisons between these receptors were made difficult due to the different targets and solvents employed. Single crystal X-ray diffraction analysis of receptor **463** and methyl phenylphosphonic acid confirmed complex formation and revealed the presence of three hydrogen bonds.

The phosphate binding properties of a bispyridine quinoxaline derivative were reported by Kruger and co-workers in 2001.[523] The protonated form of receptor **464** (**464**-H^+) displayed luminescence that was quenched by a variety of anions added as the TBA salts in acetonitrile. The proton was thought to be held between the pyridine units through a strong intramolecular hydrogen bond. This, it was suggested, would serve to increase the planarity of the receptor and enhance the preorganization of the anion binding cleft. Dihydrogenphosphate was the most strongly bound of the anions tested (including hexafluorophosphate and the halides), being bound with a K of 2.15×10^4 M^{-1} in acetonitrile. However, the emission intensity was greatly decreased in aqueous solutions and receptor **464**-H^+ was not a useful anion sensor in this medium.

Lu and co-workers developed the tetrathiofulvalene (TTF) amide system **465** with the goal of producing an electrochemical sensor.[524] Proton NMR spectroscopic studies and computational modeling supported a 1:2 dihydrogenphosphate:**465** binding stoichiometry. It also provided evidence for a complex in which each receptor contributes to three kinds of hydrogen bonds involving the pyridine nitrogen, amide NH, and the TTF C=C-H units. Cyclic voltammetry (CV) experiments carried out in dichloromethane revealed significant perturbation upon the addition of dihydrogenphosphate, while no significant change was observed in the presence of acetate, sulfate, or the halides (all species studied as the corresponding TBA salts). This lack of response in the case of competing species provides support for the high selectivity of this receptor for dihydrogenphosphate.

In early work, various heterocycles, tested as off-the-shelf, naked-eye detectable anion sensors by Sessler and Miyaji, were applied to phosphate detection.[525] Of the indicator systems tested, compounds **466-475** all displayed a visible colorimetric change in dichloromethane upon the addition of dihydrogenphosphate as the TBA salt. For example, receptor **466** displayed a dramatic yellow to purple color change in the presence of 100 molar equivalents of dihydrogenphosphate. A color change was also seen with fluoride. This signal modulation was thought to be due to the formation of charge-transfer complexes as a result of hydrogen bonding. Although absolute specificity for phosphate over fluoride could not be achieved by a single readily available compound, a multi-sensor array was suggested as a possible approach to anion sensing at neutral pH.

The benzylidene malonitrile derivatives **477-478** were reported by Zhou and co-workers in 2005; these species were observed to interact via hydrogen bonding with dihydrogenphosphate, as determined from UV-Vis spectroscopic studies carried out in

dichloromethane with TBA anion salts.[526] As expected, control compound **476**, which lacks a phenol or catechol hydrogen bond donor group, did not show any spectral change upon the addition of anions. In contrast, receptor **477** displayed a visible color change in the presence of fluoride and dihydrogenphosphate with association constants of 1.24×10^4 M^{-1} and 3.8×10^3 M^{-1} being derived for these two species, respectively. These two anions could be visually differentiated, however, via the addition of a small amount of protic solvent; this served to change the spectrum of the fluoride complex but had little effect on the spectrum of the dihydrogenphosphate complex. Little or no change was observed with other anions, including chloride, acetate, and hydrogensulfate. Compound **478** also displayed a color change in the presence of dihydrogenphosphate. However, a stronger color change was observed for the acetate and fluoride anions.

The dinitrophenylhydrazone sensors **479-480**, produced by Ou and co-workers, also displayed significant color changes in the presence of dihydrogenphosphate and fluoride anions (TBA salts).[527] The interactions were followed by UV-Vis titrations in DMSO. On the basis of these studies it was determined that receptor **479** bound fluoride more strongly than dihydrogenphosphate while the reverse was true for receptors **480**. While the binding constants for dihydrogenphosphate were similar for all three **480** derivatives, the best selectivity was found with receptor **480c**. This receptor bound dihydrogenphosphate 20-fold more strongly than fluoride, displaying a dihydrogenphosphate binding constant of 2.37×10^5 M^{-1}. It is also noteworthy that receptors **480** displayed different wavelength shifts depending on the anion added, and this difference could be observed with the naked eye. Proton NMR spectroscopic studies led to the suggestion that fluoride deprotonated the receptors while dihydrogenphosphate only interacted through hydrogen bonding. Interestingly, chloride, bromide, iodide, and nitrate produced no observable change, as determined using UV-Vis or ^{1}H NMR spectroscopies.

The anion recognition properties of hydrazone were investigated by Shang and Xu in 2009.[528] UV-Vis and fluorescence spectroscopic studies in DMSO revealed interactions of dinitro-receptor **481c** with F^-, AcO^-, and $H_2PO_4^-$ anions (added as TBA salts). Upon anion binding, a bathochromic shift in absorbance maxima was observed, along with a quenching of the inherent fluorescence of receptors **481**. In addition, visual yellow-to-violet color changes were observed when F^-, AcO^-, and $H_2PO_4^-$ anions were added to receptor **481c**. At the same time, receptor **481b** displayed spectral changes only in the presence of F^- and AcO^-, and no anion interactions were observed for receptor **481a**. The increased binding ability of **481c** relative to **481a** and **481b** was attributed to an increased acidity of the hydrazone moiety, presumably due to the increased number of electron withdrawing nitro substituents. Addition of Cl^-, Br^-, and I^- anions did not lead to spectral changes for any of the studied receptors. In the case of receptor **481c**, the largest affinity constants were reported for AcO^- ($K = 2.1 \times 10^5$ M^{-1}) and F^- ($K = 4.0 \times 10^4$ M^{-1}), while $H_2PO_4^-$ was found to bind one to two orders of magnitude less well ($K = 1.3 \times 10^3$ M^{-1}). This selectivity was attributed to the high basicity of F^- and geometric complementarity of the Y-shaped acetate anion. In addition, visual color changes were observed when F^-, AcO^-, and $H_2PO_4^-$ anions were added to receptors **481b** and **481c**.

Also in 2009, Lin and co-workers reported a system in which *p*-nitrophenylhydrazone moieties were appended to a ferrocene unit in hopes of forming a cleft for anion recognition (**482**).[529] The absorption spectrum of receptor **482** was monitored upon the addition of a number of anions as the TBA salts in DMSO. A decrease in the intensity of the maximum absorption was observed upon addition of acetate, dihydrogenphosphate, hydroxide, and fluoride. No significant change was observed upon the addition of chloride, bromide, or iodide. The change in the absorption spectrum was then used to determine association constants, which were found to bind in the order of acetate > hydroxide > fluoride >

dihydrogenphosphate. A 24-fold increase in the affinity was observed for acetate (K = 3.91 × 10^4 M^{-1}) over dihydrogenphosphate (K = 1.6 × 10^3 M^{-1}) in this system. The strong preference for acetate was attributed to the increased basicity of this anion relative to dihydrogenphosphate.

Another motif that has been used for phosphate recognition is squaramide. This subunit was incorporated into receptors **483-486** by Costa and co-workers. These systems were then tested for their anion recognition ability in a colorimetric indicator displacement assay (IDA) using Cresol red as the indicator.[530] While the addition of the dianions HPO_4^{2-} and SO_4^{2-} both led to a positive IDA response, competitive UV-Vis titrations carried out in an ethanol:water mixture (9:1) confirmed that receptors **483-486** displayed selectivity toward sulfate as opposed to phosphate. In the specific case of **485**, a relative SO_4^{2-}/HPO_4^{2-} selectivity ratio of 2.28 was found under these conditions. In this case, the anions were studied as their sodium salts.

Receptors that rely on oxygen-based hydrogen bond donor units have also been reported, specifically the carbonyl and hydroxyl motifs. For example, a reaction-based approach to phosphate detection was developed by Sancenón and co-workers.[531] It relies on the use of the 1,5-pentanedione derivatives **487** and **488**. These compounds normally undergo cyclization to form a pyrylium cation at pH 2-5, a transformation that results in a color change from yellow to magenta. However, in the case of receptor **487**, the addition of ATP allowed this color change to be visible at pH 6 in 7:3 dioxane:water. Sulfate also produced a small color change, but no other anions tested, including chloride, bromide, phosphate, GTP, and ADP, produced such a visual change at this relatively high pH. In the case of **487**, a linear response range was observed from 40 ppm to 1100 ppm for ATP. With receptor **488**, dramatic visual responses were observed for sulfate, ADP, and ATP. These shifts were attributed to hydrogen bonding between the carbonyl units of the receptors and the anions, which in turn was thought to facilitate the ring closing reaction. The addition of several inorganic cations had no effect on these reactions. Nucleotides were studied as the sodium salts and inorganic anions were studied as the TBA salts.

A unique system was reported by Zhang and Wu in 2004. It relies on the use of the magnesium complex **489**, which undergoes a naked-eye detectable color change upon treatment with TBA hydrogensulfate and TBA dihydrogenphosphate in acetonitrile.[532] These findings were rationalized in terms of a combination of electrostatic interactions involving the pre-bound Mg(II) center and hydrogen bonding with the carbonyl oxygen. The binding behavior was examined using UV-Vis and fluorescence spectroscopy in acetonitrile. However, no association constant with dihydrogenphosphate was reported.

The hydroxyl group has also been investigated as a hydrogen bond donating group. For example, Davis and co-workers reported the solubilization of hydrogenphenylphosphonate ($TBA^+PhP(OH)O_2^-$) in organic solvents by a number of cholic acid derivatives (**490** - **491**) in 1998.[533] Preliminary proton NMR spectroscopic studies in benzene-d_6 revealed solubilization of the guest in the presence of cholate **490a**. In a deuterated 1:1 benzene:hexane mixture, increased solubilization was found to correlate with increasing hydroxyl substitution such that cholates **490b** > **490c** ≈ **490d** and no solubilization was observed with cholate **490e**. In addition, alkene derivative **491** was found to be as efficient as **490a** at solubilizing the phosphonate guest in benzene. These results, as well as ^1H NMR spectral shift analysis, were considered consistent with strong hydrogen bonding interactions between the anionic guest and the cholate OH groups with little participation from the carbonyl oxygen atom of cholates **490**.

In 2002, Kondo and co-workers further investigated the hydroxyl group as a hydrogen bond donor in anion recognition.[534] The anion binding ability of receptors **492** and **493** were compared through ^{1}H NMR spectroscopic experiments carried out in deuterated acetonitrile. All anions tested (TBA salts of AcO^-, $H_2PO_4^-$, HSO_4^-, Cl^-, Br^-, I^-, and ClO_4^-) displayed a 1:1 binding stoichiometry as inferred from Job plot analyses, with the exception of perchlorate for which no binding was observed. Significant shifts in the signals corresponding to the OH group were consistent with the participation of hydroxyl-derived hydrogen bonds in the recognition of anions by receptors **492**. This conclusion was further supported by the stronger binding constants determined with receptor **492** as compared to receptor **493**. The actual values were found to correlate well with the basicity of the anion in question, with a preference for acetate being reported. Binding constants for receptors **492** and **493** for dihydrogenphosphate were determined to be 1.89×10^3 M^{-1} and 2.09×10^2 M^{-1}, respectively.

4.3. Metals in Phosphate Recognition

4.3.1. Metal Cation Coordination—Metals cations are commonly found in the binding sites of phosphate-binding proteins.[535-545] Their presence and the interactions they sustain inspired many of the receptors in this section. As will be detailed in greater length below, many of these receptors effect phosphate anion recognition as the result of dative coordinative bonds between the metal cation of the receptor and a negatively charged oxygen atom present on the phosphate group of the guest of interest.[546] Although additional functionalities can be applied to increase specificity, metal coordination of the anion is generally the greatest contributor to the stability of the complexes formed from this class of receptors. As a result, the discussion will center around the metal atom employed. Further, to provide an organizational structure, the metals will be discussed in order of atomic number, at least to the greatest extent possible.

We begin our discussion with vanadium complexes, which, like many other transition metal complexes, have been employed in the membranes of ion-selective electrodes. In 2003, Ganjali and co-workers found that inclusion of vanadyl salen complex **494** in a PVC membrane allowed for the creation of a monohydrogenphosphate selective electrode.[547] A control electrode without ionophore **494** did not display a response to the anions tested (SCN^-, SO_4^-, SO_3^{2-}, ClO_4^-, I^-, Br^-, CO_3^-, tartrate^{2-}, NO_3^-, HPO_4^{2-}). UV-Vis binding studies in acetonitrile with compound **494** provided support for axial coordination of monohydrogenphosphate. No changes in the absorption spectrum were observed with the other anions tested. The electrode gave a linear response from 1.0×10^{-1} to 5.0×10^{-6} M at pH 8.2 with a response time of 25 seconds. The vanadyl salophen complex **495** was also analyzed as a component in a PVC membrane.[548] The resulting electrode proved selective for monohydrogenphosphate, displaying a similar response range to that found for the electrode based on ionophore **494**. However, it displayed an improved response time (less than 20 seconds). In addition, the lifetime of the electrode based on complex **495** was determined to be 14 weeks. Membranes containing complexes **494** and complex **495** were further used to measure successfully the percentage of monohydrogenphosphate in commercial fertilizer.

The manganese(II) dipicolylamine (Mn(Dpa)) derived receptor **496** was found to interact selectively with pyrophosphate and ATP in aqueous solution as reported by Yoon and co-workers.[549] Upon addition of manganese to the free base Dpa receptor, chelation-enhanced fluorescence quenching (CHEQ) and a yellow-to-pink colorimetric response were observed in aqueous solution at pH 7.4. A reversal of these effects (i.e., enhanced fluorescence and a corresponding opposing color change) was observed when pyrophosphate or ATP were added to the Mn^{2+} complex. Little or no change was observed upon the addition of ADP,

AMP, $H_2PO_4^-$, HSO_4^-, or $CH_3CO_2^-$. In addition, these researchers found that the measured association constant corresponding to the binding of pyrophosphate to receptor **496** (4.2×10^4 M^{-1}) was unaffected by the presence of up to 50 equivalents of $H_2PO_4^-$. While additional structural studies were recognized as necessary to determine the precise binding modes, control experiments led to the suggestion that the manganese remains coordinated to receptor **496** and that the metal center then facilitates binding of the phosphate anions. No spectral changes were observed when the free base form of receptor **496** was treated with pyrophosphate in the absence of a metal cation. It is noteworthy that while several other metal anions led to CHEQ, no spectral changes were observed when anions were added to complexes of metals other than manganese.

D. H. Kim, J. Chin, and co-workers studied the dimethylphosphate binding ability of cobalt(III) receptors **497**.[550] As determined from single crystal X-ray diffraction studies, the coordinated water of receptor **497a** was found to be displaced by the negatively charged oxygen atom of dimethylphosphate (Figure 7). Evidence for a strong hydrogen bond between the anilinic NH group of receptor **497a** and the coordinated phosphate oxygen was also found. This binding mode was further supported by ^{1}H NMR spectroscopic analysis in D_2O. Little binding was observed with receptor **497b**, presumably due to the absence of the additional hydrogen bond donor group present in receptor **497a**. In line with these observations, equilibrium constants for receptors **497a** and **497b** were found to be 210 M^{-1} and 6.2 M^{-1}, respectively, via ^{1}H NMR spectroscopy at 80 °C. Fluoride was found to bind receptor **497a** with an affinity similar to that displayed by dimethylphosphate, while chloride and bromide interacted more weakly with this complex. All anions were studied as their sodium salts.

Through a series of detailed studies, the Martell group analyzed the phosphate binding behavior of the protonated states and metal complexes of the polyamine ligands **498** and **501**.[551] The associated interactions were studied over a pH range of 2 – 11 using potentiometric methods unless otherwise mentioned. In 1992, equilibrium constants in the range of log K = 1.5 – 7.0 were measured for the interaction of phosphate and several protonated forms of macrocycle **498**.[552] The most noteworthy results of these studies involved the complex of the hexaprotonated form of compound **498** and dihydrogenphosphate and the complexes of the di- through hexaprotonated forms of compound **498** with monohydrogenphosphate. When the copper(II) complexes of receptor **498** were studied, it was found that the mononuclear complex (**499:Cu**) displayed equilibrium constants an order of magnitude higher than the free ligand **498**, at least when comparing systems bearing equivalent charges. The related dinuclear complex (**500:Cu$_2$**) displayed similar equilibrium constants (log $K \approx 4.0$) as did the diprotonated form of the mononuclear (**499:Cu**) complex which bears a like charge. Further studies were performed to investigate the binding behavior of complex **499:Cu** and the dimetalated derivative **500:Cu$_2$** with pyrophosphate.[553] In this study, complex **500:Cu$_2$** was found to interact with pyrophosphate more strongly (log K = 2.25) than the diprotonated (equally charged) form of complex **499:Cu**. Molecular mechanics calculations involving complex **500:Cu$_2$** led to the suggestion that the receptor was rather flexible and that the pyrophosphate guest resided above the plane of the ligand rather than between the two coordination sites. Similar studies analyzed the interactions between the cobalt(II) complexes of **498** (**499:Co** and **500:Co$_2$**) and dianionic phosphite and monohydrogenphosphate.[554] The equilibrium constants corresponding to the formation of phosphite complexes with receptors **498** were found to be approximately an order of magnitude higher than those for the interaction with monohydrogenphosphate. In studies of the related cobalt(II) complexes, the equilibrium constants for the interaction between the monocobalt complex **499:Co** and both dianionic phosphite and monohydrogenphosphate were found to be approximately 0.5 log units higher

than the respective equilibrium constants for the diprotonated and hence equally charged forms of macrocycle **498**.

The binding interactions between copper(II) complexes of furan-containing macrocycle **501** and its metalated species **502:Cu, 503:Cu_2** with pyrophosphate were also investigated.[555,556] Based on the results of a prior study,[179] it was concluded that coordination of copper(II), giving rise to **502:Cu** or **503:Cu_2**, led to a significant increase in the anion binding affinities. Specifically, the equilibrium constants for the copper complexes were found to be two to three orders of magnitude higher than the respective association constants for the protonated forms of receptor **501** (at equal charge). The equilibrium constant for the interaction of pyrophosphate with the dinuclear complex **503:Cu_2** (log K = 7.73) was found to be approximately 0.4 log units higher than the equilibrium constant for the equivalent interaction with diprotonated **502:Cu**.

Interestingly, the incorporation of the aromatic units appeared to significantly affect the binding affinities. For example, the equilibrium constants corresponding to the interaction of phosphate with complex **502:Cu** were found to be two-fold higher than the corresponding equilibrium constants reported for complex **499:Cu** with respect to the same anion. However, the dinuclear **500:Cu_2** complex displayed a higher affinity for pyrophosphate then did the corresponding furan-containing **503:Cu_2** complex. Single crystal X-ray diffraction analysis of the two complexes led to the suggestion that, in the absence of an anion, the distances between the two copper centers of receptors **500:Cu_2** and **503:Cu_2** were approximately equal, at least in the solid state. These results provided support for the conclusion that the steric bulk of the furan rings interfered with the binding behavior of complex **503:Cu_2**.

Later, single crystal X-ray diffraction analysis of complexes of free base furan-containing receptor **501** and dihydrogenphosphate, pyrophosphate, or triphosphate led to the suggestion that the observed anion binding reflected a favorable combination of electrostatic interactions and hydrogen bonds.[557] Solution phase studies provided support for the conclusion that monophosphate interacts less well with receptor **501** than pyrophosphate. The interaction of pyrophosphate with receptor **501** was in turn weaker than that of triphosphate at a similar level of receptor protonation. The results were in line with the notion that electrostatic interactions played a critical role in regulating the binding affinity. Interestingly, this direct correspondence with electrostatic effects did not hold true across the board for the copper(II) complexes (**502:Cu, 503:Cu_2**). In analogy to what proved true for receptor **501**, the triphosphate complexes generally proved more stable at equal protonation states than the corresponding pyrophosphate complexes. On the other hand, the greater basicity of pyrophosphate led to higher equilibrium constants with the unprotonated and monoprotonated states of **499:Cu** and with the monoprotonated state of **503:Cu_2** as compared to the respective complexes with triphosphate. Only the dinuclear complexes **503:Cu_2** were found to interact with monophosphate, and even here the association constants were found to be relatively low.

Copper(II) complexes of expanded macrocycle **504** were also found to interact with pyrophosphate.[558] In the absence of copper, analysis of the free base macrocycle **504** revealed equilibrium constants approximately two orders of magnitude lower than those observed for the pyrophosphate complexes of the furan macrocycle **501**. However, the copper(II) complexes of macrocycle **504** (**505:Cu** and **506:Cu_2**) were found to interact with pyrophosphate significantly more strongly than the respective complexes of **501**.

Similar trends were found for phenyl-containing macrocycle **507** and its metal complexes.[559] In addition, direct competition experiments led to the conclusion that the

dinuclear complex **509:Cu$_2$** interacts more favorably with pyrophosphate than it does with monophosphate near neutral pH. A slight selectivity for triphosphate over pyrophosphate by this complex was also found under these conditions. The mono- and dinuclear macrocycles (**508:Cu** and **509:Cu$_2$**) were also found to interact with ATP and ADP.[182]

In a review of the phosphate binding behavior of the protonated and copper(II) complexes of macrocycles **498, 501**, and **507**, Martell and co-workers proposed similar binding modes for all three macrocycles.[82] In the mononuclear complexes, a covalent metal bond was available, as well as up to three hydrogen bonds, modes that were thought to give rise to strong receptor:anion interactions. The dinuclear complexes occupied all of the available nitrogen sites, leaving one or two available coordination sites at each metal center. The highest binding for mononuclear complexes was achieved when the three remaining nitrogen atoms were protonated and the bridging phosphate group remained unprotonated. Presumably, this finding reflects the fact that such an arrangement serves to maximize both coulombic and hydrogen bonding interactions. The dinuclear metal complexes were found to interact with phosphate anions most strongly when the phosphate group(s) were unprotonated and when a greater number of negative charges were available (i.e., triphosphate was found to bind more strongly than pyrophosphate). Similar trends were observed for the nucleotides ATP and ADP with respect to triphosphate and pyrophosphate. For example, it was predicted that only two of the available phosphate groups of both ATP and triphosphate interacted with the macrocyclic receptors based on distances measured during single crystal X-ray diffraction analysis. This conclusion was further supported by the observation that monoprotonation of these two anions did not produce a significant change in the equilibrium constant of the complexes.

Several research groups have expanded on these studies. For example, Herman and co-workers also examined the interactions between **508:Cu** and **509:Cu$_2$** and pyrophosphate and monophosphate.[560] The use of different conditions from the Martell studies, however, precludes a direct comparison. Nevertheless, quantitatively similar trends were observed. In addition, a preference for pyrophosphate over malonate, maleate, and fumarate was observed.

Fabbrizzi and co-workers utilized receptor **509:Cu$_2$** as the host in a pyrophosphate-selective indicator displacement assay in neutral aqueous solution.[561] Three fluorometric indicators were used for these studies. The emission of all three was completely quenched when mixed with receptor **509:Cu$_2$** in buffered water (HEPES, pH 7). For example, addition of inorganic phosphate or pyrophosphate to a complex of coumarin 343 and receptor **509:Cu$_2$** led to a strong increase in emission. This displacement allowed for quantification of the total phosphate concentration. Fluorescein, on the other hand, was found to be displaced by only pyrophosphate. This produced a ca. 10-fold increase in the fluorescence intensity when pyrophosphate was tested in the 2-20 μM range. No interference was observed upon the addition of sulfate, chloride, cyanate, acetate, benzoate, azide, or inorganic phosphate. Eosine Y displayed an even stronger signal upon displacement. The equilibrium constant measured for the interaction of receptor **509:Cu$_2$** and pyrophosphate (log K = 7.2) was at least three orders of magnitude higher than the log K values measured for inorganic phosphate or other tested anions.

Marcotte and Taglietti designed the larger macrocycle **510** with the goal of selectively sensing ATP using an indicator displacement ensemble.[562] Through force field calculations, the copper-copper distance was estimated to be 10.5 Å. The authors proposed that this was both i) an appropriate distance for the two-point coordination of triphosphate and ii) a distance that was too large to allow for the two-coordinate binding of pyrophosphate without imposing severe strain. The most effective indicator for the sensing ensemble was found to

be 6-carboxytetramethylrhodamine (6-TAMRA). The binding interactions between complex **510** and inorganic phosphate, pyrophosphate, triphosphate, and their corresponding adenosine nucleotides (AMP, ADP, ATP) were analyzed at pH 7 in aqueous media. The resulting binding affinities for complex **510** and inorganic phosphate or pyrophosphate were found to be similar to the binding affinities measured for these analytes in the case of complex **509:Cu$_2$**. The binding affinity of complex **510** and triphosphate, however, was found to be slightly higher (log K = 8.0) than the binding affinity measured for complex **509:Cu$_2$** and triphosphate. The binding constants of the nucleotides and their corresponding phosphates were generally similar. An exception was observed for AMP, whose equilibrium constant with complex **510** was found to be nearly 0.5 log units higher than that for inorganic phosphate. This result led to the suggestion that the adenine plays a modest role in mediating the binding interactions of ATP and ADP but that, in the case of AMP, the base interacts with one of the copper centers. By using the indicator displacement assay described above, ATP could be selectively identified among other common neurotransmitters through visual inspection of the sample upon illumination from a simple laboratory UV lamp.

In 2003, the smaller azamacrocycle **511** (16aneN$_6$:Cu$_2$) was analyzed by Ren and co-workers for its phosphate binding properties.[563] In HEPES buffer at pH 7.4, binding constants on the order of 10^3 M^{-1} were measured for inorganic phosphate and phosphate monoesters by monitoring the copper absorption bands via UV-Vis spectroscopy. No binding interaction was detected with fluoride, nitrate, bicarbonate, or benzoate under these conditions. Single crystal X-ray diffraction analysis with phosphate monoesters revealed two phosphate bridging structures, one on each side of the macrocyclic plane (Figure 8). However, the use of different buffer systems and different measurement methods precluded a direct comparison with the previous systems.

In the same year, Anslyn and co-workers found that the copper complexes **512** and **513** allowed the binding behavior of inorganic phosphate to be followed using UV-Vis spectroscopy and without the need for an indicator.[564] Strong binding interactions were measured for complex **513** in neutral aqueous (98:2 water:methanol) solutions, with a K of 1.5×10^4 M^{-1} for inorganic phosphate. While similar binding was observed for arsenate, no appreciable binding was observed for a variety of other anions, including sulfate. Stronger binding interactions but lower selectivities were seen in the case of receptor **512**. ITC was also used to analyze the thermodynamics of binding in the case of complexes **512** and **513**. These studies provided support for the conclusion that metal ligation was the dominant contributor to the binding interaction. Interestingly, similar Gibbs free energies were measured for the interactions of both receptors with inorganic phosphate.[565] The receptor-phosphate interactions for complex **512** were found to be entirely entropy driven while phosphate interactions with complex **513** were found to be primarily enthalpy driven. These differences in the thermodynamic parameters were attributed to solvation. Since the ammonium groups of complex **512** would be more effectively solvated, the binding of phosphate would result in a higher enthalpic cost as well as a higher entropic gain as a result of the release of these solvent molecules. Conversely, the lower charge density of the guanidinium groups and the reduced flexibility of complex **513** were assumed to lower both the enthalpic loss and entropic gain upon solvent release.

Using an indicator displacement assay with carboxyfluorescein, receptor **513** was later used in the colorimetric determination of phosphate in protein-free horse serum and human saliva.[566] The concentrations of phosphate measured using receptor **513** were comparable to the concentrations determined using a commercially available kit.

The same group analyzed the highly-preorganized “clam-shell” receptor **514** to bind 2,3-bisphosphoglycerate (2,3-BPG).[567] The cavity of this copper complex also contains four

guanidinium units that were designed to provide geometric complementarity for the phosphate groups of 2,3-BPG. This receptor exhibited a strong binding affinity for 2,3-BPG (8×10^8 M^{-1} in 1:1 water:methanol at pH 7.4). Good selectivity was also observed. Guests containing a carboxylic acid and only one phosphate group displayed binding affinities that were ca. 80 - 180-fold lower. Simple carboxylate and phosphate analytes were found to be bound with affinities four to five orders of magnitude lower than those observed for 2,3-BPG. Furthermore, this high affinity receptor could be used to reduce the level of available 2,3-BPG in a solution of horse red-cell hemolysate. The reduction was measured by monitoring the oxygenation level of hemoglobin, which is known to display increased oxygen binding upon a reduction in the 2,3-BPG concentration. Specifically, addition of receptor **514** allowed the oxygenation levels of hemoglobin to be modified.

Yoon and co-workers also analyzed the pyrophosphate binding ability of the copper and zinc receptors **515** and **516**.[568] The addition of pyrophosphate to complex **515a** in aqueous solution led to both an increase in the emission intensity of the receptor and a bathochromic shift of the emission spectrum. This is especially interesting as the structurally similar Mn(II) receptor **496**, described earlier and studied by the same group, displayed an emission increase without a shift in the emission maximum. The addition of other test anions, including dihydrogenphosphate, led only to a slight quenching of the emission of **515a** in aqueous solution. Similar spectral changes were observed with the copper-containing receptor **515b**. Association constants for the interaction with pyrophosphate were determined to be 1.68×10^5 M^{-1} and 9.84×10^4 M^{-1} for complexes **515a** and **515b**, respectively, in buffered aqueous solution (HEPES, 7.4). The closely related copper-based receptor **516** exhibited fluorescence enhancement upon the addition of pyrophosphate but no change in the emission wavelength. The binding constant for the interaction between receptor **516** and pyrophosphate was determined to be 6.4×10^3 M^{-1} under the same conditions. It is noteworthy that all three receptors were found to interact selectively with pyrophosphate in 100% aqueous solution at pH 7.4. In addition, compounds **515a** and **515b** could be used as ratiometric sensors. In particular, the differential response at different wavelengths allowed for the creation of a calibration curve, which was considered to facilitate the use of these sensors in biological applications.

A simpler Cu(Dpa) system (**517**) was reported by Hong and co-workers in 2009.[569] Both UV-Vis and fluorescence indicator displacement assays were developed using pyrocatechol violet and fluorescein indicators, respectively, in 10 mM HEPES buffer at pH 7.4. These ensembles were able to detect selectively pyrophosphate over inorganic phosphate, AMP, ADP, ATP, fluoride, chloride, bromide, iodide, nitrate, and acetate.

UV-Vis titrations were used to measure a binding constant of 3.93×10^7 M^{-1} for the complexation of pyrophosphate by receptor **517**. The binding abilities of bis-Dpa metal complexes are described in more detail in the discussion of zinc-based receptors.

Phenolic receptor **518** and the corresponding 2:1 copper(II) complex **519** were reported by Lin and co-workers.[570] On the basis of UV-Vis titrations carried out in DMSO, receptors **518** and **519** were found to interact with dihydrogenphosphate. Binding constants were measured to be 2.73×10^4 and 1.15×10^4 M^{-1} for these two receptors, respectively. Both receptors, however, displayed stronger interactions with acetate than phosphate. The phosphate binding behavior of these receptors was presumably due to a combination of metal coordination and hydrogen bonding interactions involving the amide functionalities.

Fluorescent IDAs employing receptors **520** and the indicator eosin Y were developed by the Ahn group. These receptors were found to display varying selectivities for phosphorylated compounds depending on the identity of the complexed metal.[571,572] For example, the use

of Cu(II) complex **520a** allowed the selective determination of phytate concentration in aqueous media. Pyrophosphate anions were found to interact with receptor **520a** to a lesser extent. In contrast, the use of the Zn complex **520b** allowed for the detection of IP_3. In this study, metal ions not only imparted additional binding affinity but also affected the selectivity of the host complexes.

In addition to the copper and cobalt complexes previously described, polyammonium macrocycles have also been used as ligands to produce zinc-based phosphate receptors. Many of these complexes were prepared in the context of efforts to obtain phosphoester hydrolysis catalysts. Kimura and co-workers were some of the first to study the phosphate binding behavior of such zinc complexes. For example, receptor **521** was found to interact with dianionic phenylphosphate and nitrophenylphosphate with log *K* values of 3.5 and 3.1, respectively, as inferred from potentiometric titrations.[573] A significantly lower binding affinity was observed for acetate, while no appreciable affinity was seen for bis(phenyl)phosphate esters. Zinc complexes of the larger macrocycles **522** – **524** were found to bind nitrophenylphosphate with similar association constants.[574] Incorporation of a pyridine substituent yielded receptor **525**.[575] This host was able to fluorescently sense select anions in aqueous media (HEPES buffer, pH 7.0). Significant binding was observed for dicarboxylate anions, a number of thiol derivatives, inorganic phosphate, phospho(enol)pyruvate, and *O*-phospho-L-threonine. Phosphorylated derivatives displayed log *K* values from 2.8 - 3.7. The observed effects on the emission spectrum were attributed to a conformational change around the amine-pyridine bond upon anion complexation. More recently, these researchers incorporated styrene-functionalized complex **526** into polymers for the separation of nucleotides by high performance liquid chromatography (HPLC).[576] Interestingly, this receptor monomer displayed increased binding to 4-nitrophenylphosphate and dAMP compared to receptor **522**, presumably due to hydrophobic or π-surface interactions with aromatic residues present in the guests.

The bridged receptor **527** was found to bind nitrophenylphosphate with a log *K* value of 4.0, a modest increase compared to what was observed in the case of the monomer **522**(log *K* = 3.3).[577] Despite the small apparent advantage in binding nitrophenylphosphate, this ditopic receptor, as well as the related receptor **528**, could be employed in the selective binding of deoxythymidine (dT) derivatives, including anti-HIV drugs 3′ -azido-3′ -deoxythymidine 5′ -monophosphate (AZTMP) and 3′ -azido-3′ -deoxythymidine 5′ -diphosphate (AZTDP), as inferred from ITC, potentiometric, UV-Vis, and NMR spectroscopic experiments.[578] Receptors **527** and **528** were found to have higher selectivities for thymidine derivatives over other nucleotides when compared to their monomer counterpart; presumably this is due to the additional binding interactions between the dibasic phosphate and the second Zn^{2+} center. The log apparent binding constants at pH 7.6 for receptors **527** and **528** for thymidine mono- and diphosphate derivatives ranged from 5.2-6.4. Stronger binding interactions were observed with *para*-substituted receptor **528**, a finding that is ascribable to an enhanced level of geometrical complementarity.

Kimura and co-workers also reported that the tris(Zn(II)-cyclen) complex **529** is an effective receptor for C_3-symmetric organic phosphates and phosphonates in slightly acidic aqueous solutions.[579] Potentiometric titrations of receptor **529** and phenylphosphate were consistent with the formation of 1:1 host:guest complexes with the strongest interaction (log *K* = 6.6) being observed at slightly acidic pH. The reduced association at higher pH was attributed to competitive binding of hydroxide anions to the Zn(II) centers. Receptor **529** also bound nitrophenylphosphate, glucose-1-phosphate and phenylphosphonate with the relative affinities mirroring the substrate order of basicities. No evidence of binding interactions was found with phosphodiester monoanions. Phosphorous NMR spectroscopic titrations revealed that the interaction between phenylphosphate and the free-base polyammonium rings was

negligible. This finding provided support for the conclusion that the phosphate associations resulted from interactions with the three zinc(II) ions. Further studies focused on the design of an IP_3 sensor.[580] In the context of this effort, receptor **529** was found to display strong binding to *cis*, *cis*-1,3,5-cyclohexanetriol triphosphate (CTP_3), an achiral model system for IP_3. A log apparent association constant of 8.0 for the **529**:CTP_3 complex at pH 7.4 was determined through pH-metric titrations. In efforts to develop a luminescent sensor, the phenyl core of receptor **529** was replaced with a tris(2,2′ -bipyridyl)ruthenium unit (**530**). Single crystal X-ray diffraction analysis of receptor **530** revealed a bifacial arrangement of the zinc(II) cyclen moieties, with three zinc centers making up each face. A Job plot analysis was performed for the binding of CTP_3 to receptor **530** through ^{1}H NMR spectroscopy and revealed a 1:2 host:guest stoichiometry (D_2O, pD = 7.4). Based on these results, it was proposed that each face of the receptor bound one CTP_3 molecule through coordination to the three zinc centers of that face. A strong blue shift and increase in intensity of the emission band were observed upon the addition of CTP_3 to a solution of receptor **530** in 10 mM HEPES at pH 7.4. The enhancement of the emission intensity was attributed to the increased rigidity of the complex relative to the free receptors. Receptor **530** was further able to distinguish CTP_3 from mono- and diphosphates (inorganic phosphate, phenylphosphate, glucose-6-phosphate, *cis*-1,3-cyclohexanediol diphosphate, and fructose-1,6,-diphosphate). The presence of a 10-fold excess of inorganic phosphate did not affect the luminescence sensing of CTP_3 by this receptor. Analysis by pH-metric titration allowed for the determination of a log apparent association constant of 19.0 for the 1:2 **530**:CTP_3 complex. The addition of IP_3 also led to an increase in emission intensity, albeit a less dramatic increase than that observed for CTP_3.

The ditopic zinc receptor systems **531** were developed by Benniston, Peacock, and co-workers. These systems were designed to facilitate interactions between the zinc-azamacrocycles and inorganic phosphate as well as interactions between the crown ether moiety and sodium or potassium cations. In preliminary studies with receptor **531a**, sodium and potassium phosphate were found to bind more strongly to receptor **531a** than to the zinc-azamacrocycle alone in aqueous media near neutral pH.[581] As might be predicted by the size of the crown ether, potassium phosphate was bound more effectively ($K = 9.3 \times 10^4$ M^{-1}, pH 7.4, HEPES buffer) than sodium phosphate ($K = 4.93 \times 10^4$ M^{-1}). The proposed ditopic binding mode was further supported by the absence of any appreciable interaction with $NaClO_4$ or $KClO_4$. Interestingly, ITC studies revealed that binding to the simple zinc-azamacrocycle **522** was exothermic while binding to receptor **531a** was endothermic. This led to the suggestion that binding to this ditopic receptor is driven largely by entropy. More detailed ^{1}H NMR spectroscopic analyses revealed that the addition of Zn^{2+} ions strongly perturbed the signals of the azamacrocycle but produced only minor changes to the signals associated with the crown ether. Such observations support the conclusion that the zinc ion was bound primarily to the azamacrocycle.[582] Phosphate interactions were also analyzed for receptor **531c**, a zinc(II) bimetallic complex and the copper(II) receptor **531b**. UV-Vis titrations of receptor **531c** with monohydrogenphosphate supported the formation of 1:2 host:guest complexes; however, association constants could not be determined from ITC studies, presumably due to competing aggregation phenomena. No phosphate interactions were observed for receptor **531b**.

Zn^{2+}-cyclens were employed in material-based molecular recognition by Kalinina, König, and co-workers. In 2007, monotopic (**532**) and ditopic (**533**) Zn^{2+}-cyclen amphiphiles were used in the formation of films with characteristics of both self-assembled monolayers and Langmuir-Blodgett films, which were immobilized on a gold surface.[583] On the basis of surface plasmon resonance (SPR) measurements in aqueous solution (pH 7.5) it was concluded that films based on the monotopic **532** displayed much greater sensitivity for inorganic phosphate (10^{-8} M) than uracil (10^{-4} M). SPR measurements in solutions of

uridine and adenosine nucleotides led to the suggestion of monovalent nucleotide-receptor interactions with **532**. Films based on bis(Zn^{2+}-cyclen) complex **533**, however, were significantly more sensitive to nucleotides than **532**-based films. This sensitivity was attributed to divalent intramolecular interactions in which the phosphate moiety and the nucleobase each interact with one of the two Zn^{2+}-cyclen rings.

In 2009, this recognition chemistry was applied to self-assembled vesicular receptors based on **534** and **535**.[584] These materials were formed from diacetylene surfactants, which self-assemble into vesicles that can be photopolymerized to form polydiacetylene (PDA) in situ. Vesicles based on monotopic **534** displayed a colorimetric response (blue-to-red) upon addition of ATP and pyrophosphate anions in buffered aqueous solution (HEPES pH 7.2). No color change was observed upon addition of halide anions, dihydrogenphosphate, acetate, AMP, or ADP. These anion-binding interactions were also monitored through UV-Vis spectroscopy, which allowed for the determination of apparent binding constants for ATP and pyrophosphate to **534**-functionalized vesicles (log *K*(ATP) = 2.2; log *K*(pyrophosphate) = 2.5). Stronger binding interactions were observed with vesicles based on ditopic **535** functionalization (log *K*(ATP) = 2.7; log *K*(pyrophosphate) = 3.2), presumably due to divalent interactions between these anions and the bis(Zn^{2+}-cyclen) complex. The copper(II) complex **536** was also evaluated for a response to anions under these conditions. Interestingly, **536**-functionalized vesicles displayed a change in absorption spectra only with pyrophosphate among the anions studied (log *K*(pyrophosphate) = 2.6). These trends were also found in paper test strip-based experiments. Interestingly, light microscopy images led to the conclusion that ATP and pyrophosphate caused aggregation of **535**-functionalized vesicles under these conditions, but inorganic phosphate did not.

The Lin group investigated the binding behavior of ligand **537** and its metal complexes (**538**) with adenosine nucleotides.[585] As seen with many of the previously described polyammonium macrocycles, the binding affinities with receptor **537** increased with increasing degree of protonation as inferred from potentiometric titration (pH 2-10.5). This receptor bound ATP > ADP > AMP in accordance with the relative anionic charge of the analytes. The ternary complexes formed by combination of ligand **537** with Zn^{2+} or Cd^{2+} (giving complexes **538**) were also found to interact with ATP. Phosphorous NMR spectroscopic studies led to the conclusion that only the terminal phosphate of the ATP substrate interacts with the metal centers.

Another ditopic Zn(II) complex (**539**) was prepared by Kwong and co-workers.[586] This receptor was proposed to interact with anions via a combination of metal-ligand and hydrophobic interactions. Fluorometric studies in water:methanol (1:4) at pH 7.4 revealed strong fluorescent quenching of receptor **539** by carboxylates, with less quenching observed in the presence of the dihydrogenphosphate, nitrate, bicarbonate, bromide, and iodide anions.

Bencini and Bianchi analyzed the multifunctional receptor **540**.[587] Proton and ^{31}P NMR spectroscopic studies revealed strong binding to ATP. Both terminal phosphate groups of ATP were found to interact with the metal center. In addition, π-surface interactions were postulated to exist between the tripyridine unit and the adenine base.

The heteroditopic zinc-containing receptor **541** was prepared and analyzed by Gunning. This receptor displayed positive cooperative binding behavior for phosphate anions under physiological conditions.[588] Proton NMR spectroscopic titrations carried out in HEPES buffer at pH 7.4 allowed binding constants of 3.90×10^4 for $Na[H_2PO_4]$, 5.10×10^4 for $K[H_2PO_4]$, and 3.13×10^4 M^{-1} for $Li[H_2PO_4]$ to be calculated. The affinities of these

phosphate salts were found to correlate well with the known affinities of the respective cations for the crown ether moiety.

In later studies, Bencini and Bianchi demonstrated that the zinc(II) phenanthroline complexes **542:Zn** and **543:Zn_2** interacted with bis(4-nitrophenyl)phosphate (BNPP) in CD_3OD as inferred from ^{31}P NMR spectroscopic studies.[589] Significantly stronger binding was observed with the ditopic receptor **543:Zn_2**, presumably due to the chelation ability of the dinuclear complex. Phosphorous and ^{1}H NMR spectroscopic titrations provided support for the proposed formation of 1:1 complexes as well as the presence of π-surface interactions between the phenanthroline groups and the *p*-nitrophenyl moieties of BNPP. These complexes were also found to effect phosphoester hydrolysis, with ditopic **543:Zn_2** displaying a rate six-fold higher than that of the monotopic receptor **542:Zn**.

The bis-(cobalt(II) dipicolylamine) complex **544a** was originally developed by J. Chin and co-workers for use as a phosphate hydrolysis catalyst.[590] Using the same organic frame, but with coordinated Zn(II) cations instead of Co(II) (complex **544b)**, D. H. Kim and Han were able to develop a colorimetric indicator displacement assay for inorganic phosphate in aqueous media.[591] The displacement of pyrocatechol violet from receptor **544b** in HEPES buffered water at pH 7.0 with inorganic phosphate led to significant spectroscopic changes. A drastic visible color change, from blue to yellow, was also observed. An association constant of 1.1×10^5 M^{-1} was measured for receptor **544b** and inorganic phosphate through ITC experiments. Interestingly, little or no response was observed with other anions, including acetate, carbonate, nitrate, azide, perchlorate, sulfate, fluoride, chloride, and bromide. Receptor **544b** was later found to bind AMP but not 3′,5′-cAMP.[592] Based on this selectivity, the researchers were able to develop a visual assay of cyclic nucleotide phosphodiesterase (PDE) activity. In this assay, a solution of 3′,5′-cAMP and receptor **544b** was added to a solution of PDE. PDE then effected the conversion of 3′,5′-cAMP to AMP, and the presence of receptor **544b** allowed for real-time monitoring of the increasing AMP concentration. More recently, R. Smith and co-workers developed a pyrophosphate-selective indicator displacement assay based on receptor **544b**.[593] Eleven commercially available indicators were screened for spectroscopic changes upon the addition of **544b** in a solution of aqueous HEPES buffer at pH 7.4. Indicators with binding affinities that fell between the affinities for inorganic phosphate and pyrophosphate were identified. When complexed to receptor **544b**, these indicators (bromo pyrogallol red, mordant blue 9, and zincon) displayed a selective response to the addition of pyrophosphate over inorganic phosphate. Binding constants were not reported using this method.

Hamachi and co-workers have extensively pursued zinc(II) dipicolylamine (Zn(Dpa)) as a useful molecular recognition motif for phosphates, particularly phosphorylated proteins and peptides. The underlying host:guest interactions presumably derive from metal-ligand coordination chemistry. The Zn(Dpa) unit has also been studied as a selective phosphate binding motif. For example, receptor **545** was found to interact selectively with inorganic phosphate and methylphosphate over carbonate, acetate, nitrate, sulfate, azide, and chloride in aqueous media at pH 7.2.[594] Furthermore, the addition of ATP to receptor **545** resulted in fluorescence enhancement. An affinity constant of 2.2×10^6 M^{-1} was measured for the **545**:ATP complex. Significantly weaker binding behavior was observed with AMP and ADP, and no binding behavior was observed for 3′,5′-cAMP. The selectively of receptor **545** for ATP was attributed to the higher charge of the triphosphate. Phosphorous NMR spectroscopic studies provided support for the conclusion that receptor **545** bound the two terminal phosphate units of ATP. Reasonable selectivity for ATP over other nucleoside triphosphates was also observed.

A more application driven study by the same researchers relied on the use of receptor **545** in an assay of glycosyltransferase activity.[595] These studies first confirmed the selectivity of receptor **545** for phosphate monoesters over phosphate diesters. This selectivity permitted for the formation of 5′-uridine diphosphate (UDP) to be monitored in the presence of the starting glycosylated nucleotide. This allowed the glycosylation reaction to be monitored without having to modify the enzyme or its substrates.

Analysis of the anion binding behavior of receptor **546** revealed a similar selectivity trend as that previously observed with receptor **545**.[596] Binding constants on the order of 10^4-10^5 M^{-1} were measured for receptor **546** with inorganic phosphate, adenosine nucleotides, and a variety of phosphate monoesters. The binding interactions were again monitored via fluorescence titration. No change in the emission spectrum of receptor **546** was observed upon additional of 3′,5′-cAMP or dimethylphosphate. Furthermore, a mono-Zn(Dpa) anthracene (**547**) did not undergo a change in fluorescence in the presence of these phosphate species. Displacement experiments with this mono-Zn(Dpa) receptor led to the determination of binding constants 1-2 orders of magnitude lower than those observed with the bis-Zn(Dpa) analogues **545** and **546**. Proton NMR spectroscopic studies and single crystal X-ray diffraction analysis of receptor **546** and phenylphosphate supported equal participation of the two Zn(Dpa) units, with each metal center interacting with a phosphate oxygen atom (Figure 9). The fluorescent enhancement observed upon phosphate anion addition to these receptors was attributed to an increase in the extent to which the second zinc atom was complexed to the Dpa ligand in the presence of phosphate. It was proposed that this increased metal-ligand interaction serves to reduce the inherent PET quenching of the anthracene fluorescence by the Dpa amine ligand.

In further application-based studies by these researchers, receptors **545** and **546** were found to bind phosphorylated peptides over non-phosphorylated peptides under neutral aqueous conditions.[597] Binding constants for the interactions between **545** and **546** with **548a** and **d** were determined through fluorescence spectroscopy and were found to range from 10^4 - 10^7 M^{-1}. In contrast, no binding behavior was observed for the non-phosphorylated sequence **548g** or for a phosphorylated sequence bearing positively charged arginine residues (**548c**). The observed charge selectivity was attributed to charge repulsion between the cationic zinc centers and the arginine side chains. When the phosphorylated peptides **548a** and **d** were added to 9-Zn(Dpa)-anthracene (**547**), the observed binding constants were two to three orders of magnitude lower than what was observed for the ditopic receptors. The reduced binding affinities seen for the mono-Zn(Dpa) complex were consistent with the conclusion that ditopic chelation played a critical role in the binding abilities of receptors **545** and **546**. Further binding analyses with a larger set of phosphorylated peptides (**548a-f**) supported the conclusion that the affinity was strongly dependent on the overall charge of the peptide, with the strongest affinities being recorded for negatively charged sequences **548a**,**b** ($K \approx 10^6 - 10^7$ M^{-1}).[596] More detailed ITC studies revealed that the binding behavior was entropy driven, presumably due to solvation effects. Taking advantage of the receptor:peptide interactions, these researchers were able to show that receptor **546** could be used to monitor phosphatase activity via changes in the fluorescence emission intensity in the case of various peptide substrates. Other applications of this class of receptors included fluorescent staining of phosphoproteins on an SDS-PAGE gel[598] and the disruption of phosphoprotein-protein surface interactions.[599] Many of the above studies have been reviewed by Ojida and Hamachi.[600,601]

Hamachi and co-workers also investigated the utility of bipyridine bis(ZnDpa) receptors **549** and **550** as cross-linkers in solutions of hyperphosphorylated peptide sequences.[602] These interactions were attributed to the stabilization of helix formation as a result of the simultaneous binding of multiple phosphate residues enforced by the complexes. This

stabilization was monitored using circular dichroism (CD) spectroscopy. Fluorescence quenching of the receptors in the presence of the peptides was also observed. No significant conformational change was observed when receptors **549** and **550** were presented with monophosphorylated peptide sequences, or when mono-Dpa analogues were presented with the hyperphosphorylated sequences. These results provided support for the proposed cross-linking interactions.

Other fluorophore scaffolds for the Zn(Dpa) subunit were investigated later in the context of this work. For example, the acridine-based receptors **551** and **552** were synthesized. These systems displayed a reduction in emission intensity and a blue-shift in the emission wavelength in the presence of various nucleotides in HEPES buffer at pH 7.2.[603] Binding affinities in the range of 10^6 -10^7 M^{-1} were measured for tri- and diphosphates in this aqueous medium, while much lower affinities were observed for nucleotide monophosphates, cyclic monophosphates, and monohydrogenphosphate. Little to no response was observed with non-phosphate anions. The spectroscopic changes seen in the presence of phosphates were attributed to a change in the coordination mode of zinc upon the addition of the phosphate anions. Hamachi and co-workers proposed that in the absence of anions the zinc coordinates to the nitrogen atom of the acridine as well as to the nitrogen atoms of the Dpa units. These interactions led to an increase in the emission intensity and a red-shift in the acridine fluorescence spectrum. This effect was reversed in the presence of the di- or triphosphates. Presumably, these species coordinate to the Dpa units and reduce coordination to the acridine nitrogen atom. These sensors were then used to monitor ATP and ADP hydrolysis as well as glycotransferase activity.

A xanthone-based bis-ZnDpa scaffold (**553**) was also investigated.[604] Under similar conditions as used for the acridine-based studies, receptor **553** was found to interact with nucleotide tri- and diphosphates. Slightly lower binding affinities (10^5 – 10^6 M^{-1}) were measured with receptor **553** as compared to receptors **551** and **552**. Similar selectivities were observed. The interaction of receptor **553** with ATP was also studied by ITC. These studies revealed that the binding process was both enthalpically and entropically driven. Spectroscopically, emission changes were observed at three separate wavelengths upon addition of phosphate anions to receptor **553**. Again, a rearrangement of the zinc coordination environment was proposed as the source of the observed spectral changes. More recently, a xanthene-based scaffold (**554**) was explored as part of continued efforts to improve the sensitivity of this class of receptor.[605] Receptor **554** was found to exhibit unexpectedly low fluorescence in aqueous solution. Single crystal X-ray diffraction analysis of receptor **554** revealed a structure in which a water molecule coordinated to both zinc centers had added into the xanthene chromophore. High-resolution fast atom bombardment (FAB) mass spectrometry of the receptor also supported this addition reaction. The resulting disruption of the conjugation was proposed as the cause of the low emission intensity. This reaction was found to be reversible through pH titrations. A strong increase in fluorescence was observed upon the addition of ATP to receptor **554** at pH 7.4 (50 mM HEPES, 10 mM NaCl, 1 mM $MgCl_2$). It was suggested that the coordination of ATP by the zinc centers shifted the equilibrium away from the water adduct. A strong binding constant ($K = 1.3 \times 10^6$ M^{-1}) and high sensitivity (< 10^{-6} M ATP) were observed in further fluorescence experiments. Job plot analyses were consistent with a 1:1 host:guest stoichiometry for ATP. Similar binding constants were observed with other nucleotides (ADP, GTP, CTP, UDP) and IP_3. A significantly higher binding constant was measured for the pyrophosphate complex ($K = 4.0 \times 10^7$ M^{-1}), presumably due to the higher charge density of this anion. Little to no change in the emission intensity was observed upon the addition of monophosphate species (HPO_4^{2-}, 3′,5′-cGMP, 3′,5′-cAMP) and other small inorganic anions (AcO^-, SO_4^{2-}, NO_3^-, HCO_3^-). This receptor was also applied as a stain for ATP in human cells.

Hamachi and co-workers have also studied the ability of this class of receptors to sense phosphate when incorporated in a hydrogel. In preliminary studies, the incorporation of di-Zn(Dpa) receptor **545** into a semi-wet hydrogel was investigated.[606] The hydrogel material was based on a glycosylated amino acid-type hydrogelator, which was transparent enough to allow supramolecular binding events to be monitored in the gel matrix. For example, a binding constant of 1.1×10^5 M^{-1} for receptor **545** and inorganic phosphate was inferred from fluorescence titrations in a hydrogel matrix. This value was three-fold lower than the binding constant reported for **545** and inorganic phosphate in aqueous solution. Fluorescence enhancements were also observed upon addition of phenylphosphate and phosphotyrosine to the **545**-hydrogel. Addition of sulfate, however, did not lead to a change in the emission spectrum. Further studies examined the response of receptors **545, 555**, and **556** in hydrogel materials to a wider variety of anions.[607] Receptor **545** displayed a binding constant of greater than 10^6 M^{-1} for ATP when studied through fluorescence titrations in the hydrogel matrix. No changes in the emission spectrum were observed for sulfate, nitrate, acetate, azide, bromide, chloride, or fluoride. Receptor **555**, containing an environmentally sensitive dansyl fluorophore, displayed differing responses to phosphate anions when in the hydrogel. For example, a blue-shift in emission maxima and an increase in emission intensity was observed upon addition of phenylphosphate. On the other hand, addition of ATP, inorganic phosphate, and phosphotyrosine led to a red-shift in emission maxima and a decrease in emission intensity. The binding constants for receptor **555** with ATP and phenylphosphate were measured to be 1.8×10^5 M^{-1} and 7.2×10^3 M^{-1}, respectively. Interestingly, receptor **555** did not display a response to anions in aqueous solution or when immobilized in an agarose gel. Using confocal microscopy, fluorescent receptors **555** and **556** were observed to travel from the aqueous solution to hydrophobic fibers in the gel matrix in the absence of anions. However, the addition of ATP or phenylphosphate altered the hydrophobicity of these fluorescent receptors, causing the complexes to move in the opposite direction.

Hong and co-workers utilized Zn(Dpa) units to create the azophenol receptor **557** that was designed for the visual sensing of pyrophosphate.[608] While monovalent anions caused no change in the absorption spectrum, the addition of pyrophosphate led to a strong bathochromic shift. This change could be observed visually by a change in the solution color from yellow to red over the pH range 6.5-8.3. A strong binding affinity was observed, with an association constant of 6.6×10^8 M^{-1} being recorded in HEPES buffer at pH 7.4. Single crystal X-ray diffraction analysis of the complex led to the conclusion that the anion bridges the two zinc centers in the solid phase. The visual color change was attributed to the decrease in the association between the phenolic OH and the zinc centers that takes place upon anion coordination. A red absorbance shift and strong increase in emission intensity were observed for receptor **558** in the presence of pyrophosphate.[609] A binding constant of 2.9×10^8 M^{-1} was determined under conditions similar to those used to analyze receptor **557** (HEPES buffer, pH 7.4). Much smaller spectroscopic changes were observed with ATP, ADP, and AMP as compared to what was seen with pyrophosphate. Based on this selectivity, it was proposed that this sensor could be useful in bioanalytical assays, particularly ones that require the measurement of small amounts of pyrophosphate in the presence of ATP.

In efforts to improve the binding affinity of these receptors, amide substituents were included in the design of receptor **559**.[610] The interaction of this receptor with pyrophosphate was followed through an indicator displacement assay using pyrocatechol violet in 10 mM HEPES buffer at pH 7.4. An extremely high binding affinity was inferred, and the binding constant could only be determined through competitive binding experiments with receptor **558** ($K = 5.39 \times 10^{10}$ M^{-1}). Job plot analysis confirmed a 1:1 host:guest stoichiometry. No change in the absorption spectrum was observed upon the addition of

inorganic phosphate, fluoride, chloride, bromide, acetate, bicarbonate, sulfate, azide, nitrate, perchlorate, or citrate anions. A single crystal X-ray diffraction analysis of the **559**:pyrophosphate complex revealed chelation of the anion by both metal centers and the formation of hydrogen bonds with all four amide units. Notably, a 100-fold increase in binding affinity in aqueous media was achieved by the incorporation of four hydrogen bond donor units.

A FRET-based assay was achieved by attaching one unit of a fluorophore pair to the bis(Zn(DPA)) host and phosphate guest, respectively (**560, 561**).[611] In this case, methyl red was appended to the recognition unit (**560**) while fluorescein was appended to phosphate (**561**). Fluorophore **561** then served as the indicator in a displacement assay with host **560**, in which decomplexation of the indicator decreased the FRET efficiency of the system. This signal could be used to detect a number of anions, in the order pyrophosphate > ATP > ADP > AMP ≈ inorganic phosphate > acetate ≈ fluoride in 10 mM HEPES buffer at pH 7.4. A binding constant of 10^8 M^{-1} was estimated for the **560**:pyrophosphate complex using this method.

While little activity was seen for monomers of other Zn(Dpa) complexes, the pyrene-based receptor **562** was found to form dimers with pyrophosphate and ATP.[612] This dimerization could be observed through excimer formation in aqueous solution (HEPES buffer, pH 7.4). The addition of monovalent anions, including hydrogenphosphate and AMP, did not lead to spectroscopic changes. While no binding constants were reported, a significantly more intense excimer emission was observed for pyrophosphate than for ATP. More recently, these researchers reported the binding of FAD by simple receptor **563**.[613] This receptor served to increase the innate fluorescence of FAD, and this emission increase was used to determined a binding constant of 1.11×10^5 M^{-1} in water (10 mM HEPES, pH 7.4). Little change was observed in the emission of FMN upon addition of this receptors. In efforts toward a biological application, receptor **563** was used to treat human white blood cells, which are known to contain high levels of FAD. This allowed for imaging of these cells through flow cytometry.

Smith and co-workers analyzed Zn(Dpa) sensors for biological applications. In preliminary studies, receptor **545** (described previously) was found to bind phosphatidylserine (PS), an anionic phospholipid, on cell surfaces.[614] The binding behavior was monitored through an increase in fluorescence intensity. No change in the emission spectrum was observed when receptor **545** was treated with zwitterionic phospholipids or when a mono-Zn(Dpa) sensor (**547**) was treated with PS. Receptor **545** was then used to detect apoptotic cells, which are commonly identified by the presence of PS on the cell surfaces. No cell internalization or cytotoxicity was detected. A library of functionalized Zn(Dpa) sensors were then analyzed in hopes of obtaining a red-shifted excitation wavelength; this was done via the incorporation of a 7-nitrobenz-2-oxa-1,3-diaza-4-yl (NBD) fluorophore. [615] Among the several sensors containing this latter moiety, the bis-Zn(Dpa) system **564** proved to be the most effective for the detection of PS-containing vesicles. Interestingly, fluorescence enhancement was only observed for PS located on vesicle surfaces. Again, no vesicle internalization or increased permeability was observed. Receptor **564** was able to detect vesicles containing as little as 5% PS. The sensing mechanism was attributed to the sensitivity of the NBD fluorophore to environmental polarity.

Fluorescent indicator displacement assays were also analyzed for use in the detection of PS.[616] Two Zn(Dpa) receptors described previously (i.e. **544b** and **563**) were incorporated into displacement assays that were based on the use of a coumarin (**565**) as the indicator in aqueous media at pH 7.4. The bis-Zn(Dpa) receptors again displayed selectivity for hydrogenphosphate and pyrophosphate over other anions. Receptor **563** was found to bind

pyrophosphate more strongly than it bound hydrogenphosphate. The association constants for these two substrates were $1.5 \times 10^{7-}$ M^{-1} and 7.3×10^5 M^{-1}, respectively. Interestingly, receptor **563** was found to bind both phosphate species more strongly than receptor **544b**. This increased binding affinity was attributed to the lower net positive charge of receptor **544b** as a result of the phenolate anion. Analysis of the *para*-substituted derivate of **563** did not reveal any binding interactions with phosphate derivatives, again emphasizing the importance of the bridging coordination of the two zinc centers in these systems. Such displacement assays were then applied to vesicles containing PS.[617] In studies with pyrocatechol violet as the indicator, only receptor **544b** proved responsive to low (5%) concentrations of PS in the vesicles. Receptor **563**, however, proved to be the most responsive receptor when using fluorescence indicator **565**; this resulted in detection limits as low was 5% PS. No displacement was observed with monodisperse PS.

A macrocyclic peptide was employed as a scaffold for a bis-Zn(Dpa) pyrophosphate sensor by Jolliffe and co-workers.[618] This gave rise to receptor **566**. In combination with this receptor, indicator **565** was used to achieve fluorescence sensing in aqueous media (HEPES buffer, pH 7.2). It was proposed that the larger spacing between the two zinc centers in this macrocycle-containing framework would lead to greater selectivity for pyrophosphate than had been observed with the previously described bis-Zn(Dpa) receptors. A high association constant (log $K = 8.0$) was indeed obtained for receptor **566** and pyrophosphate. The selectivity for pyrophosphate was good, with pyrophosphate being bound approximately two orders of magnitude more tightly than ATP, ADP, or citrate. No other anions appeared to bind this receptor. The observed selectivities were similar to those observed with the previously investigated bis-Zn(Dpa) systems. The larger scaffold, therefore, increased the association constant for pyrophosphate but did not significantly affect the overall selectivity.

In addition to their work with receptor **515** described earlier, the Yoon group investigated different scaffolds for preparing bis-Zn(Dpa) systems. Simple acridine receptor **567** displayed differing spectral responses for inorganic phosphate and pyrophosphate, respectively, in aqueous solution (HEPES buffer, pH 7.4).[619] Specifically, emission enhancement was seen upon addition of inorganic phosphate to receptor **567**. In contrast, quenching was observed when exposed to pyrophosphate. Addition of either anion led to a bathochromic shift in the emission maximum. The quenching mechanism with respect to pyrophosphate addition was assumed to be consistent with that previously observed for related receptors **551** and **552**, in which the anions coordinate to the Dpa units and reduce coordination to the acridine nitrogen atom. On the other hand, the emission increase seen upon the addition of inorganic phosphate was attributed to the formation of an additional hydrogen bond between the acridine nitrogen and the bound phosphate. Binding constants of 4.85×10^7 M^{-1} and 9.36×10^4 M^{-1} were determined for receptor **567** with pyrophosphate and inorganic phosphate, respectively. No spectral change was observed upon the addition of a variety of other anions.

Sensor **568** was also prepared by Yoon and co-workers. This system proved to be reasonably selective for pyrophosphate based on the formation of 2 + 2 complexes that induced excimer emission.[620] An association constant of 4.1×10^5 M^{-1} was determined in aqueous solution (HEPES buffer, pH 7.4). The only response among the other anions tested was a slight increase in emission upon addition of ATP. Despite this response, pyrophosphate could be reliably detected in the presence of 10 molar equiv. of ATP.

Das and co-workers analyzed the interaction of the mono-Zn(Dpa) receptor **569** with phosphate derivatives.[621] Receptor **569** was found to bind selectively dihydrogenphosphate among other monovalent anions in acetonitrile ($K = 5.62 \times 10^5$ M^{-1}). A striking yellow to red color change was also observed upon addition of dihydrogenphosphate. The binding

behavior of inorganic phosphate and other phosphate derivates was then tested in aqueous solution (HEPES buffer, pH 7.2). Under these conditions, responses were only observed upon the addition of ATP, ADP, and CTP, as inferred from red-shifted absorbance bands. Association constants for these three anions were determined to be 1.13×10^3 M^{-1}, 2.50×10^2 M^{-1}, and 7.72×10^2 M^{-1}, respectively. The largest shift (ca. 20 nm) was observed for ATP. ATP was also the only anion to produce a visual color change from yellow to pink. The addition of AMP, pyrophosphate, and inorganic phosphate did not give rise to any appreciable spectral changes. The stronger binding of ATP as compared to CTP was attributed to the weaker electron donating character of CTP as a result of its constituent pyrimidone moiety. This receptor was later utilized as a non-toxic stain against ATP production in yeast and bacterial cells.[622]

In 2009, Guo, He, and co-workers reported triphosphate and pyrophosphate sensor **570**.[623] This receptor incorporated a 4-amino-7-aminosulfonyl-2,1,3-benzoxidiazole fluorophore that served as a visible light excited reporter unit. A five -six-fold emission enhancement was observed upon the addition of one equivalent of triphosphate or pyrophosphate to receptor **570** in aqueous media (10 mM HEPES, 1% DMSO, pH 7.2). The strong change in emission intensity was attributed to a chelation effect in which the polyanionic guests were bound by both Zn(Dpa) centers. No change in emission was observed in the presence of inorganic phosphate, acetate, nitrate, chloride, carbonate, sulfate, perchlorate, or fluoride. The importance of the second Zn(Dpa) binding site was supported by the lack of response of receptor **571** to any of the studied anions under the same conditions. The anion binding properties of receptor **572**, in which the Zn(Dpa) units are directly conjugated to the fluorophore) were also investigated. This receptor did not respond to the addition of any anions, presumably due to a reduced zinc-binding ability of the Dpa as a result of the electron-withdrawing sulfonamide substituent. No binding constants were reported in this study.

Qian, Tang, and co-workers studied a bis(2-benzimidiazolylmethyl)amino group (see **573**) as a replacement for the Dpa unit of receptor **544b** in hopes that steric interactions between the benzimidazole units would increase the metal-metal distance.[624] This increase was expected to facilitate selective pyrophosphate recognition in aqueous media. Following the synthesis of receptor **573**, an indicator displacement assay was developed using fluorescein as a fluorescent indicator. Addition of pyrophosphate to the **573**:fluorescein ensemble led to displacement of the indicator as inferred from an increase in fluorescence intensity (10 mM HEPES buffer, pH 7.4). No change in fluorescence was observed upon the addition of oxalate, inorganic phosphate, acetate, hydrogensulfate, fluoride, chloride, iodide, or bromide to the ensemble. Titrations with pyrophosphate led to the determination of a stability constant of 1.3×10^5 M^{-1} and a stoichiometry of 1:1 through Job plot analysis. It was thus concluded that receptor **573** is reasonably selective for pyrophosphate.

Receptors containing ammonium-expanded analogues of the Dpa systems were reported by Pina, García-España, and co-workers.[625] Among these is the anthracene-based receptor **574**. This system displayed a significant decrease in emission intensity in the presence of triphosphate, pyrophosphate, iodide, citrate, and cyanurate (pH 8, 0.15 M NaCl). Each of these anions were bound with similar affinity (log *K* 5.2 - 5.8), and very little change in fluorescence was observed upon the addition of inorganic phosphate, fluoride, or D, L-isocitrate. At the same time, addition of triphosphate, pyrophosphate, citrate, and D,L-isocitrate to dansyl-based receptor **575** led to an overall increase in emission intensity (pH 7.5, 0.15 M NaCl). No change in emission was observed with the other anions tested. This receptor displayed more selective binding than receptor **574**. Significantly higher values were reported for triphosphate and pyrophosphate (log $K = 4.9$ and 4.1, respectively) than

citrate and D,L-isocitrate (log K = 2.7 and 2.1, respectively). This study highlights the importance of the reporter moiety in these systems.

In 2007, Guo, Wang, and co-workers described the dihydrogenphosphate sensing abilities of a tetranuclear zinc(II) complex based on a cresolic ligand (**576**).[626] A single crystal X-ray diffraction analysis of complex **576** revealed a 4:2 metal:ligand structure in which all of the ligand heteroatoms are bound to pentacoordinate zinc(II) centers. This receptor was found to be strongly fluorescent in unbuffered methanol. Addition of NaH_2PO_4 led to a dramatic decrease in fluorescence intensity while the addition of Na_2CO_3, $NaHCO_3$, $NaNO_3$, $NaNO_2$, NaF, NaCl, NaBr, NI, or Na_2SO_4 led to rather small changes in the emission spectrum. Proton NMR spectroscopic experiments supported a binding mechanism in which the dihydrogenphosphate anion displaced the central phenol oxygen from the zinc(II) center. It was proposed that this displacement disrupted the ligand-to-metal charge transfer in the native complex, resulting in decreased emission. Evidence for both 1:1 and 1:2 host:guest binding stoichiometries was found, and an overall association constant was estimated to be 1.0×10^5 M^{-1}.

Polyamine-phenol-based zinc complexes (**577** and **578**) were developed as anion receptors by Fusi, Micheloni, and co-workers in 2009.[627] Potentiometric studies in aqueous solution (0.15 M NaCl) revealed the formation of 1:1 complexes with the receptors and both inorganic phosphate and pyrophosphate. At near-neutral pH, a strong preference was observed for pyrophosphate by **577** and for inorganic phosphate by **578**. This selectivity was attributed to the respective Zn-Zn distances in each receptor. The longer distance in receptor **577** was thought to better accommodate pyrophosphate while the more compact **578** system could chelate inorganic phosphate more effectively. This trend was further supported by ^{1}H NMR spectroscopic experiments in D_2O (HEPES buffer, pH 7.4). Fluorescence studies with receptor **577** revealed a quenching effect upon addition of inorganic phosphate but an increase in emission intensity upon addition of pyrophosphate (HEPES buffer, pH 7.4). Little change in the emission spectrum was observed upon the addition of anions to receptor **578**. The development of an indicator displacement assay based on pyrocatechol violet allowed for the determination of binding constants with inorganic phosphate, pyrophosphate, ATP, and glucose-6-phosphate guests in the HEPES buffer system at pH 7.4. In line with the potentiometric and ^{1}H NMR spectroscopic studies, the strongest complexes were formed between receptor **577** and pyrophosphate and receptor **578** and inorganic phosphate, respectively (log $K \approx 4.4$ for both complexes). In addition, receptor **577** was found to bind ATP strongly (log K = 3.8) while receptor **578** was found to bind glucose-6-phosphate strongly (log K = 3.5). The selectivity observed for these latter guests was again attributed to the specific inter-Zn distances of the two receptors. No interactions were observed with chloride, bromide, perchlorate, nitrate, or sulfate in these systems.

A unique bimetallic zinc receptor (**579**) was developed by Churchill and co-workers.[628] The chiral, lysine-based ligand allowed for the detection of anions through fluorescence, absorption, and CD spectroscopy in aqueous media (10 mM HEPES, pH 7.4). A bathochromic shift in the absorption peak was observed upon the addition of pyrophosphate (40 nm), ATP (36 nm), and ADP (1 nm). No significant changes were found for fluoride, bromide, iodide, acetate, bicarbonate, inorganic phosphate, AMP, or 3′,5′-cAMP. The use of pyrocatechol violet indicator in a displacement assay allowed for the visual detection of pyrophosphate, ATP, and ADP. Fluorescence quenching was also observed for these three anions (pyrophosphate, ATP, ADP), and binding constants were calculated using this method. Pyrophosphate and ATP exhibited similar binding affinity ($K \approx 4 \times 10^5$ M^{-1}) while ADP bound with significantly lower affinity ($K = 9.14 \times 10^3$ M^{-1}). The stronger binding interactions with pyrophosphate and ATP were attributed to the greater electronic charge density of these anions relative to ADP. Phosphorous NMR of the three respective

complexes displayed large shifts of the bound guests compared to the free anions. Only the signal for the α-P center (the phosphorous atom closest to the sugar unit) of ATP did not shift, which led to the conclusion that the β- and γ-P centers were more involved in complex formation. Importantly, receptor **579** allowed for the selective detection of pyrophosphate in the presence of excess chloride and inorganic phosphate.

In 2007, a zirconium-based anion receptor (**580**) was developed by Wang, Wu, and co-workers.[629] In this receptor design, anion binding to the zirconium center would displace the indicator (*4′-N,N*-dimethylamino-6-methyl-3-hydroxylflavone) and thus cause a change in the emission intensity of the indicator. In accord with such expectations, an increase in emission intensity of approximately 1.5-fold was indeed observed upon the addition of dihydrogenphosphate, acetate, and chloride, albeit a larger increase (6-fold) was observed upon the addition of fluoride. These experiments were conducted in a HEPES buffer at pH 7.6, and anions were added as their TBA salts. No binding constants were reported.

Molybdenum has also been incorporated into synthetic phosphate receptors by Pérez and co-workers.[630] A specific combination of amide functionality and molybdenum metal-carbonyl fragments was used to produce receptors **581**. These systems are of interest because they might allow anion binding to be monitored via IR spectroscopy. In this report, however, dihydrogenphosphate was only analyzed through ^{1}H NMR spectroscopy in DMSO-d_6. Nevertheless, these experiments allowed for the measurement of binding affinities. Binding constants for receptor **581a** and **581b** with dihydrogenphosphate were measured to be 856 M^{-1} and 257 M^{-1}, respectively. Little selectivity was observed among the anions analyzed.

Steed and co-workers have utilized ruthenium(II) as both a scaffold and a recognition site in receptor **582**.[631] In this receptor, an amino-ferrocene cleft was assembled through ruthenium(II)-pyridine coordination. Anion binding studies were performed using ^{1}H NMR spectroscopy with NO_3^-, AcO^-, ReO_4^-, $CF_3SO_3^-$, $H_2PO_4^-$, Cl^-, and HSO_4^- as the TBA salts in $CDCl_3$. Receptor **582** was found to bind $H_2PO_4^-$ with a binding constant of 550 $M^{-1}$for the 1:1 complex. However, selectivity in this system was observed for NO_3^- and HSO_4^-. Addition of Cl^- and AcO^- anions led to displacement of the pyridine ligands from the ruthenium(II) center.

Palladium and platinum metals have also found a place in phosphate anion recognition. For example, Vilar and co-workers reported the anion binding ability of dipalladium thiolate complexes functionalized with urea units.[632,633] Proton NMR spectroscopic titrations in DMSO-d_6 of receptor **583a** with TBA anion salts revealed the following trend in binding strength: dihydrogenphosphate > phenylphosphate > bromide > chloride > hydrogensulfate. While the strongest binding was observed for dihydrogenphosphate (log K = 3.5), little selectivity was recorded between this anion, phenylphosphate, and bromide. Replacing the phenyl substituents with ethyl groups (**583b**) did not improve the selectivity. The anion binding ability of these receptors was attributed to a combination of hydrogen bonding interactions with the urea moieties and electrostatic interactions between the anion and the metal center. It was also proposed that the dipalladium thiolate backbone preorganized the receptor cavity. These receptors were also observed to decompose in solution over time. As result, platinum derivatives (**584**) were investigated in efforts to increase stability.[634] A slight change in the trend in binding affinity was observed (dihydrogenphosphate ≈ phenylphosphate > acetate > hydrogensulfate > halides ≈ nitrate) through ^{1}H NMR spectroscopic studies in DMSO-d_6. The highest selectivity for dihydrogenphosphate was reported for receptor **584a**, and the highest binding affinity for this anion was reported with receptor **584c** (log K = 3.7). No decomposition was observed for receptors **584**. A qualitative indicator displacement assay in CH_3CN:CH_2Cl_2 solution was also developed by first coordinating the indicator methyl red to receptor **584c**. Significant displacement was

observed upon addition of dihydrogenphosphate and acetate to this complex through UV-Vis spectroscopy. This displacement was visible to the naked eye as an orange-to-yellow color change.

Platinum was utilized by Loeb and co-workers to organize urea-functionalized ligands for anion binding as in receptor **585**.[635] Proton NMR spectroscopy in DMSO-d_6 led to the conclusion that the recognition units in **585** existed in the 1,2-alternate, 1,3-alternate, or cone conformations depending on the anion added (all as the corresponding TBA salts). While the halides (Cl^-, Br^-, I^-) were complexed in a 1,2- or 1,3-alternate conformations, sulfate and dihydrogenphosphate were complexed in a cone conformation. The binding constants for the oxoanion complexes were found to be higher than could be determined using this method ($K > 10^5\ M^{-1}$), even in the highly competitive solvent DMSO. Single crystal X-ray diffraction analysis of the **585**:SO_4^- complex led to the suggestion the observed strong binding was due to a combination of metal chelation and hydrogen bonding interactions between the anions and all eight NH groups of the complex. It was proposed that these interactions served to anchor the guest within the host cavity.

Kikuchi and co-workers analyzed the phosphate recognition of receptor **586**, a system designed to combine the sensitivity of coumarin fluorescence with a Cd(II)-azamacrocycle.[636] In neutral aqueous solution, Cd(II) was coordinated by the four nitrogen atoms of the macrocycle and the aromatic amino group of the coumarin subunit. While Cu(II) and Zn(II) complexes were also investigated, only the Cd(II) complex was found to quench appreciably the fluorescence of the indicator. Potentiometric titrations supported the conclusion that Zn(II) cation did not coordinate to the coumarin indicator while the Cd(II) center did. Addition of anions to the Cd(II) complex **586** led to a shift in the excitation spectrum. Presumably, this shift is due to displacement of the coordinated coumarin group by the anion. The spectroscopic shifts were used to determine binding affinities. Both pyrophosphate and citrate displayed dissociation constants in the 10^{-5} M range in HEPES buffer at pH 7.4. Monoanions (iodide, bromide, chloride, and inorganic phosphate), however, were characterized by much weaker interactions. Nucleotides also bound with strong to moderate affinities, although the various cyclic nucleotides that were tested were found to bind weakly if at all. The latter selectivity allowed receptor **586** to be used as the reporter group in a PDE assay, in a similar manner to the **544b**-based assay previously described.

The Anslyn group designed and synthesized a cadmium(II) boronic acid-trispyridyl receptor (**587**) with the goal of binding carboxy- and phosphosugars.[637] UV-Vis displacement experiments in methanol:water (3:1) allowed for the measurement of binding affinities. Association constants of log $K = 3.95$ and 4.76 were reported for AMP and for ribose 5-phosphate, respectively. The strongest affinity, however, was observed for carboxylated sugars. The binding interactions were proposed to derive from a combination of boronic acid-sugar bonding and metal-anion complexation.

Tin, particularly in the guise of organotin compounds, has a long history of use in the area of phosphate-selective polymer electrodes. In one of the first successful studies, Arnold and Glazier incorporated bis(*p*-chlorobenzyl)tin dichloride into a PVC membrane.[108,638] This electrode proved selective for dibasic phosphate at pH 7.0 over acetate, sulfate, chloride, bromide, nitrate, and iodide. The detection limit was found to be 3.3×10^{-5} M with a linear response range from 2.2×10^{-4} M to 1.2×10^{-2} M. The electrode lifetime was reported to be at least 28 days. Further studies revealed that electrodes containing bis(*p*-methylbenzyl)tin and dibenzyltin derivatives lost much of the previously observed selectivity for dibasic phosphate seen in the case of the bis(*p*-chlorobenzyl)tin systems. Importantly, however, these electrodes remained selective for dibasic phosphate over chloride, which is generally

considered as the most competitive contaminant in real world systems due in part to its high concentration.[639] The observed selectivity trend led to the suggestion that electron-withdrawing substituents on the benzyl ring increased the selectivity for dibasic phosphate over other anions, presumably through an increase in tin-carbon hyperconjugation. The interaction of bis(*p*-chlorobenzyl)tin dichloride with several other anions was also studied. For example, bis(*p*-chlorobenzyl)tin dichloride electrodes were found to be as selective for thiocyanate as they were for dibasic phosphate. This chlorobenzyl derivative was also observed to interact with adenosine nucleotide derivatives in the order ATP > ADP > AMP > 3′,5′-cAMP. However, a slight selectivity for dibasic phosphate over these nucleotides was maintained. On the other hand, this electrode was found to be more selective for benzoate, salicylate, and pyrophosphate than dibasic phosphate while less selective for arsenate and fluoride.

Arnold and co-workers analyzed the response of a bis(*p*-fluorobenzyl)tin dichloride membrane with the hope of attaining an even greater phosphate selectivity.[640] As expected, the resulting electrode proved selective for dibasic phosphate over thiocyanate, iodide, bromide, nitrate, acetate, and chloride. A strong correlation between the detection limit and the Hammet constant of this and the previously studied benzene substituents (*p*-methylbenzyl, benzyl, and *p*-chlorobenzyl) was observed. Both the fluoro derivative and the chloro derivative, however, were found to have an even stronger response for tribasic citrate than dibasic phosphate.

Simon and co-workers further probed the interaction of anions with trialkyl and dialkyl tin complexes via ^{119}Sn NMR spectroscopy in $CDCl_3$.[641] For example, tributyltin chloride underwent a greater chemical shift upon addition of dihydrogenphosphate than upon addition of hydrogensulfate (TBA salts). These studies supported the proposal that tetracoordinate tin complexes are formed through ion exchange. Dioctyltin dichloride, however, was observed to form 1:2 complexes with dihydrogenphosphate (Kryptofix® 222/potassium salt) at low concentrations of anion. The host became oligomeric and/or polymeric upon the addition of more than 0.5 equiv. of phosphate. The chemical shifts of this tin derivative were consistent with the formation of a pentacoordinate tin species.

The multivalent tin receptor **588** was investigated by Chaniotakis and co-workers.[642] When incorporated into a PVC membrane, this receptor displayed a selective response for inorganic phosphate over a variety of other anions, including salicylate, perchlorate, and thiocyanate. The observed selectivity correlated more closely to the metal coordination ability of the different anions than with the Hofmeister series. The lifetime of these electrodes, however, was less than 24 hours. A series of ditopic tin receptors (**589a-i**) were also studied in an effort to optimize the substitution pattern and fine tune the distance between the metal centers.[643] In general, the best phosphate selectivity was observed for receptors with tin centers containing one electron withdrawing organic substituent and two halide substituents, presumably due to the increased Lewis acidity at the tin atom. In addition, linkers containing an odd number of carbon atoms appeared to complex phosphate more successfully. The binding behavior of receptors **589a, 589g, 589j, 589k**, and **589l** were analyzed by both ^{119}Sn NMR spectroscopy and potentiometric methods.[111] On the basis of the potentiometric studies, it was concluded that organic substituents that were electron-withdrawing in nature (Ph, **589a, k**, l) displayed much better responses to inorganic phosphate than those lacking such substituents. On the other hand, a receptor that was more lipophilic (octyl, **589j**) also displayed an improved response. The latter effect was attributed to improved partitioning of the carrier into the membrane. Both the inorganic phosphate response and the selectivity were found to decrease with increasing carbon spacer length (**589a** > **589k** > **589l**). Tin-119 NMR spectroscopic studies in CH_2Cl_2 confirmed that the phosphate selectivity of the receptor **589a** based electrode, the best of the systems analyzed,

was likely due to an increase in the stability constant of the receptor:phosphate complex. However, the lifetime of these electrodes was found to be on the order of 3 to 20 days, too short for repeated, long-term use.

Yu, Liu, and co-workers investigated divalent tin systems, such as bis(tribenzyltin) oxide, as ion selective electrode sensor elements.[644,645] The corresponding electrode proved selective for dibasic phosphate over nitrate, chloride, acetate, and sulfate. Similar responses were observed, however, for iodide and bromide. An electrode lifetime of approximately one month was reported.

More recently, Suzuki and co-workers examined the effects of anionic additives on trialkyl/aryltin chloride electrodes.[646] In preliminary studies, tributyltin chloride was incorporated into a PVC membrane containing sodium tetrakis[3,5-bis(trifluoromethyl)-phenyl]borate (NaTFPB) as an additive. The presence of the additive led to a high phosphate response at pH 7.0. The percentage of additive as well as the tin substitution (butyl, octyl, or phenyl) was then optimized. The most successful combination in terms of both selectivity and response proved to be tributyltin chloride mixed with NaTFPB at 25 mol % relative to tin ionophore. This result was tentatively attributed to facilitated chloride release arising from coordination of the additive to the tin center, and an increase in the Lewis acidity of the metal.

In addition to transition metals and main group cations, lanthanides (Ln) have also proven successful in the detection and complexation of phosphate species. Lanthanide-based receptors typically rely on the change in emissive properties of the cation that arises when anions displace water molecules from the metal center. For example, addition of anions to the chiral Eu and Tb complexes **590** (Ln = Eu(III), Tb(III)) results in changes in the circularly polarized luminescence (CPL) of the receptor in aqueous media buffered to pH 7.4.[647,648] Apparent binding affinities for monohydrogenphosphate were estimated at log K = 4.15 or greater for all four complexes (**590a**,**b:Eu** and **590a,b:Tb**). The phosphate affinities were significantly higher than those measured for bicarbonate, acetate, and lactate. Control experiments provided support for the suggestion that the dianionic ligand is bound to the metal center in a monodentate fashion. In general, the methylated ligand complexes (**590b**) proved to interact with anions more strongly than complexes of receptor **590a**. Presumably, this reflects differences in the coordination environment of the two ligands. In addition, the terbium complexes of general structure **590** were found to respond to anions more effectively than the corresponding europium complexes. This trend was postulated to be due to differences in the pK_a values of the metal centers. The closely related complex **591** was later bound to gold nanoparticles for improved luminescent sensing.[649] Addition of diketone antenna compound **592** to these particles led to a strongly luminescent complex in HEPES buffer at pH 7.4. Displacement of the antenna by a variety of anions led to emission quenching. However, only addition of FMN resulted in almost complete quenching of the luminescence, allowing selective detection of this anion over AMP, ADP, ATP, 3′,5′-cAMP, NADP, inorganic phosphate, and a number of carboxylates.

The Anslyn group designed and synthesized the multifunctional receptor **593** that contains an *N*-oxide bipyridine europium complex and an ammonium “pinwheel” functionality.[650] Host **593** was found to form a 1:1 complex with 2,3-BPG in 50% methanol/acetonitrile. The complex was thought to be stabilized through a europium-phosphate interaction acting in combination with ammonium-phosphate and ammonium-carboxylate interactions. The europium:2,3-BPG interaction was the one considered responsible for the fluorescence quenching. From the change in emission, a binding constant of 6.7×10^5 $M^{-1}$was calculated for this solvent system. Binding studies in pure methanol supported the formation of 2:1 **593**:2,3-BPG complexes. This solvent-dependent stoichiometry was attributed to the

complexation of each of the two phosphate groups by the europium center of a different receptor as a result of a reduction in the ammonium-anion binding interaction. The proposed model was supported by several subsequent experiments. For example, the removal of the ammonium centers in receptor **593** also led to a 2:1 binding stoichiometry, even in the original methanol/acetonitrile solvent system. Compounds containing one phosphate group and one carboxylate group also demonstrated 2:1 binding behavior. Furthermore, phenylphosphate was found to have a slightly reduced binding affinity of 2.0×10^5 M^{-1} relative to 2,3-BPG, a finding that was attributed to coordination of the monoanion at only the europium center. These results provided support for the conclusion that the three anionic groups of 2,3-BPG were indeed bound to the three cationic centers of host **593** in the methanol/acetonitrile solvent mixture. However, the addition of water to the solvent system led to a quenching of the fluorescence, preventing the use of receptor **593** in aqueous media.

Ziessel and co-workers found that europium and terbium complexes of receptors **594** and **595** displayed emission changes upon the addition of inorganic phosphate and ATP in aqueous media. In preliminary studies, both the absorption and emission of complexes **594** were observed to undergo changes upon the addition of inorganic phosphate or ATP in aqueous media at pH 7.0 (Tris/$HClO_4$ buffer).[651] No spectral changes were observed upon the addition of AMP or ADP. Consistent with what was seen with system **593** above, spectral analysis supported the displacement of water from the inner coordination sphere of the lanthanide. In addition, a peak corresponding to the ternary **594:Eu**:ATP complex was observed in the ESI-mass spectrum. The nature of the anion interactions in **594:Eu** were further probed using optical spectroscopy; these interactions were also analyzed using computational studies based on density functional theory.[652] In the computational studies, mono-, di-, and triphosphate anions were generally found to be bound to the europium center in a multi-dentate fashion. Addition of water to the theoretical monohydrogenphosphate complex led to monovalent complexation. Upon binding, these anions displaced one or more of the ligand nitrogen atoms depending on the binding affinity of the guest. Based on these computational studies, it was proposed that increasing the lanthanide complexation ability of the ligand would enhance the selectivity of the system. To test these predictions, the alkyl nitrogen moiety was replaced by a phenylphosphine group and the methylene spacers were removed yielding receptor **595**. With this new system, a 20-fold increase in binding affinity of the ligand to the europium metal was observed relative to **594**. The binding behavior of anions toward **595:Eu** was analyzed through UV-Vis, emission and luminescence lifetime measurements in a Tris/HCl buffer at pH 7.0. No spectral changes were observed with the addition of nitrate or AMP. However, ATP, ADP, and monohydrogenphosphate were found to bind. Similar association constants ($\log K \approx 5.5$) were recorded for all three anions, although the spectral changes were more pronounced with ATP and monohydrogenphosphate than with ADP. The increase in luminescence lifetimes for these complexes was attributed to a decrease in the nonradiative decay pathways available with coordinated water molecules in the absence of the anion. While stronger binding affinity was observed with **595:Eu** relative to **594:Eu**, little change in selectivity was observed.

While many lanthanide-based anion receptors have focused on differences in emission intensity, the Eu(III) complex **596** displayed differential UV-Vis spectral shifts when exposed to phosphate anions in aqueous media at pH 7.4.[653] In preliminary studies, a pink to blue color shift was observed upon the addition of monohydrogenphosphate or pyrophosphate but not upon the addition of a variety of other inorganic anions. The detection limit for monohydrogenphosphate, even in the presence of other anions, was determined to be 6.0×10^{-7} M at this pH. A 2:1 anion:receptor binding stoichiometry was determined for monohydrogenphosphate and receptor **596**, as was the second association constant (3.2×10^5 M^{-1}). The association constant for the 1:1 complex with pyrophosphate

was determined to be 5.9×10^3 M^{-1}. When nucleotides were analyzed, moderate responses were observed for AMP and ADP, while a strong new absorption band was observed upon addition of ATP ($K = 2.2 \times 10^5$ M^{-1}). These results highlight the sensing power of lanthanide cations. In appropriately designed complexes, these metals will give rise to distinct spectral responses to different substrates that are thought to reflect slight differences in the overall coordination environment.

A number of diketone antibiotics have also been used as ligands in europium(III) sensing complexes. For example, C. Jiang and co-workers reported that the addition of ATP to a europium(III):doxycycline complex (**597a**) led to a strong increase in the luminescence intensity.[654] This change was particularly apparent in aqueous ammonium chloride at pH 10. The emission intensity was found to correlate linearly with the ATP concentration in the range of 1.00×10^{-7} - 2.00×10^{-6} M^{-1}. ATP detection was found to be independent of several possible intereferents found in bodily fluids and could be applied to commercial ATP samples. An analogous complex with oxytetracycline (**597b**) displayed similar spectroscopic changes but an increased linear concentration range (8.00×10^{-8} - 1.50×10^{-6} M).[655] These researchers also reported the detection of NADP using a tetracycline ligand (**597c)** at concentrations as low as 6.9×10^{-8} M at pH 7.6. [656] Duerkop and co-workers studied the effect of inorganic phosphate on the luminescence of the europium(III) tetracycline complex (**597c**).[657] Quenching was observed to be linear with phosphate concentration over the range of 5×10^{-6} - 7.50×10^{-4} M at neutral pH. This signal was also subject to little interference by common cations and anions. Schäferling and Wolfbeis studied the effect of several other phosphate derivatives on the luminescence of complex **597c**.[658] In this case, quenching was found to be strongest with ATP and GTP, followed by pyrophosphate > AMP > ADP ≈ 5′-guanosine diphosphate (GDP) ≈ 3′,5′-cAMP. This selectivity allowed for the use of complex **597c** to monitor kinase assays in real time.

C. Jiang and co-workers also developed a terbium(III)-based luminescence sensor for ATP by using a norfloxacin ligand (**598**).[659] Coordination of the norfloxacin ligand led to an emissive complex in aqueous solution, presumably due to energy transfer processes from the ligand to the terbium(III) center. Addition of ATP to the Tb^{3+}:L complex in Tris-HCl buffered aqueous solution at pH 7.4 further increased the emission intensity. It was proposed that multi-dentate coordination of ATP to the terbium(III) center displaced coordinated water molecules, which are known to quench lanthanide luminescence. The change in signal intensity was found to vary linearly with the concentration of ATP over the 1.0×10^{-6} M - 1.60×10^{-5} M range, and a detection limit of 4×10^{-8} M was reported. Less than 10% interference was measured for a range of metal ions, proteins, and metabolites expected to be found in biological fluids. This system was further used to quantify the levels of ATP in commercial drug samples.

A different lanthanide-based system was reported by Yang and co-workers, who combined ytterbium and pyrocatechol violet in a 2:1 ratio to form receptor **599**.[660,661] This complex proved successful in the detection of phosphate-derivatives in aqueous media (HEPES buffer, pH 7.0). In preliminary studies, the addition of ATP or monohydrogenphosphate led to the decomplexation of pyrocatechol violet and concurrent formation of 1:1 Yb:anion complexes. The resultant optical changes were observed both visually and spectrophotometrically. While several other lanthanide and transition metals were analyzed, only ytterbium proved successful in these assays. Little to no interference was observed in the presence of other anions, even when the latter were added at a 1000-fold excess relative to the two test phosphate anions. Other nucleotides resulted in a much lower response. Spectrophotometric titrations with ATP and monohydrogenphosphate resulted in conditional binding constants of 5.85×10^{15} M^{-1} and 2.0×10^{15} M^{-1} for the interaction of these two

anions to this ytterbium complex. The sensitivity of this system was found to lie in the 10^{-5} M range in HEPES buffer.

The uranyl cation has also been extensively exploited to produce phosphate receptors, particularly by Reinhoudt and co-workers. In early studies involving complexes of this high valent actinide cation, receptors **600a-c** and **601a-b** were made and tested; these receptors were found to be selective for dihydrogenphosphate in acetonitrile / 1% DMSO as inferred from conductometric studies.[662] The highest association constants were observed with receptors **600b** and **600c** ($K = 2.5 \times 10^6$ M^{-1} and 5.0×10^6 M^{-1}, respectively). The efficiency of these receptors was attributed to the presence of anion-binding amide groups and increased preorganization. These complexes were also observed by ^{31}P NMR spectroscopy and FAB mass spectrometry. In addition, high selectivities over chloride, hydrogensulfate, nitrite, and thiocyanate were reported. Further studies focused on receptors **601a-d**.[663] Single crystal X-ray diffraction analysis of receptor **601b** and $H_2PO_4^-$ was performed. The resulting structure revealed the expected two apical oxygen bonds on the uranyl cation, as well as coordination to the four positions of the salophene ligand. An additional coordination site at the uranium cation was seen to be filled by an oxygen atom of the phosphate. Hydrogen bonds were also observed between the phosphate oxygen atoms and the methoxy substituents of the ligand. Single crystal X-ray diffraction analysis of receptor **601d** and $H_2PO_4^-$ revealed similar uranyl coordination and clear hydrogen bonding interactions between the dihydrogenphosphate and the amide units. Several trends in the anion binding properties of these compounds were further analyzed using conductometry. The incorporation of the nitro groups in receptor **601c**, for example, increased the binding affinity for chloride and nitrite but not dihydrogenphosphate. While receptors **602** and **603** contained additional hydrogen bonding units as compared to receptor **601a-c**, weaker complexation constants were observed with dihydrogenphosphate. This effect was attributed to the proposed smaller cavity size of these latter receptors. Receptors **600a, 600b, 600f**, and **604**, however, displayed an increase in complex stability due to the presence of additional amide units. Complexation studies in DMSO/water mixtures revealed weak binding interactions.

Lipophilic receptors **605** were incorporated into PVC membranes cast on chemically modified field effect transistors (CHEM-FETs).[664] These FETs were found to sense dihydrogenphosphate with selectivity observed relative to nitrate, bromide, chloride, and sulfate. The lowest detection limit (1.6×10^{-4} M) was observed with receptor **605b**, which may form hydrogen bonds between the methoxy groups and the phosphate. A reduced selectivity for dihydrogenphosphate over nitrate was observed with receptors **605b** and **605c** as compared to receptor **605a**. Presumably, this reflects the electron-donating nature of the substituents on the former systems. An investigation of these compounds in ion-selective electrodes at varying pH led to the suggestion that the hydrogen bonding available in receptor **605b** led to an increased selectivity over the hydroxide ion as compared to receptors **605a** and **605c**.[665] More detailed studies compared the response of receptors **605a** and **605b** to receptors **601a** and **601b** in phosphate selective electrodes.[666] The latter set displayed reduced phosphate selectivity, presumably due to reduced solubility in the PVC membrane. The best selectivity and response in these electrodes was observed with receptor **605a**. An analysis of ion selective electrode durability was also carried out. It was revealed that receptor **601e** had a selectivity that was comparable to that of receptor **605a**, but with a lifetime that was improved by up to two months.[667] Similar trends but lower lifetimes (2 weeks) were observed with the CHEMFETs.[668]

Recently, Mandolini and Rissanen analyzed receptor **601f**, which possesses bulky tert-butyl substituents, and macrocyclic receptor **606**.[669] As inferred from UV-Vis titrations in DMSO, receptor **601f** is characterized by significant fluoride selectivity. In addition, the interaction

of receptor **601f** with hydrogenphosphate (log $K = 2.32$) was found to be significantly weaker than that found with receptor **601a** (log $K = 4.00$). The reduced binding of receptor **601f** was attributed to steric hindrance due to the *tert*-butyl substituents, which inhibits complexation. The small cavity of the receptor **606** led a to selectively toward fluoride over other anions. All anions were studied as the TBA salts.

Reinhoudt and co-workers also analyzed several uranyl receptors for their ability to function as ion pair receptors and transporters. Ditopic receptor **607d** was designed to extract an ion pair, in this case KH_2PO_4, from an aqueous layer into apolar media.[670] Proton NMR spectroscopic experiments carried out in DMSO allowed for the determination of a complexation constant of 1.1×10^3 M^{-1} for the interaction of receptor **607d** with $H_2PO_4^-$. These experiments also provided support for the presence of hydrogen bonding interactions between the anion and the amide moiety of the receptor. A similar association constant for the anion was determined through cyclic voltammetry. This latter method also permitted the determination of an association constant for potassium of 1.0×10^2 M^{-1}. Complexation of the cation:anion pair by receptor **607d** was observed through FAB mass spectrometry, thus providing direct evidence of effective ion pair extraction. The calixarene-salen compounds **608** were also found to function as ditopic receptors.[671] These receptors were found to bind dihydrogenphosphate, with association constants of 3.5×10^2 M^{-1} and 3.9×10^2 M^{-1} being recorded for receptors **608a** and **608b**, respectively. Peaks corresponding to the ion pair NaH_2PO_4:**608b** complex were also seen in the FAB mass spectrum.

The ability of these receptors to transport ion pairs across membranes was also analyzed. Receptors **607** were tested for their ability to transport KH_2PO_4 but were found to only be effective when used in combination with calixarene **609**.[672] Receptors **607e-g, 610**, and **611** were studied for their ability to transport $NPr_4H_2PO_4$ over NPr_4Cl through a supported liquid membrane.[673] Receptors **610** and **611** provided for the selective co-transport of chloride over phosphate. The receptors **607e** and **607f**, however, allowed for the selective co-transport of dihydrogenphosphate, as predicted. A 2:1 carrier:anion ratio was observed for the latter dihydrogenphosphate complexes, presumably due to the presence of the sulfamido moieties.

Receptors **612** and **613** incorporate thymine bases, a moiety included in order to promote specific ditopic binding of AMP.[674] Proton NMR spectroscopic experiments in DMSO supported a ditopic binding mode. Binding constants for the AMP complexes of receptors **612** and **613** were determined to be 75 M^{-1} and 1.2×10^3 M^{-1}, respectively, in this solvent. The relatively low binding interaction observed with receptor **612** was attributed to steric crowding as well as to the presence of only one open coordination site. Both host:guest complexes were also observed through FAB mass spectrometry.

4.3.2 Metal Cations as Non-coordinating Reporter Groups—As seen in many of the previously discussed examples, metal cations have played an important role in anion complexation. In this section, metal ions that act largely as non-coordinating reporter sites will be reviewed, particularly metallocene as an electrochemical reporter and $[Ru(bpy)_3]^{2+}$ as a fluorescent reporter.

Metallocene as an electrochemical reporter: Reversible electrochemical behavior is generally seen for metallocene (Mc)/metallocenium (Mc^+) redox processes. These couples can be affected by the presence of anions. Generally, anion binding interactions result in cathodic shifts of both the oxidation and reduction peaks of the Mc/Mc^+. These shifts are attributed to the interaction of the neutral receptor with the anion as well as the stabilization of the metallocenium species by coordination of the anion.[675] The magnitude of the shift is correlated with several factors, including the strength of the receptor-anion interaction as

well as the distance between the bound anion and the metallocene unit. Insolubility of the metallocenium:anion ion pairs in organic media, however, can lead to adsorption of the receptors onto the electrode. This adsorption leads to irreversible and/or poorly characterized electrochemical spectra. In addition, irreversible ligand oxidation can also interfere with this sensing method.

In 1993 Beer and co-workers began an investigation of the binding behavior of ferrocene and cobaltocene-appended phosphate receptors. Many of the systems developed by this group relied on the phosphate binding functionalities discussed in previous sections. The key difference was the use of a metallocene subunit as an electrochemical reporter group. In early studies, the amide-based receptor **614**, bearing two kinds of metallocenes, was found to form 1:1 complexes with dihydrogenphosphate by ^{1}H NMR spectroscopy in acetonitrile.[676] In cyclic voltammetric (CV) experiments, both the ferrocenyl and cobaltocenium centers were observed to undergo cathodic shifts upon addition of dihydrogenphosphate, chloride, and hydrogensulfate (TBA salts) in acetonitrile. Similar results were obtained in studies with receptors **615a, 616, 617**, and **618**. Among this set of receptors, the tripodal receptor **617** displayed the largest cathodic perturbation in the presence of dihydrogenphosphate. Interestingly, no perturbation of the dihydrogenphosphate signal was observed in the presence of a ten-fold excess of hydrogensulfate or chloride anions for receptors **616, 617**, and **618**. Further investigation of the phosphate binding behavior of receptors **615a** and **616** revealed a discrepancy in the selectivity observed through CV and ^{1}H NMR spectroscopic methods. Based on ^{1}H NMR spectroscopic analyses, a significantly stronger interaction with hydrogensulfate than dihydrogenphosphate in acetonitrile was inferred.[677] In contrast, a stronger electrochemical response for dihydrogenphosphate over hydrogensulfate was inferred through CV studies. This disparity was attributed to an increased participation of the ferrocene moiety in the electrochemical experiments as compared to the NMR spectroscopic studies. The researchers concluded that complex stabilities were not a reliable indicator of electrochemical response.

The simple cobaltocene amide receptors **619** also displayed a selective electrochemical response towards dihydrogenphosphate (TBA salt) in acetonitrile.[678] The association constant corresponding to the interaction between dihydrogenphosphate and receptor **619b** was measured to be 3.2×10^2 M^{-1} in DMSO-d_6 via ^{1}H NMR spectroscopy.

The binding behavior of various analogues that contain several other recognition moieties was analyzed in order to probe further the inherent anion selectivity of this class of receptors.[679] The amine- and amide-based receptors **615a, 615b, 620**, and **621** displayed low dihydrogenphosphate binding affinities and selectivities as inferred from both ^{1}H NMR spectroscopic and CV measurements carried out in chloroform and acetonitrile using several test TBA anion salts. In separate electrochemical studies, the amidopyridine receptors **616** and **622** displayed the strongest response to dihydrogenphosphate as compared to other anions; however, these receptors did not produce reversible redox waves.

Interestingly, ^{1}H NMR spectroscopic analyses provided support for significantly different binding modes for hydrogensulfate and dihydrogenphosphate in their interactions with receptor **622**. Hydrogensulfate was considered to interact more significantly with the amine side than the ferrocene unit of the receptor, presumably due to its ability to act as a proton donor to the terminal amine. The opposite binding mode was observed with the more basic dihydrogenphosphate anion. In addition, only the addition of dihydrogenphosphate resulted in a bathochromic shift of the d-d transition absorption band. Hydrogensulfate, however, was able to inhibit the UV-Vis spectral response to dihydrogenphosphate binding. The mono-functionalized receptor **616** did not display any anion-based UV-Visible spectroscopic response.

The anion binding behavior of urea receptors **623** and **624** was also analyzed via ^{1}H NMR spectroscopic studies carried out in acetonitrile-d_3 with TBA anion salts.[680] Selectivity for chloride and dihydrogenphosphate was observed, presumably based on steric effects. For example, receptors with bulky *tert*-butyl ester substituents (**623b** and **624b**) displayed chloride selectivity while their hexyl-substituted counterparts displayed the more common dihydrogenphosphate selectivity. Furthermore, stronger binding constants were observed with difunctionalized receptors **624** over monofunctionalized receptor **623**. Reversible CV responses were reported for both classes of receptors in the presence of dihydrogenphosphate, acetate, and chloride.

Efforts were also made by Beer and co-workers to achieve phosphate complexation in competitive solvents such as DMSO, methanol, and water. For example, the polyammonium receptors **625** and **626** displayed cathodic responses to monohydrogenphosphate and ATP in aqueous media near pH 7.[681,682] The thiourea receptor **627a** was able to bind dihydrogenphosphate (TBA salt) in DMSO ($K = 2.6 \times 10^2$ M^{-1}). At the same time, guanidinium receptor **627b** was observed to complex pyrophosphate (TBA salt) in a 2:1 receptor:anion ratio in 1:1 MeOH:H_2O ($K = 4.6 \times 10^3$ M^{-2}).[683] While binding constants were determined by ^{1}H NMR spectroscopy, nearly concordant results were obtained using CV measurements.

Martínez-Máñez and co-workers also prepared a series of polyammonium-functionalized ferrocenes **628-639** and investigated the ability of these receptors to recognize phosphate anions in aqueous media. The largest responses were observed at lower pH when the protonation state of the amines was maximized. Low selectivities were generally observed. For instance, receptors **628** displayed a redox response to ATP, inorganic phosphate, sulfate, and nitrate in aqueous media.[684] Among the series of receptors **628**, the strongest response was observed with receptor **628b**. This receptor also proved somewhat selective for ATP over inorganic phosphate and sulfate at a pH of 4.9 in 7:3 THF:water. Macrocyclic receptor **629** displayed a low response under these conditions, which was attributed to the steric bulk of the ferrocene units when compared to the unfunctionalized azamacrocycle. More detailed studies of receptor **628b** were performed through both potentiometric and CV methods.[685] Potentiometric studies in 7:3 THF:water confirmed that receptor **628b** forms complexes with ATP and inorganic phosphate over a wide pH range. Using this method, the concentration of ATP could be determined in the presence of inorganic phosphate or sulfate. On the other hand, a preference for hydrogensulfate over dihydrogenphosphate was observed through CV studies with receptor **628b** in dry acetonitrile with TBA anion salts.

A comparison of the binding behavior of receptors **625a, 628a, 630, 631a**, and **631b** revealed differing selectivities in aqueous media.[686,687] Potentiometric studies supported the conclusion that for most species the sulfate complex dominated at lower pH ranges while the inorganic phosphate complex dominated at neutral and basic pH. This trend was also reflected in CV studies. Indeed, receptor **631b** could be used to detect inorganic phosphate quantitatively at pH 7, even in the presence of competing sulfate or nitrate ions. Little inorganic phosphate interaction was observed with receptor **628a**, supporting the suggestion that the cyclic receptors may bind the anion more closely to the ferrocene moieties. Receptors **631a** and **631b** displayed a strong response to ATP and ADP, as well as a weaker response to AMP.

The effect of polyoxo ligands as well as the number of appended ferrocene units was studied by comparing several receptors in water (**633** - **635**) and in a dioxane-water mixture (7:3, **629** and **632**).[688] Under these conditions, receptor **629** displayed a stronger electrochemical response for inorganic phosphate (pH 6-7) than receptor **632**, presumably due to the increased number of nitrogen atoms in receptor **629**. While receptors **633** and **634** each

contain four nitrogen atoms, a higher binding affinity for ATP was observed for **634** in potentiometric studies. Molecular modeling led to the suggestion that receptor **634** could adopt a conformation allowing all four nitrogen atoms to interact with the phosphate. Complexes with receptor **633** were presumed to be stabilized by interaction with only two nitrogen atoms. Similar trends were observed in the case of ATP complexation; however, these receptors displayed modest electrochemical responses to inorganic phosphate and ATP. The binding behavior of receptors **629** and **632** was then analyzed in acetonitrile using TBA anion salts. In this solvent, a selective response to dihydrogenphosphate over hydrogensulfate was observed.

Further studies compared the binding behavior of receptors **628b, 629, 632**, and **636-638** in acetonitrile using TBA anion salts.[689] In these studies, no electrochemical shifts were observed upon addition of chloride or bromide anions. Only receptor **628b** displayed a change in the ferrocene oxidation potential upon addition of hydrogensulfate. Dihydrogenphosphate addition resulted in more pronounced shifts in both the oxidation and reduction waves. Receptor **629** displayed the strongest interaction with dihydrogenphosphate. In general, greater responses were observed with those receptors containing more amine groups, presumably due to increased proton transfer from the anion. On the other hand, the number of ferrocene centers did not appear to impact the CV spectrum directly. Selectivity studies revealed that the dihydrogenphosphate responses of receptors **629, 636**, and **637** were not affected by the presence of a ten-fold excess of chloride, bromide, or hydrogensulfate anions.

Receptor **639** was designed to provide both an electrochemical and fluorescent response to anions. However, it was found to be insensitive to the presence of anions in acetonitrile except as its copper(II) complex (**639:Cu**).[690] This latter complex displayed an electrochemical response to both fluoride and dihydrogenphosphate, as well as a fluorescence response to dihydrogenphosphate, nitrate and fluoride (all anions added as TBA salts). Log *K* values determined from fluorescent titrations were reported as 4.2-4.5 for these three anions, leading to the conclusion that this receptor is not particularly selective. It is important to note that many of the receptors in these studies displayed electrochemical signals in response to transition metal cations as well as to anions.

Pyrrolic ferrocene receptors were first reported by Sessler and co-workers in 1998.[691,692] Preliminary studies compared the anion binding properties of **640a** to that of its acyclic counterpart **641**. This was done using both ^{1}H NMR spectroscopy and electrochemical analyses (both carried out in acetonitrile using various TBA anion salts). The two pyrrolic systems were found to bind chloride and dihydrogenphosphate in preference to bromide and hydrogensulfate. In addition, a slight preference for dihydrogenphosphate over chloride was observed with receptor **640a**. Fluoride was bound too strongly for an association constant to be determined by NMR spectroscopic methods, but Job plots supported a 2:1 guest:host stoichiometry. All other anions were found to form 1:1 complexes. The stronger binding of **640a** was attributed to the macrocyclic effect of preorganization. Both receptors produced electrochemical signals, with the strongest response reported for dihydrogenphosphate followed by fluoride and chloride.

Further studies examined derivatives **640b-d**, although solubility problems required the use of dichloromethane / 2% DMSO as the solvent. This precluded a direct comparison with **640a**. The highest binding affinity was observed for receptor **640d**, a finding that provides support for the conclusion that the additional oxygen atoms present in this system participate in hydrogen bonding with the anion, thereby increasing the stability of the complex. A similar trend was inferred from the electrochemical studies, although little difference was observed in the responses of **640c** and **640d**. This latter result led to the suggestion that

additional factors, such as the proximity of the bound anion to the ferrocene unit, greatly affected the electrochemical response.

Gale and co-workers found that that cleft-like pyrrole-ferrocene compounds **642-645** also function as phosphate sensors under conditions of electrochemical analysis.[693,694] The association constants for these receptors with a variety of anions (fluoride, chloride, bromide, dihydrogenphosphate, hydrogensulfate, and benzoate as their TBA salts) were first determined via ^{1}H NMR spectroscopy titrations carried out in dichloromethane. Based on these analyses, it was concluded that the conjugated receptors **642** and **643** bind the targeted anions more strongly than receptors **644** and **645**. Selectivity for fluoride among other anions was observed with all four receptors. The highest association constant for dihydrogenphosphate was measured with receptor **643** ($K = 2.96 \times 10^2$ M^{-1}). Distortion of the redox wave was observed for receptors **642** and **644** in the presence of dihydrogenphosphate. CV studies with receptors **643** and **645**, however, led to the observation of strong, measurable electrochemical responses for dihydrogenphosphate.

Gale and co-workers also investigated a small library of amide and urea ferrocene receptors (**646-651**).[695] In this case, ^{1}H NMR spectroscopic titrations carried out in DMSO-d_6 / H_2O (0.5%) with TBA anion salts proved consistent with the presence of dihydrogenphosphate-receptor interactions. However, as a rule, receptors **646-651** displayed a preference for carboxylate anions over dihydrogenphosphate. Electrochemical studies supported the conclusions drawn from the NMR spectroscopic studies. Receptors **648-651** were found to produce the highest electrochemical response upon anion addition.

Moutet and co-workers also investigated the interactions of dihydrogenphosphate and ATP with a variety of amide and cationic ferrocene receptors. In preliminary studies, the amide-bipyridine receptor **652a** was observed to bind dihydrogenphosphate in dichloromethane as inferred from both ^{1}H NMR spectroscopic and CV experiments using TBA anion salts.[696] The NMR spectroscopic studies provided support for strong anion-amide interactions. This conclusion was further confirmed by the finding that weak or essentially non-existent binding was seen in the case of receptors **652b-d**. No interaction with the bipyridine unit was observed by ^{1}H NMR spectroscopy. While evidence of fluoride, chloride and hydrogensulfate complexation was also obtained from CV experiments, the strongest and most well-characterized response was observed for dihydrogenphosphate. The bipyridine-free amide receptors **653-657** displayed electrochemical responses to dihydrogenphosphate and ATP.[697] The binding behavior of receptors **653-656** with dihydrogenphosphate was analyzed in dichloromethane (**653, 654** and **656**) or acetonitrile (**655**) through ^{1}H NMR spectroscopic titrations. A strong perturbation of the chemical shift of the amide protons provided support for the conclusion that hydrogen bonding at these sites was the primary binding force leading to complex formation. Interestingly, both types of amide protons in receptor **656** were found to shift upon treatment with dihydrogenphosphate. Monitoring these shifts allowed a binding constant of 89 M^{-1} to be determined for dihydrogenphosphate. Similar binding constants were observed with fluoride anion. However, in this case the iron center was considered to be more involved in binding than the amide groups. In dichloromethane, receptor **654** was found to bind dihydrogenphosphate and fluoride more strongly than receptor **653**. This finding serves to highlight the importance of the amide substituents. Receptor **654** was also found to have a greater affinity for the dihydrogenphosphate and fluoride anions than receptor **655**, an observation that was attributed to macrocycle **655** being too small and rigid to accommodate well the phosphate anion. It should be noted, however, that receptor **655** was studied in acetonitrile while receptor **654** was studied in dichloromethane. Receptor **655** also displayed a low electrochemical response. Interestingly, both **656** and **657** produced well-characterized electrochemical responses when exposed to dihydrogenphosphate and ATP. However,

receptor **657** did not display evidence of binding as inferred from ^{1}H NMR spectroscopic analyses. Nevertheless, taken in concert these results led to the suggestion that the cyclotriveratrylene scaffold and additional ferrocene units in receptors **656** and **657** create phosphate binding sites appropriate for amperometric sensing. Little electrochemical response was observed for these receptors in the presence of hydrogensulfate, and fluoride produced irreversible waves.

Well-characterized electrochemical behavior with dihydrogenphosphate in dichloromethane was seen for the simple ammonium receptor **658**.[698] However, little response was observed in methanol. Presumably, this is due to the counteracting effects of ion pair solubilization and solvation of the anion. In these experiments, all anions were studied as the TBA salts. The cationic viologen receptors (**659, 660** and poly-**661**) were observed to be efficient electrochemical sensors for ATP, even in aqueous media.[699] While the redox activity of the viologen was not well behaved, monitoring the ferrocene activity in unbuffered water revealed a strong interaction with ATP and a smaller interaction with hydrogensulfate, dithionite, and phenylphosphate. Interestingly, no response was observed with dihydrogenphosphate, sulfate, hydrogenphosphate, trifluoroacetate, fluoride or chloride. Receptor **660** displayed the greatest and most selective ATP response. This finding was attributed to the presence of additional interactions between the viologen and adenine moieties, as well as to an increased level of preorganization. Interestingly, films of poly-**661** could be deposited on electrode surfaces and the resulting systems were found to display a selective response for ATP that could be measured quantitatively.

Molina and co-workers studied several urea substituted ferrocene compounds, including the ditopic receptors (**662**) and the cyclophane-type receptors (**663-664**). The ditopic receptor **662** was designed to bind concurrently both anions and cations through interactions with the urea moieties and the crown ether moieties, respectively.[700] Indeed, both dihydrogenphosphate ($K = 1.5 \times 10^4$ M^{-1}) and fluoride ($K = 1.5 \times 10^3$ M^{-1}) were found to interact with the urea groups as judged from ^{1}H NMR spectroscopic titrations carried out in chloroform using TBA anion salts. Potassium cation was then able to bind the resulting **662**:$H_2PO_4^-$ complex with an association constant of 7.3×10^3 M^{-1} in this solvent. A strong, selective electrochemical response was observed for dihydrogenphosphate in dichloromethane (a different solvent than the chloroform used in the NMR spectroscopic studies). A lower response, however, was observed in the presence of the potassium cation. The authors attributed this variation to a conformational change that occurs upon the binding of potassium, a movement that was thought to bring the two crown ether arms closer together, thereby making the urea groups less accessible to the anion. The cyclic receptors **663** and **664** were both found to bind dihydrogenphosphate with association constants of 7.2×10^2 M^{-1} (chloroform) and 4.5×10^2 M^{-1} (DMSO), respectively, as inferred from ^{1}H NMR spectroscopic studies (studied as the TBA salts).[701] While fluoride was also found to interact with these receptors, chloride, nitrate, and hydrogensulfate did not produce any spectral changes. Although the binding behavior of receptor **664** in a much polar solvent supported stronger complexation, electrochemical studies revealed the formation of an unidentified new species after oxidation in the absence of anion, presumably due to an irreversible chemical reaction following electron transfer (EC mechanism). Receptor **663**, however, gave a well-behaved cathodic shift and demonstrated a strong selectivity for dihydrogenphosphate over other anions. Molecular modeling of the two receptors led to the conclusion that intramolecular hydrogen bonding interactions served to stabilize cavities that are appropriate for dihydrogenphosphate.

Later studies analyzed the anion binding of the urea receptors **665-669**.[702] This series of receptors allowed for fluoride and dihydrogenphosphate detection using ^{1}H NMR spectroscopy and electrochemical methods. Furthermore, the incorporation of fluorescent

moieties allowed for the use of receptor **670** as a fluorescent sensor. This receptor was able to recognize hydrogenpyrophosphate, ATP and ADP, and signal their presence via fluorescence and UV-Vis spectroscopies as well as electrochemical methods.[703] In the case of ATP and ADP, hydrogen bonding interactions were considered to be most important in terms of stabilizing complex formations. However, in the case of hydrogenpyrophosphate, the induced spectroscopic and electrochemical changes were thought to reflect simultaneous hydrogen bonding and deprotonation events. Fluorescence titrations with receptor **670** in acetonitrile allowed binding constants of 4.5×10^5 M^{-1} and 1.4×10^4 M^{-1}, to be determined for ATP and ADP, respectively. An apparent association constant for hydrogenpyrophosphate of $\approx 10^4$ M^{-1} was also calculated. Naked-eye detection was achieved via the incorporation of colorimetric units into the guanidinium receptor **673** as part of a larger study including receptors **671-674**.[704] Electrochemical studies revealed the ability of each these receptors to detect fluoride, acetate, hydrogensulfate, dihydrogenphosphate, and hydrogenpyrophosphate. ITC studies in DMSO allowed for the determination of association constants on the order of 10^4 M^{-1} for dihydrogenphosphate in the case of receptors **671, 672**, and **674**. Furthermore, naked-eye detection proved possible with receptors **671-674** upon addition of the fluoride, acetate, dihydrogenphosphate and hydrogenpyrophosphate. Anions were studied as the TBA salts in these studies.

Recently Torroba and Riant have prepared and studied the N,N-diethylthiobarbituric receptors **675-679**.[705] In this case, UV-Vis and ^{1}H NMR spectroscopic studies carried out in acetonitrile supported the conclusion that the dihydrogenphosphate, benzoate, acetate, fluoride and cyanide anions (TBA salts) interacted with both the thiobarbituric unit and the ferrocene moiety of the receptors. However, dihydrogenphosphate was found to bind to a much lower extent than the other studied anions. While no binding constant could be measured for dihydrogenphosphate, naked-eye detection proved possible with receptor **675** at anion concentrations of 10^{-5} M. These receptors were also found to complex transition metal cations.

Several other more specialized ferrocene receptors have also been reported. For example, the binding behavior of the phosphorous containing macrocycle **680** was analyzed by Mathieu, Delavaux-Nicot, and co-workers.[706] In this case, a strong cathodic shift was observed by CV in acetonitrile upon the addition of dihydrogenphosphate (TBA salt). Smaller shifts were observed for hydrogensulfate and chloride. The addition of up to two equivalents of the latter anions did not affect the phosphate-induced signal. Molecular modeling supported the conclusion that the cavity of receptor **680** was compatible with the dihydrogenphosphate anion in terms of size and shape.

Wang and co-workers recently reported a bis-amidoferrocene receptor containing an ethylene glycol linkage (**681**).[707] Electrochemical analyses were conducted in dichloromethane with 0.1 M [TBA-BF_4]. CV curves revealed the growth of a new oxidation peak after the addition of 0.5 equivalents of dihydrogenphosphate (presumably added as the TBA salt) relative to the ferrocene unit. Above two equivalents of dihydrogenphosphate, no change in the oxidation peak was observed. This behavior was attributed to the formation of a 2:1 host:guest complex below 0.5 equivalents of anion, and these complexes did not exhibit dramatically altered electrochemical activities. Between 0.5 and 2 equivalents of anion, 1:1 host:guest complexes were formed, and these complexes led to the formation of a new oxidation peak. After 2 equivalents of anion were added, it was proposed that 1:2 host:guest complexes predominated in the solution.

Many examples of metallocene-based phosphate receptors derived from a calix[4]arene core have been reported by Beer and co-workers. In early studies, receptor **682** was found to complex dihydrogenphosphate in DMSO-d_6 as judged from ^{1}H NMR spectroscopic

titrations ($K = 2.8 \times 10^3$ M^{-1}).[708] Chloride and bromide, however, were bound even more strongly. Precipitation of the host:guest complexes prevented an analysis of these systems by electrochemical methods. Increasing the bulkiness of the lower rim substituents (**683** and **684**) led to similar association constants but produced better selectivities for dihydrogenphosphate in the case of receptor **684**.[709] The bridged receptor **685** was found to bind dihydrogenphosphate slightly more strongly than **684** ($K = 6.38 \times 10^3$ M^{-1}in DMSO-d_6).[710,711] Receptors **683-685** were also observed to interact with a variety of carboxylate anions. While receptor **683** displayed the lowest binding affinity of the series, it also displayed the only high selectivity for dihydrogenphosphate. Reversible cathodic shifts were observed for receptors **683-685** in acetonitrile with the magnitude of the response in line with the observed stability constants recorded in DMSO-d_6. As with receptor **618** described earlier, receptors **686** and **687**, derived from calixarenes substituted on the lower rim, gave rise to strong cathodic shifts in the presence of dihydrogenphosphate (TBA salt), at least in organic media.[712] Overall, calixarenes of this type proved useful as scaffolds for creating amide-metallocene anion receptors since, by and large, these compounds displayed both high stability constants and good electrochemical responses. Oddly, receptor **688** did not display a response to anions, perhaps due to the presence of competing steric or intramolecular hydrogen bonding interactions. Thus, the specifics of the design are seen to be important in terms of receptor performance.

More recently, Beer and co-workers reported calixarenes functionalized at the upper rim with amido- and ureidoferrocene units (**689** - **691**).[713] These receptors were found to bind benzoate, chloride, and dihydrogenphosphate through ^{1}H NMR spectroscopic studies in 1:1 deuterated acetonitrile:DMSO. The spectra obtained were consistent with the presence of hydrogen bonding interactions with the urea and amide units, respectively, at the upper rim of the macrocycle. Significantly stronger binding constants were observed with tetraurea receptor **690** than di-substituted receptor **689**, presumably due to increased hydrogen bonding interactions with the additional recognition moieties. Tetraurea receptor **690** and tetraamido receptor **691** displayed similar association constants with dihydrogenphosphate ($K = 150$ M^{-1} and 120 M^{-1}, respectively); however, receptor **690** displayed a greater selectivity for dihydrogenphosphate over benzoate and chloride. Cyclic and square-wave voltammetry studies revealed the presence of a new peak upon addition of dihydrogenphosphate to all three receptors while only peak shifts were observed with benzoate and chloride (1:1 CH_2Cl_2:CH_3CN). As a result, the authors suggested that the new peak resulted from the kinetically slow binding of dihydrogenphosphate relative to the cyclic and square-wave voltammetry time scale. All anions were studied as the TBA salts.

In 2008, Lemaire and Saint-Aman synthesized receptors **692-693** in which the ferrocene moieties were connected to calixarene units through urea or amide groups.[714,715] Electrochemical analyses, as well as ^{1}H NMR spectroscopic studies, carried out in dichloromethane led to the conclusion that receptors **692-693** interact with dihydrogenphosphate (TBA salt); however, the resulting binding constants were rather low (e.g., $K = 36$ M^{-1} for receptor **692a**). In a unique variation on the calixarene ferrocene paradigm, Guo and co-workers developed the thiacalix[4]arene-based receptor **694**.[716] In this case, electrochemical studies carried out in dichloromethane:acetonitrile revealed a cathodic shift that was correlated with formation of a dihydrogenphosphate (TBA salt) complex.

The effect of organizing electrochemically active receptors on surfaces was also investigated by the Beer group.[717] Solution studies of the free receptor **695** and its surface bound analogue **696**, carried out via ^{1}H NMR spectroscopy in acetonitrile/dichloromethane with TBA anion salts, led to the suggestion that these receptors formed much stronger anion complexes than the previous systems. Interestingly, electrochemical analyses revealed an

irreversible phenomenon in the case of receptor **695** but well-behaved redox behavior for receptor **696**. This latter system was found to display selectivity for dihydrogenphosphate. Good selectivity was also observed when receptors **696** and **697** were absorbed onto gold monolayers. An increase in the cathodic shift was observed for the surface-bound version of **696** relative to what was observed for this same receptor in acetonitrile/dichloromethane solution. Such an observation provides support for the conclusion that absorption to the surface amplified the signal, presumably by preorganizing the system. Both the surface and solution based forms of receptor **696** were found to be free of interference for dihydrogenphosphate recognition at up to 100 equivalents of halide.

Amidoferrocene moieties have also been attached to dendrimers for electrochemical anion sensing, particularly by Astruc and co-workers. Early studies in 1997 investigated the electrochemical behavior of dendrimers bearing 3, 9, 18, and 36 amidoferrocene units, of which two are shown (**698a** (9 units) and **698b** (18 units)).[718] These studies revealed a clear "dendritic effect" in which an increase in the number of arms, and therefore amidoferrocene units, led to an increase in anion association as inferred from ^{1}H NMR spectroscopic and CV studies with TBA anion salts. With high generation dendrimers (e.g., 36 units), however, saturation was reached. Presumably, this reflects the fact that many of the ferrocene units were no longer available to the anion. In the case of the amino dendrimers analyzed in this study, the highest anion response was observed with the system bearing 18-amidoferrocene units. For this receptor, a new wave was observed in the presence of dihydrogenphosphate in dichloromethane. Apparent association constants with values > 10^5 M^{-1} were calculated for the interaction of dendrimers **698a** and **698b** with this anion. In addition, strong selectivity for dihydrogenphosphate over hydrogensulfate, chloride, and nitrate was observed.

Further studies compared the incorporation of Cp (**699a**) *vs.* Cp* (**699b**) substituted amidoferrocene ligands attached to a polyamine core.[719] Dendrimers of generation 1-5 were studied, with generation 3 (G3) shown here (**699**). Interestingly, the anion response of the Cp* derivatives (e.g., **699b**) proved to be longer lived and more reversible than that of the unsubstituted Cp congeners (e.g., **699a**). The improved behavior of the Cp* receptors was attributed to the enhanced stability and increased lipophilicity that are characteristic of Cp* ligands. Furthermore, appreciable phosphate selectivity was observed, with little interference from chloride or hydrogensulfate. The strongest apparent association constant for dihydrogenphosphate interactions was estimated to be 7×10^4 M^{-1} (DMF) with the fourth generation (G4) Cp* functionalized ferrocenium dendrimer. While no dendritic effect was observed for the Cp* substituted dendrimers in dichloromethane, a modest dendritic effect was reported for analyses in DMF. Anions were studied as the TBA anion salts.

In 2003, Astruc and co-workers reported a series of polyamine dendrimers that were functionalized through phenol-N hydrogen bonds with silicon linkages to the amidoferrocene.[720,721] Receptor **700** represents the first generation dendrimer (G1), which bears 12 amidoferrocene groups. Electrochemical studies in dichloromethane based on G1-G4 dendrimers revealed a positive dendritic effect for dihydrogenphosphate (studied as its TBA salt). No dendritic effect was reported for ATP, however, possibly due to the increased steric restraints of anion complexation. Addition of dihydrogenphosphate or ATP produced new waves in the cyclic voltammogram of these dendrimers in dichloromethane.

Dendrons similar to those above have also been attached to gold nanoparticles in an effort to create unique dihydrogenphosphate electrochemical sensors.[722-725] With these surface-supported systems, a new redox wave was again observed upon addition of dihydrogenphosphate. Electrochemical titrations supported a 1:1 ferrocene: dihydrogenphosphate stoichiometry. Interestingly, a 2:1 ferrocene: anion ratio was observed with ATP. The ferrocene signals associated with this response proved to be independent of

the concentrations of the ferrocene ligand on the gold surface. The response was also independent of the length of the carbon spacer in the case of the linear amidoferrocene ligands. An increased response was seen when electron-withdrawing groups were present on the Cp rings. This result provides support for the presence of amide-anion hydrogen bonding interactions. A decreased response was observed when the Cp rings were substituted with electron-donating groups. A small interference from hydrogensulfate and chloride was observed in these systems. All studies were performed in dichloromethane with TBA anion salts.

Astruc and co-workers also showed that the derivatized nanoparticles could be adsorbed to a Pt electrode surface to produce a phosphate responsive electrode. These electrodes were able to sense dihydrogenphosphate and ATP over hydrogensulfate and chloride in dichloromethane. The facile synthesis of these nanoparticle systems and the similarity in their response to the free dendrimer systems lends support to the conclusion that this multi-faceted approach to electrochemical sensor development could prove to be of general utility.

Recently, Astruc and co-workers prepared the organometallic cluster dendrimers **701** and **702** and showed that these macromolecules could be used to recognize ATP with selectivity over dihydrogenphosphate.[726] In CV studies carried out in dichloromethane, dendrimer **701** was found to display larger cathodic shifts in the presence of ATP than did dendrimer **702**. This difference in dendrimer response was attributed to the shorter distance between the metallic fragments in dendrimer **701**. The incorporation of dendrimer **702** onto a Pt electrode also allowed for selective ATP detection.

Other researchers have also analyzed dendritic systems. For example, Losada, Cuadrado, and co-workers developed silicon-amine based dendrimers incorporating simple (non-amido) ferrocene units (e.g., **703**).[727] These dendrimers displayed an electrochemical response to the presence of anions (TBA salts) in both dichloromethane solution and when immobilized on an electrode. Strong but irreversible electrochemical responses were observed for dihydrogenphosphate, even in the presence of other anions. Dendrimers without the inner amine groups displayed a much smaller response to anions. As a result, the inner amine groups of the dendrimer were considered to be a crucial determinant of the selective recognition shown by dendrimers based on **703**, presumably as the result of favorable hydrogen bonding interactions with the anion.

In another example, Cuadrado and co-workers reported a strong response for dihydrogenphosphate in DMSO when G1-G4 polyamine dendrimers appended with urea-ferrocene units were analyzed.[728] Receptor **704** represents the fourth generation dendrimer containing 32 urea-ferrocene units. Despite the high polarity of the solvent, submillimolar concentrations of dihydrogenphosphate could be selectively detected in the presence of chloride and hydrogensulfate when the latter anions were present at equal concentrations to dihydrogenphosphate (all anions studied as the TBA salts).

$[Ru(bpy)_3]^{2+}$ as fluorescence and electrochemical reporter: The unique $[Ru(bpy)_3]^{2+}$ unit can serve as both an electrochemical and a luminescent reporter group. When functionalized with amide or other hydrogen bonding moieties, an increase in emission of the MLCT band is generally observed in the presence of anions. These effects are not observed with unfunctionalized $[Ru(bpy)_3]^{2+}$. It has been proposed that the observed increase in emission is a consequence of the bound anion serving to rigidify the receptor and thus inhibiting vibrational and rotational relaxation modes of nonradiative decay. As observed with the ferrocene systems, the binding affinities can be tuned quite dramatically through an appropriate choice of the spacer/bridging moieties. Similar effects have been observed in

many case where $Re(bpy)(CO)_3Cl$ units were used to replace the $[Ru(bpy)_3]^{2+}$ moieties. However, these latter systems will not be discussed in detail here.

Beer and co-workers were among the first to report applications of this unique signaling unit. The researchers focused first on bipyridyl cores bearing amine and amide substituents. Many of these systems displayed good selectivities for phosphate containing analytes. For instance, in the case of receptor **705a** ^{1}H NMR spectroscopy and CV studies carried out in acetonitrile revealed a selective response to dihydrogenphosphate, even in the presence of hydrogensulfate and chloride.[729] It was proposed that anion coordination primarily involves interactions with the amide-bipyridyl functionality. A similar selectivity was observed with the calixarene-based receptor **706**.[709] This latter system displayed a binding constant of 4.4 $\times 10^3$ M^{-1} with dihydrogenphosphate in DMSO-d_6 as determined from ^{1}H NMR spectroscopic analyses. This binding affinity was similar to that reported for the cobaltocene analogue **685**, described earlier in the section. The response of receptor **705a** to dihydrogenphosphate, as well as that of receptors **687b** and **707** - **710**, was further investigated through ^{1}H NMR spectroscopic, electrochemical and optical techniques.[730] All receptors in this study displayed a dihydrogenphosphate association constant that was at least 10-fold higher than the corresponding association constant for chloride anion as inferred from ^{1}H NMR spectroscopic analyses carried out in DMSO-d_6. Receptor **707b** displayed a much stronger binding response than receptor **708**, a finding that provided further support for the importance of amide hydrogen bonding interactions in moderating the anion recognition process. Increased binding affinities were also seen as the degree of macrocyclic preorganization increased. Specifically, binding affinities were found to follow the sequence **707c** < **709** and **710** < **705a** for dihydrogenphosphate in DMSO-d_6.

Phosphate selectivity was also observed through electrochemical experiments carried out in acetonitrile with receptors **705a, 707c, 709**, and **710**. Here, a strong selectivity for dihydrogenphosphate over hydrogensulfate, chloride, and bromide was observed for each of the four studied receptors. Notably, the response of receptor **705a** to dihydrogenphosphate was not affected by the addition of up to 10 equivalents of hydrogensulfate and chloride anions. Optical studies with receptors **705a, 707c**, and **710** revealed an increase in the intensity of the MLCT absorption band in the presence of dihydrogenphosphate. In acetonitrile solution, a bathochromic shift in the emission band as well as an increase in the quantum yield was observed upon the addition of dihydrogenphosphate. These changes in emission spectral features were attributed to the increased structural rigidity of the anion:receptor complex as compared to the free receptor. All anions were studied as their TBA salts.

The hybrid ferrocene receptor **711** was found to stabilize a strong 2:1 guest:host complex when exposed to dihydrogenphosphate (TBA salt) as inferred from both ^{1}H NMR and UV-Vis spectroscopic studies carried out in acetonitrile.[94] While the presence of the ferrocene moiety served to quench the emission of the $[Ru(bpy)_3]^{2+}$ subunit in the free receptor, a strong emission intensity was observed in the presence of dihydrogenphosphate. Addition of chloride or hydrogensulfate did not affect the luminescence, presumably due to weaker host-guest interactions with these two anions.

Dimeric derivatives **712** were also found to be selective for dihydrogenphosphate over chloride and bromide.[731] The anion binding ability of these receptors was studied by ^{1}H NMR spectroscopy in DMSO-d_6 using TBA anion salts. Anion binding affinity was found to be highly dependent on the nature of the linker, the bipyridyl substituent, and the metal center. For example, the *meta*-substituted **712a** bound dihydrogenphosphate two orders of magnitude more strongly than *para*-substituted **712b**, and the ethylene-substituted **712c** displayed very low anion binding affinity. In addition, ester-containing receptor **712d** was

found to bind dihydrogenphosphate more strongly than methyl-derivative **712f**. The different metal centers studied bound dihydrogenphosphate in the order Os > Ru > Re. Of the combinations studied, the highest binding affinity was found for receptor **712h** ($K > 3.0 \times 10^5$ M^{-1}).

Macrocyclic derivatives of receptor **707c** (**713**) were found to interact more strongly with dihydrogenphosphate (TBA salt) than the linear analogues ($K > 10^5$ M^{-1} *vs.* 1.3×10^3 M^{-1} for **713** and **707c**, respectively, in DMSO-d_6).[732] Smaller macrocycles such as receptor **714**, however, proved too sterically hindered to allow for appropriate hydrogen bonding interactions. These receptors displayed only weak affinities ($K < 10^3$ M^{-1} for $H_2PO_4^-$ in DMSO-d_6).

The use of the polyammonium receptors **715** allowed these kinds of analyses to be extended into aqueous media. These systems were found to interact strongly with dihydrogenphosphate and ATP over the pH range 2-10, as observed through potentiometric titrations.[733] Interestingly, luminescence *quenching* was observed upon the addition of dihydrogenphosphate and ATP in these systems (pH = 6). The mechanism of this response was not determined. In a mixed acetonitrile:water (9:1) medium, the charged imidazolium receptors **716** and **717** were found to display a significant luminescence enhancement in the presence of ATP. [734] Association constants could not be determined for this anion. On the other hand, receptor **717** displayed an association constant of 1.4×10^3 M^{-1} with dihydrogenphosphate as measured by emission titrations in 9:1 acetonitrile:water. However, it is to be noted that under these conditions, much stronger binding affinities were observed for the chloride, bromide, and acetate anions.

The anion binding properties of receptor **718**, a quinone derivative, and receptor **719** were analyzed by ^{1}H NMR spectroscopy in DMSO-d_6.[735] The dihydrogenphosphate binding affinity was found to be similar for both receptors ($K \approx 2 \times 10^2$ M^{-1}) under these conditions. Notably, both receptors proved selective for acetate and chloride over dihydrogenphosphate. All anions studied as the TBA salts.

Ditopic receptors, such as compound **720**, were also investigated by Beer and co-workers. Receptor **720** was found to bind TBA dihydrogenphosphate ($K = 9.0 \times 10^2$ M^{-1} in DMSO).[736] In addition, a clear ability to complex K^+ into the crown ether moieties was observed using ^{1}H NMR spectroscopy. The presence of K^+ reduced the affinity for dihydrogenphosphate, presumably due to a conformational change that precluded the formation of optimal anion-receptor hydrogen bonds. The anion binding behavior of this receptor could also be observed through an increase in the $Ru(bpy)_3$ luminescence intensity.

The Watanabe group analyzed the anion complexation of metalloreceptor **721**. This system contains two bis(acylaminoimidazoline) subunits in addition to a $Ru(bpy)_3$ core.[737] The guanidinium units of this receptor displayed largely reduced basicities ($pK_a \approx 2, 4$) as compared to traditional guanidinium groups. Thus, in contrast to what was true for many other systems, the guanidinium units were initially neutral in these studies. On the other hand, ^{1}H NMR spectroscopic studies revealed that protonation of **721**, as opposed to complexation, takes place when treated with diphenylhydrogenphosphate (DPHP) or dibenzyl hydrogenphosphate (DBHP) in acetonitrile. In contrast, when acetone was used as the solvent the formation of a 1:1 inner-cleft complex was observed in the presence of DBHP. Complex formation was observed in both solvents in the presence of anionic diphenylphosphate (DPP). The nature of these interactions was further probed using absorbance and luminescence spectroscopy in acetone. Since no responses were observed during control experiments with $Ru(bpy)_3^{2+}$, the changes when receptor **721** was exposed to this set of phosphate anions were attributed to specific chemical differences (complex

formation, deprotonation) and not counter anion exchange or solvent polarity differences. Addition of DBHP produced a red-shift and decreased the intensity of the MLCT band as well as the luminescence of receptor **721**. These responses were attributed to the increase in the positive charge at the guanidinium moiety, presumably through proton transfer, that occurs upon complex formation. Addition of DPP produced a slight blue-shift and served to increase intensity of the MLCT band and the overall luminescence. The enhancement of the emission response was attributed to an increase in the rigidity of the receptor. From the observed anion-induced UV-Vis absorption changes, binding constants of 3.3×10^4 M^{-1} and 4.8×10^3 M^{-1} were calculated for DPP and DBHP, respectively. The decreased affinity seen for DBHP was attributed to electrostatic repulsion between the cationic ruthenium center and the incipient positive charge produced on the guanidinium binding site of receptor **721** during complexation. Notably, strong spectral responses were observed in relatively polar solvents even in the absence of a positive charge on the guanidinium moiety. In these studies, the anions were added as the tetraethylammonium salts.

Several other research groups have also used the $Ru(bpy)_3$ reporter unit to probe phosphate binding interactions. For example, Smith and co-workers developed the ditopic receptor **722**, which was designed to bind phosphorylated sugars through anion-amide and diol-boronic acid interactions.[738] Strong binding ($\log K \approx 3$) was observed for fructose-6-phosphate, glucose-1-phosphate and galactose-6-phosphate in water as inferred through the enhancement in the emission spectrum. Interestingly, the binding behavior of fructose alone was found to be greatly increased when sodium phosphate was used as the buffer. A cooperative binding model was proposed in which the phosphate anion formed hydrogen bonds with both the amide units and the alcohols of the saccharide.

Another set of $Ru(bpy)_3$-based phosphate anion receptors was introduced by Vos and co-workers. These researchers compared the emission behavior of receptors **707c, 723**, and **724** in the presence of dihydrogenphosphate (TBA salt) in acetonitrile.[739] Emission intensity and lifetime increases were observed for all three receptors up to the point where 2.5 equivalents of dihydrogenphosphate had been added. When greater quantities of anion were added, receptor **724** displayed an unexpected decrease in the emission intensity. Similar binding was, to a first approximation, observed for both the bipyridine receptor **707c** and the phenanthroline receptor **723**. An extreme sensitivity to water was also observed.

Lin and co-workers also explored the use of phenanthroline ligands. These researchers developed colorimetric sensor **725**, which contains a functionalized phenanthroline core with nitrophenylhydrazine substituents as the L groups.[740] Naked-eye and UV-Vis studies led to the suggestion that receptor **725** formed a complex with dihydrogenphosphate (TBA salt) in DMSO. However, a stronger binding behavior was seen with acetate.

The dithiocarbamate ruthenium receptors **726-727** were found to function as anion redox sensors.[741] In this case, the addition of TBA dihydrogenphosphate produced a cathodic shift in the ruthenium-centered redox couple in acetonitrile. Proton NMR spectroscopic titrations in DMSO-d_6:CD_3CN (1:1) provided support for the electrochemical results. Binding constants of 70 M^{-1} and 45 M^{-1} for receptors **727a** and **727b**, respectively, were reported.

Recently, Sun and co-workers studied the quinoxaline(sulfonamide) receptors **728-729** and related metalloreceptors **730-731**.[742] Changes in the acidity of the N-H sulfonamide proton appeared to be the largest determinant of anion binding behavior within the limited set of receptors **728** and **729**. Metal coordination to receptors **728** and **729** to yield **730** and **731**, respectively, increased the association constants by one to two orders of magnitude for the studied anions (fluoride, cyanide, acetate, dihydrogenphosphate, and chloride as the corresponding TBA salts). UV-Vis and ^1H NMR spectroscopic studies carried out in

different solvents provided support for the conclusion that all of the receptors subject to study were bound to dihydrogenphosphate through hydrogen bonding interactions.

Metal dipyrrolyl-bipyridine receptors **732-733** were prepared by Sessler and co-workers.[743,744] Relative to other test anions studied, the ruthenium receptor **732a** was found to display the highest affinity toward dihydrogenphosphate (as the TBA salt). A binding constant of 1.04×10^5 M^{-1} was obtained through fluorescence titrations carried out in DMSO.

The anion binding abilities of similar systems were studied by Lin and co-workers in 2009.[745] UV-Vis titrations in dry DMSO were carried out in the case of receptors **734** - **735** by adding the TBA salts of fluoride, chloride, bromide, iodide, acetate, hydroxide, and dihydrogenphosphate to solutions of the receptor. Association constants in the range of 10^2 - 10^4 M^{-1} were reported for binding to dihydrogenphosphate, although a preference was observed for fluoride and acetate. Little to no spectroscopic changes were observed upon the addition of halide ions. In general, the benzene-substituted compounds **734b** and **735b** bound anions more strongly than did the toluene derivatives **734a** and **735a**. The relatively lower binding affinity of the toluene-functionalized receptors was attributed to the electron donating ability of the methyl group relative to the hydrogen atom of the benzene derivatives.

Dimetallic systems were analyzed as possible sensors for phosphate by Martínez-Máñez and co-workers. In preliminary studies, receptor **736** was found to serve as a selective fluorescent sensor for ATP in 7:3 acetonitrile:water at slightly acidic pH values (4-6).[746] No changes in the emission spectrum were observed upon the addition of chloride, sulfate, or inorganic phosphate. Further studies examined the dimetallic complex produced upon coordination of Cu^{2+} to the cyclam unit of ligand **736**.[747] For example, in acetonitrile:water (7:3), the stability constant between [**736:Cu**)]$^{4+}$ and ATP was determined to be log K = 6.10 through potentiometric titrations. CV studies supported the conclusion that non-coordinating anions (ATP, ADP, GMP, sulfate, and inorganic phosphate) interacted with the **736:Cu** receptor through electrostatic (Coulombic) interactions rather than metal-anion coordination. A larger shift was observed in the CV spectrum of coordinating anions such as chloride, bromide, and hydroxide.

4.4. Macrocycles

Macrocycles offer several advantages as phosphate receptors. One advantage derives from the effective preorganization of the constituent binding moieties. A variety of macrocycle classes have been analyzed, including calixarene, calixpyrrole, porphyrin, and other polypyrrolic macrocycles. Each class exhibits different geometrical organization and in some cases metal-coordination propensities.

Calixarenes can be functionalized at either or both the upper and lower rim with a variety of substituents. Bulky functionalities on either rim are often used to lock the structures in the cone or other conformations. In an early example, Reinhoudt and co-workers functionalized the upper rim with sulfonamide groups to produce receptors such as **737**.[748] While receptors of this type were found to display selectivity for hydrogensulfate, receptor **737** was also found to interact with dihydrogenphosphate. An association constant of 3.5×10^2 M^{-1} in chloroform was determined through ^{1}H NMR spectroscopy. Loeb and Cameron examined amide functionalized calixarene **738**, which displayed 1:1 binding with a variety of anions as inferred from ^{1}H NMR spectroscopic analyses carried out in $CDCl_3$.[749] A preference was observed for Y-shaped anions over tetrahedral anions such as dihydrogenphosphate, for which an association constant of 22 M^{-1} was calculated. Both studies relied on TBA anion salts.

Schneider and co-workers later introduced trimethyl ammonium groups onto the upper rim and demonstrated that various functionalized calix[4]arene (e.g., **739, 740**), calix[6]arene (e.g., **741a**) and calix[8]arene (e.g., **741b**) derivatives interact with nucleotides in water as inferred from ^{1}H NMR spectroscopic studies.[750] The calculated binding constants for receptors **739** and **740** were similar (e.g., 11×10^3 M^{-1} *vs.* 7×10^3 M^{-1}, respectively, with ATP), but the larger macrocycles (**741a, 741b**) displayed significantly higher binding affinities (ca. 7×10^4 M^{-1} with ATP). These studies, along with molecular modeling, revealed several interesting trends. In accord with studies previously discussed, each ion pair interaction was calculated to contribute approximately 5 kJ/mol of binding energy. The authors concluded that these ion pairing interactions, however, disrupted the insertion of the nucleobase into the calixarene cavity. The increased binding affinity of larger macrocycles was thus attributed to more complete insertion of the nucleobase into the macrocyclic cavity. Little selectivity was observed among the different nucleobases. These receptors were also found to interact with double stranded DNA.

A comparison of cone and 1,3-alternate binding behavior was conducted by Lhoták, Stibor, and co-workers using the urea receptors **742a** and **743a**.[751] While receptor **742a** was predicted to interact with two anions, a 1:1 binding stoichiometry was observed for most anions as deduced from ^{1}H NMR spectroscopic titrations carried out in deuterated chloroform/acetonitrile (4:1). The associated affinity constants proved similar to those determined in the case of receptor **743a**. Association constants for dihydrogenphosphate were found to be approximately 2×10^3 M^{-1} for both receptors (i.e. **742a** and **743a**), and little anion selectivity was observed. The putative lack of binding to the second site of receptor **742a** was attributed to a negative allosteric effect in which widening of one side of the receptor caused the other side to be compressed. It was proposed that compression of the second site rendered it unsuitable for anion binding.

An extensive ^{1}H NMR spectroscopic study was used to analyze receptors **742-747**.[752] These studies led to the conclusion that neither cone nor partial cone conformations displayed a preference for dihydrogenphosphate over other anions in deuterated chloroform:acetonitrile (4:1). At the same time, receptors **742b** and **742d** with 1,3-alternate conformations were found to exhibit slightly greater affinities for dihydrogenphosphate as compared to other anions under these conditions (e.g., $K(H_2PO_4^-) = 3.2 \times 10^3$ M^{-1} and $K(PhCO_2^-) = 2.1 \times 10^3$ M^{-1} for receptor **742b**). All anions were studied as their TBA salts.

The amide-containing receptor **748** was synthesized by Chen and Huang. This system is characterized by the use of a functionalized anthracene to strap the upper rim of a calix[4]arene with a fluorescent substituent.[753] Fluorescence quenching was observed upon the addition of dihydrogenphosphate (TBA salt) in acetonitrile. A higher degree of quenching, however, was observed upon addition of acetate. Thus, this system, although interesting, is not selective for phosphate.

Chawla developed the upper rim functionalized neutral semicarbazone calixarene **749**.[754] UV-Vis and NMR spectroscopic studies confirmed that receptor **749** is highly selective for dihydrogenphosphate (TBA salt). A binding constant of 1890 M^{-1} was measured in DMSO.

A number of studies have also focused on the analysis of lower rim-functionalized receptors. Nam and co-workers examined the urea-functionalized receptors **750** and **751**. The three calix[6]arenes (receptors **750**) were found to bind dihydrogenphosphate (TBA salt), albeit with different affinities. The strongest binding interaction was observed with receptor **750a**, which displayed a binding constant of 8.3×10^2 M^{-1} in deuterated dichloromethane as deduced from a ^{1}H NMR spectroscopic titration.[755] This receptor, however, displayed a much stronger affinity for chloride than dihydrogenphosphate. Methylation of the remaining

alcohol groups yielded receptor **750b**. This substitution pattern was found to result in lower binding constants, presumably due to reduced hydrogen bonding interactions. The incorporation of thioureas (**750c**) also served to reduce the phosphate binding affinity relative to **750a**. The weaker observed binding behavior was attributed to increased intermolecular interactions as a result of the enhanced acidity. The same kinds of relative enhancements were seen in the case of the calix[4]arene derivatives (**751**); again, the urea derivatives proved to be better receptors. On the other hand, with these smaller macrocycles, an increased binding affinity was seen for the methylated receptor **751b** relative to alcohol substituted receptor **751a**.[756] This effect was attributed to increased intramolecular hydrogen bonding of the hydroxyl groups in the smaller macrocycles in the absence of anions. No selectivity for dihydrogenphosphate was observed. Nam, Jeon, and co-workers also developed an electrochemical calix[4]arene derivative (**752**) containing quinone moieties as well as urea functionalities.[757] Proton NMR spectroscopic studies in $CDCl_3$ displayed an increased binding affinity for dihydrogenphosphate ($K = 1.0 \times 10^3$ M^{-1}) relative to the more traditional calixarene receptor **751b** ($K = 2.5 \times 10^2$ M^{-1}). This increased binding affinity was attributed to an additional hydrogen bonding interaction between the hydrogen of the anion and the oxygen of the quinone moiety of receptor **752**. Receptor **752** bound anions in the order of hydrogensulfate > dihydrogenphosphate > acetate > chloride > bromide > perchlorate. Cathodic shifts were observed through CV upon addition of these anions to a solution of receptor **752** in acetonitrile. The magnitude of the shift followed a similar binding trend as that observed in the NMR spectroscopic studies. All anions were studied as their TBA salts.

In another example, Beer and co-workers functionalized the lower rim of calix[4]arene to produce the positively charged pyridinium-containing receptor **753**. This system was found to form 2:1 anion:receptor complexes in DMSO with TBA anion salts.[758] On the basis of ^1H NMR spectroscopic titration studies, a binding constant of 4.5×10^4 M^{-2} for the interaction with dihydrogenphosphate was calculated. Selectivity for dihydrogenphosphate over chloride, bromide, and hydrogensulfate was also observed. Furthermore, receptor **753** was found to give rise to an electrochemical response to dihydrogenphosphate.

Moderate dihydrogenphosphate selectivity was also observed with receptor **754**, which contains positively charged phosphonium groups.[759] Using ^1H NMR spectroscopy, Nam, Vicens and co-workers were able to measure a binding constant of 7.5×10^2 M^{-1} in chloroform using the TBA salt.

Highly selective dihydrogenphosphate binding in acetonitrile was achieved with the fluorescent calixarene-based receptor **755** developed by Chen and Chen.[760] A strong increase in the emission intensity, along with a small blue-shift in the fluorescence maximum, was observed upon the addition of dihydrogenphosphate. This spectral change was attributed to inhibition of PET quenching arising either from an increase in the hydrogen bonding interactions or enhancements in the rigidity of the receptor. On the basis of fluorescence titrations, a 1:2 receptor:anion stoichiometry for dihydrogenphosphate and a binding constant of 5.4×10^9 M^{-2} was inferred. Strong selectivity over a variety of other anions was observed, including a 2700-fold greater binding affinity for dihydrogenphosphate as compared to fluoride. Proton NMR spectroscopic studies in chloroform/DMSO led to the suggestion that phosphate binding derived from multiple hydrogen bonding interactions, including those with the amides, sulfonamides, hydroxyl groups, and various CH protons. These multi-point interactions were proposed to be the most likely source of the observed selectivity. The same researchers later examined the calixarene-based receptors **756**, which were functionalized with chromogenic azophenol groups as sensing units at the upper rim and with hydrogen bonding units on the lower rim.[761] These receptors were found to bind dihydrogenphosphate, as well as fluoride and

acetate, in acetonitrile. A color change of light yellow to blue was observed upon anion addition. The absorption spectrum of receptor **756b** was found to shift to different wavelengths upon the addition of dihydrogenphosphate, fluoride, and acetate, thus allowing selective detection of all three anions. Binding constants with dihydrogenphosphate were found to be on the order of 10^4 M^{-1} for all three receptors. Interestingly, the carboxylate groups at the lower rim were thought to play a critical role in dihydrogenphosphate binding, but not for the recognition of acetate or fluoride. These studies were carried out using the TBA salts of the anions in question.

An alternative approach to the colorimetric detection of dihydrogenphosphate was introduced by Gunnlaugsson and Matthews.[762] These researchers utilized receptor **757**, which incorporates two amidourea substituents at the lower rim. UV-Vis titrations in DMSO with TBA anion salts led to the conclusion that this receptor interacts with both dihydrogenphosphate and hydrogenpyrophosphate (log $\beta = 4.86$ and 5.72 for these two substrate, respectively). A higher affinity, however, was observed for fluoride. Color changes from yellow to purple were observed upon the addition of fluoride or hydrogenpyrophosphate TBA salts.

The use of receptor **758**, which incorporates four amido urea units, led to increased binding constants.[763] For example, in the case of receptor **758a** log *K* values near 5 for were observed for acetate, fluoride, dihydrogenphosphate, and pyrophosphate (TBA salts) were recorded in DMSO as deduced from UV-Vis spectroscopic titrations. Fluoride or pyrophosphate addition resulted in a strong clear-to-red color change in solutions of receptor **758b**. On the other hand, acetate or dihydrogenphosphate addition resulted in weaker, yellow color changes. However, a 1:1 binding stoichiometry was seen only in the case of pyrophosphate. Proton NMR spectroscopic studies of receptor **758b** confirmed that the amidourea moieties participated in anion binding.

Beer and co-workers prepared calixarenes **759** and **760** as potential ditopic anion-cation receptors. Receptor **759** was designed to interact with anions at the amide functionality of the lower rim while complexing cations within the crown ether unit.[764] After formation, the potassium and ammonium complexes of receptor **759** were found to bind a variety of anions (add as the TBA salts) as revealed by ^{1}H NMR spectroscopic studies carried out in acetonitrile. With this system, the strongest interaction was seen with dihydrogenphosphate. The binding affinity was too high to be determined using NMR spectroscopic methods but was estimated to be greater than 10^4 M^{-1}. The free base receptor **759**, on the other hand, did not bind anions. In the case of receptor **760**, it was the calixarene moieties that were designed to function as the cation binding sites.[765] The interaction of **760** and control compound **761** with various anions was analyzed using ^{1}H NMR spectroscopy in DMSO-d_6. Compound **760** displayed a preference for dihydrogenphosphate over acetate, benzoate, and chloride. The association constant corresponding to the interaction between receptor **760** and dihydrogenphosphate was measured to be ca. 700 M^{-1} in this solvent. Interestingly, acetate was found to interact with receptor **761** more strongly than did dihydrogenphosphate. This led these researchers to suggest that the calixarene moiety contributes to phosphate binding even in the absence of a cation. Studies of receptor **760** as a potential ditopic binding agent were only carried out using halide anion salts.

More application-driven studies of calixarene receptors have also been preformed. For example, Roundhill and co-workers compared the dihydrogenphosphate extraction ability of the amide and amine calix[4]arene derivatives **762** and **763**.[766] These researchers found that the amine derivatives **763** were more efficient at extracting phosphate from a neutral aqueous phase into an organic layer (chloroform). This effect was attributed to the enhanced

interactions between the phosphate and the charged amines present in the protonated form of **763** as compared to the neutral amide receptor **762**.

In a different study, Gupta and co-workers developed a phosphate selective electrode based on the calix[6]arene-derived ionophore **764**. The resulting ISEs displayed a working concentration range of 1.77×10^{-5} to 0.1 M for monohydrogenphosphate with a response time of 20 seconds.[767] Good selectivity was achieved over a variety of anions. A slight interference was observed for ClO_4^-. The lifetime was found to be approximately one month. Furthermore, phosphate measurements made using this calixarene-based electrode in field samples were comparable to values obtained with a traditional molybdenum assay. In another example, receptors **765** were analyzed by Arrigan and co-workers after incorporation into a PVC-based ISE.[768] The resulting electrodes were found to be selective for monohydrogenphosphate in aqueous media over a concentration range of 5.0×10^{-5} to 1.0×10^{-1} M.

Alterations made to the core structure of calixarenes, such as varying the linkages between the phenyl rings, have also been explored. For example, Anslyn and co-workers designed the expanded azacalixarene **766**. This receptor is characterized by several hydrogen bonding sites, as well as a cationic and hydrophobic core.[769] Through a fluorescent indicator (HPTS) displacement assay, receptor **766** was observed to interact with IP_3, fructose-1,6-diphosphate, and gluconic acid in aqueous media buffered to pH 7.2 with HEPES. The highest binding affinity was measured with IP_3 (2.4×10^4 M^{-1}). Proton NMR spectroscopic studies with fructose-1,6-diphosphate led to the conclusion that the most important binding interactions involve the secondary amines of the core, rather than the primary amines at the upper rim.

Akkaya and co-workers achieved a cationic core by replacing the phenyl rings of a traditional calixarene with pyridinium units (**767**).[770] As in the previous Anslyn system, HPTS was used as the fluorescent indicator in a displacement assay for the sensing of polyanions. The anion binding interactions of receptor **767** were studied through fluorescence titration in a 3-(N-morpholino)propanesulfonic acid (MOPS) buffer at pH 7.5. This assay was found to be selective for ATP ($\log K = 2.87$) over ADP, AMP, NADP, NAD, pyrophosphate, citrate, succinate, and fumarate ($\log K = 0.03$ - 0.51). The observed selectivity was attributed to the increased charge of ATP relative to the other tested anions.

Receptor **768**, a functionalized cavitand reported by Diederich and Sebo, was also found to be an effective anion receptor.[771] For instance, ^{1}H NMR spectroscopic titrations revealed that receptor **768** interacted with a variety of nucleotides at pH 8.3 in D_2O. An increase in the binding affinity was observed with increasing anion charge (AMP < ADP < ATP). Furthermore, a slight selectivity for AMP ($K = 1.0 \times 10^4$ M^{-1}) over other nucleotide monophosphates was found. Analysis using both the NMR spectroscopic chemical shifts and molecular modeling led to the conclusion that ion pairing and hydrogen bonding interactions were largely responsible for the observed binding. Selectivity was attributed to hydrophobic interactions such as inclusion of the nucleotide base within the receptor cavity.

The fluorescent cavitand **769** was investigated later by Yoon, Lee, and co-workers.[772] In this case, relatively minor fluorescence changes were observed in the presence of pyrophosphate and dihydrogenphosphate in 40% aqueous DMSO. However, significant fluorescence quenching was observed upon the addition of GTP ($K = 7.38 \times 10^4$ M^{-1}). Much lower binding affinities were observed for CTP and ATP. Phosphorous NMR spectroscopic studies led to the suggestion that the receptor interacts most strongly with the phosphate sites of the nucleotide.

In another study involving the use of a modified calixarene core, Lhoták and co-workers designed the ureido-thiacalixarenes **770** and **771**.[773] In this case, ^{1}H NMR spectroscopic studies carried out in a chloroform : acetonitrile mixture (4 : 1) revealed hydrogen bonding interactions between various TBA anion salts (e.g., chloride, bromide, iodide, cyanide, benzoate, nitrate, dihydrogenphosphate, and hydrogensulfate) and the urea moieties. Stronger interactions were observed with the bis-urea receptor **771** than receptor **770**. However, in neither case was significant selectivity observed for dihydrogenphosphate, although this analyte did display an association constant of 1.5×10^4 M^{-1} with receptor **770**. Attempts to measure the binding constants for receptor **771** were hampered by the formation of species with mixed anion:receptor stoichiometries.

Cyclodextrins (CyD's) are also species that contain cone-shaped cavities and surfaces that can be functionalized to promote selective anion binding behavior. As a general rule, the resulting scaffolds are water-soluble while still providing a hydrophobic inner cavity. As early as 1970, Hoffman and Bock studied the complexation of nucleotides by unsubstituted cyclodextrins through UV-Vis spectroscopy in a buffered aqueous solution (0.01 M Tris, 0.1 M NaCl, pH 7.6).[774] Under these conditions, significant spectral shifts were observed upon the addition of a variety of adenine derivatives as well as inosine monophosphate (IMP) to cycloheptaamylose (β-CyD). Little change was observed upon the addition of these guests to cyclohexaamylose (α-CyD) or upon the addition of CMP, GMP, UMP, or 2′-deoxy-5′-thymidine monophosphate (dTMP) to either CyD. Spectral changes of adenine derivatives with β-CyD increased in the order adenine < adenosine < 5′-ADP < 5′-ATP < 5′-AMP. Among a number of adenosine monophosphates (3′-AMP, 5′-AMP, 2′,3′-cAMP, 2′-AMP), a significantly stronger signal was observed upon addition of 3′-AMP. An equilibrium constant of 22 M^{-1} was measured for the 3′-AMP:β-CyD complex. Formoso reported similar binding trends with β-CyD through the use of circular dichroism (CD) spectroscopy in a sodium phosphate buffered solution at pH 7.[775,776] Using this method, a 1:1 binding constant of 41 M-^{1} was measured for the complex of β-CyD and 5′-AMP at 25°C. Studies at various temperatures allowed for the calculation of thermodynamic parameters. Interestingly, the measurement of a favorable enthalpy (ΔH° = -4.9 kcal/mol) and an unfavorable entropy (ΔS° = -8.7 e.u.) was inconsistent with the dominance of hydrophobic host:guest interactions, which would be expected to display favorable entropy and opposing or negligible enthalpy.

In 1979, Knowles and co-workers functionalized *O*-methylated α-CyD with ammonium groups (**772**) and demonstrated the selective binding of benzylphosphate in aqueous media.[777] Binding affinities were measured through the competitive displacement of nitrophenol as monitored by UV-Vis spectroscopy. A dissociation constant of 3.1×10^{-2} M was reported for benzylphosphate. It was suggested that the benzyl group binds inside the cavity while the ammonium groups, all charged at pH 7, would interact with the phosphate oxygen atoms. The proposed binding model was supported by the observation of strong selectivity for benzylphosphate over benzyl alcohol and inorganic phosphate. Furthermore, substantially lower binding affinities were observed with a monoammonium derivative analogous to **772** (i.e., **773**). While the binding behavior of receptor **772** and benzylphosphate at pH 5.5 and 9.5 was also analyzed, the strongest binding interaction between these two compounds was observed at pH 7.

Schneider and Eliseev later investigated the binding of amino-β-CyD's **774** and **775** with a variety of nucleotides.[778-780] In this case, ^{1}H NMR spectroscopic experiments carried out in water at pD 6.0 revealed binding interactions with inorganic phosphate, ATP, ribophosphates, and a variety of nucleotide monophosphates. The strongest binding behavior, as predicted based on electrostatic interactions, was observed between receptor **775** and ATP ($K = 3.24 \times 10^6$ M^{-1}). Chemical shift analysis and 2-D NMR spectroscopic

experiments led to the suggestion that the nucleobase of AMP was partially included within the CyD cavity along with the ribose unit. The ribose unit was also proposed to participate in hydrogen bonding interactions with the CyD. These interactions, along with the approximately 4 salt bridges that were expected to be formed with the ammonium groups of this receptor, were suggested to be largely responsible for the observed binding affinity.

Thatcher and co-workers examined the binding of a variety of 4-isopropylphenylphosphate esters (**776**) to the ammonium-functionalized CyD's **777a, 777b**, and **778a** using UV-Vis spectroscopy.[781] In the case of compound **776d**, ^{31}P NMR spectroscopy was used. A number of trends were observed in aqueous media (pH 7.3, Tris buffer). For instance, when compared to unsubstituted β-CyD, receptor **777a** displayed a significantly higher binding affinity only for dianions **776c** and **776d**. As this increase was not observed for various monoanions, these researchers suggested that the interactions of monoanions **776a** and **776b** remained largely hydrophobic in nature. In addition, a lower affinity for the binding of the phenylphosphate derivatives **776a-c** to receptor **777b** relative to the larger receptor **777a** was observed. Proton NMR spectroscopic studies confirmed the inclusion of the phenyl ring within the CyD cavity of both receptors. Interestingly, the free energy of the complexation proved to be 1-1.5 kcal/mol weaker for both receptors than what would be expected based on a combination of the presumed hydrophobic and electrostatic interactions. This disparity was attributed to the exclusion of the propyl group of the guest into the aqueous media on the other side of the ring as a result of the strong electrostatic interactions between the phosphate and ammonium groups. Molecular modeling and a slow observed dissociation rate supported this conclusion.

Darcy and co-workers studied the binding behavior of the CyD derivative **778a** with a variety of phosphates via potentiometric titrations and ^{1}H NMR spectroscopic measurements.[782] Log association constants as high as 9.57 were measured with receptor **778a** and ATP. A comparison of equally charged anions revealed that stronger complexes were formed with guests containing a hydrophobic moiety for cavity inclusion (AMP and *p*-nitrophenylphosphate) than those that lacked such a feature (e.g., ribose-5-phosphate). Proton NMR spectroscopic experiments supported this conclusion and led to the suggestion that the ribose unit was not included within the cavity unless it was part of a cavity-bound nucleotide.

Seto and co-workers reported that receptor **778a** inhibited phosphatase activity by binding arylphosphate esters.[783] In later studies, these researchers analyzed the effect of buffers on the binding behavior of both receptors **778a** and **778b** in aqueous media at pH 7.0 using both ^{1}H and ^{31}P NMR spectroscopy.[784] Negatively charged buffers were found to compete with arylphosphate esters by binding to the receptors, a phenomenon that reduced the apparent association constant. On the other hand, positively charged buffers served to enhance the binding affinity, sometimes up to 4 orders of magnitude over negatively charged buffers. Similar association constants were observed with both receptors **778a** and **778b** for *p*-nitrophenylphosphate and phosphotyrosine. In contrast, an appreciable difference was observed with N-acetylphosphotyrosine methyl ester. This ester was found to be bound to receptor **778a** with an association constant of 6.1×10^5 M^{-1} and to receptor **778b** with an association constant of 1.5×10^5 M^{-1} in Tris buffer. This difference in affinities was attributed to the larger size of the N-acetylphosphotyrosine methyl ester anion, which makes it a better match for the larger cavity in CyD **778a**. Dynamic NMR spectroscopic experiments revealed that the buffer identity had a strong effect on the on rate, leading to complex formation but little effect on the corresponding dissociation rate.

Smith and co-workers later used β-CyD's as scaffolds to probe the thermodynamics associated with the binding of aryl phosphate monoesters to receptors bearing guanidinium

(**779b, 780b**), as opposed to ammonium (**779a, 780a**), substituents.[785,786] The thermodynamic parameters were determined in water at pH 7.00 using ITC. Free energies of association of approximately 3-4 kcal/mol were generally observed but significant differences were observed in the enthalpic and entropic contributions. Ammonium compounds were observed to be characterized by greater entropic contributions, while enthalpic contributions were more important in the case of the guanidinium species. This led to the conclusion that guanidiniums provided stronger interactions but only when properly oriented.

Binding events were also probed in the case of phosphotyrosine using indicator displacement assays and fluorescence titrations in aqueous media at pH 7. These studies revealed that monosubstituted β-CyD derivative **781** did not bind phosphotyrosine. However, the more highly substituted CyD's **780a** and **780b**, were found to bind phosphotyrosine with binding affinities of 350 M^{-1} and 310 M^{-1}, respectively. Inclusion of the aromatic ring within the macrocyclic core of these latter two receptors was inferred based on an observed increase in the quantum yield of phosphotyrosine in the presence of receptor **780a** or **780b**.

Yuan, Fujita, and co-workers recently reported that ATP is complexed well by the guanidinium substituted receptor **782**.[787] For instance, at a pD of 5 in D_2O, the formation of the ATP:**782** complex was found to be irreversible under conditions of NMR spectroscopic analysis. This irreversibility prevented the determination of an association constant. Proton and ^{31}P NMR spectroscopic analysis, however, did lead to the conclusion that the ATP was most likely bound through all three phosphate groups, as well as via partial inclusion of the adenine moiety within the core. Unfortunately, ITC analyses of the interaction between ATP and receptor **782** were complicated by the formation of other weak complexes. Nevertheless, these researchers suggested that binding would be driven in part by a strong entropic driving force, which was thought to reflect the extensive desolvation that would be expected upon analyte binding. Weaker ATP binding was observed in the case of the amine receptor **777a**.

Calixpyrroles have also been investigated for their anion binding abilities. These species form complexes with Lewis basic anions in organic media. Anion complexation is believed to occur primarily through hydrogen bonding interactions between the anion and the pyrrolic NH units. Early efforts to develop calixpyrroles as anion receptors were spearheaded by Sessler and co-workers who synthesized a variety of derivatives in order to tune the selectivity of the basic calixpyrrole skeleton. In the original reports, the octamethyl calixpyrrole **783** was found to display a selectivity for fluoride anion (TBA salt) in dichloromethane ($K > 10^4$ M^{-1}) as judged from ^1H NMR spectroscopic titrations. A relatively low binding affinity for dihydrogenphosphate ($K = 97$ M^{-1}) was measured in this solvent under analogous conditions.[788] Bromide substitution at the pyrrole β positions (**784a**) increased the binding affinity to 6.5×10^2 M^{-1} but did not alter the previously observed selectivity.[789] The binding affinity of dihydrogenphosphate in acetonitrile (0.5% water) was found to be slightly higher for receptor **783** ($K = 1.3 \times 10^3$ M^{-1}) than that observed in dichloromethane.[465] Under the same conditions, an increase in the binding affinity was observed for the octafluoro-substituted system **784b** ($K = 9.1 \times 10^3$ M^{-1}). However, the use of different solvents precluded a direct comparison of the binding behavior of receptors **784a** and **784b**.

The Sessler group also examined the binding behavior of various fluorinated calix[n]pyrroles (**784b** and **785**) by means of ITC analyses carried out in DMSO with TBA anion salts. In general, higher binding affinities for phosphate anions (1.7×10^4 M^{-1} for **784b**, 9.6×10^3 M^{-1} for **785a**, and 1.5×10^4 M^{-1} for **785b**) were found relative to what was seen for the unsubstituted receptor **783** (5.1×10^3 M^{-1}).[790] Substitution with aryl groups at

the *meso* positions (**786**), on the other hand, did not increase binding affinities or change the selectivity relative to what was seen for the parent calix[4]pyrrole receptor **783**.[791,792]

The anion affinity of calixpyrrole **783** was also investigated by Schmidtchen and co-workers using ITC.[793] Although both the counter cation (e.g., K^+-cryptand *vs.* TBA) and solvent (CH_3CN *vs.* CD_2Cl_2 in the original study) were changed, several differences in the anion binding behavior were noted relative to what was reported originally by Sessler and co-workers based on NMR spectroscopic studies. For example, no preference in selectivity for fluoride over chloride was observed, and the binding affinities for Cl^- and $H_2PO_4^-$ under these disparate conditions proved higher than recorded previously. Specifically, a binding constant of 1.5×10^4 M^{-1} was determined with dihydrogenphosphate by Schmidtchen using ITC. Quite recently, a partial reconciliation of these results was made, with the conclusion being drawn that non-first order effects, including solvation, ion pairing, and solvent dependence are more important than originally assumed.[331,794,795]

The use of receptor **783** to create a rudimentary fluoride anion sensor using a displacement assay with *p*-nitrophenolate and UV-Vis readout was reported by Gale, Sessler, and Twyman.[796] Recently, Machado and co-workers exploited calix[4]pyrrole in displacement assays for naked-eye anion detection with Brooker's merocyanine dye in acetonitrile.[797] The dye solution, which is violet in color, becomes orange upon the addition of receptor **783**. Using this approach, fluoride, and to lesser extent chloride and dihydrogenphosphate, could be visually detected via a reversal of this color change (anions added as the TBA salts).

In work that was seminal in its time, Miyaji, et al. exploited calixpyrrole to create the first naked-eye detectable chemosensor for anions. This was done by linking anthraquinone chromophores to a calixpyrrole core to create receptors **787a** and **787b**. These systems permitted the visual detection of fluoride, chloride and dihydrogenphosphate in dichloromethane.[798] Receptor **787c** was observed to bind anions by ^1H NMR spectroscopy but did not undergo a colorimetric change upon the addition of anions. An alternate substitution with anthracene at the β positions (**788**) allowed sensing through anion-induced fluorescence quenching.[799] Interestingly, a slight selectivity for dihydrogenphosphate over chloride was observed in acetonitrile for receptors **788**. Binding affinities were found to be stronger than those previously observed (log $K = 4.20$ and 3.56 for **788a** and **788b**, respectively). The higher binding constant for receptor **788a** was attributed to electronic communication between the substituent and the pyrrole nitrogen. The fluoride anion was found to be bound with the greatest affinity among the test set of anions explored, whereas no binding behavior was observed for hydrogensulfate or bromide (all anions studied as their TBA salts).

In an effort to improve the phosphate anion selectivity, fluorophores were attached through sulfonamide and thiourea groups; this led to receptors **789**.[800] It was expected that the additional hydrogen bonding sites would also participate in anion binding interactions. Indeed, support for this model was obtained through ^1H NMR spectroscopic analyses. The additional binding interactions led to selectivity for dihydrogenphosphate and pyrophosphate over chloride in acetonitrile/water mixtures. Selectivity over fluoride was not observed. The presence of water was found to be necessary to achieve appreciable anion binding, presumably as a consequence of preventing receptor aggregation. In these studies, 0.01% water was used for the studies involving receptors **789a** and **789b**, and 4% water was used for those involving receptor **789c**. At this latter concentration of water, receptor **789c** was found to bind pyrophosphate with a binding constant of $\geq 10^6$ M^{-1} as inferred from fluorescence quenching experiments. This receptor was able to operate as a fluorescent sensor in acetonitrile solutions containing up to 20% water. These studies used TBA anion salts.

Anion detection by supramolecular self-assembly was explored by Shao and co-workers in the context of calixpyrroles.[801] The addition of any one of the studied receptors (**783, 790-792**) to a solution of chloranil in chloroform led to the formation of a supramolecular structure with charge-transfer character, resulting in a blue colored solution. The resulting systems could then be employed in naked-eye detection of anions, with addition of TBA fluoride and TBA dihydrogenphosphate producing a visual and reversible blue-to-orange color change. UV-Vis spectroscopic studies revealed that the absorption spectrum of the receptor-chloranil-anion solutions were distinct from that of chloranil alone. This result led to the suggestion that the system was more complicated than a simple displacement assay.

Sessler, Lee and co-workers further developed a chromogenic calix[4]pyrrole system in which a dipyrrolylquinoxaline moiety was incorporated as a “strap” across the macrocycle core (**793**).[802] Significant changes in the absorption spectrum were observed upon the addition of various anions as their TBA salts in a solution of 97:3 CH_3CN:DMSO. Binding affinities increased with decreasing anion size (fluoride > chloride > bromide > acetate > dihydrogenphosphate. ITC experiments were in good agreement with the absorbance-based studies. Anion-dependent visual color changes were also observed. While dihydrogenphosphate displayed the lowest binding affinity ($K = 1.13 \times 10^3$ M^{-1}), addition of this anion displayed the largest visual color change. This large color change was attributed to a different binding mode for dihydrogenphosphate complexation relative to that of other anions.

Several other calixpyrrole derivatives were explored by Sessler and co-workers in the context of pursuing various applications of anion recognition. For instance, several functionalized calix[4]pyrrole derivatives were prepared and used to create the calixpyrrole modified silica gels **794b** and **795b**, which were employed in the separation of dihydrogenphosphate from a variety of other anions by HPLC.[803] ATP, ADP, and AMP could also be separated. It was suggested that the modest anion binding behavior of the parent calixpyrroles **794a** and **795a** facilitated the separation of anions. The low binding affinity was expected to lead to more rapid exchange as compared to a receptor with high affinity.

The membrane transport ability of receptor **783** was measured through incorporation into a PVC-based ISE.[804] For reference, the transport was compared to that of pyridine derivatives **796** and **797**. An ISE based on receptor **783** was found to be selective for inorganic phosphate over halides from pH 3.5-9.0. In addition, a low response to fluoride was measured. The selectivity trend observed for this ISE was thus the reverse of what was in organic media. This result was attributed to the high hydration energy, and therefore slow kinetics, of fluoride in such systems. ISE's incorporating receptors **796** and **797** also displayed selectivity for inorganic phosphate. The response was most intense at low pH, presumably due to protonation of the pyridine moieties. A modification of this system was developed by Shishkanova and co-workers.[805] In these studies, PVC membranes derived from receptor **783** were coated with a conducting polymer, polyaniline (PANI). These membranes demonstrated an improvement in anionic sensitivity, particularly towards highly hydrated guests, such as fluoride and dihydrogenphosphate. The improved response was attributed to an increase in the transfer of anions from the sample into the PVC matrix as a result of the conducting polymer.

Sessler and co-workers also analyzed the membrane transport of receptors **798** and **799** using both aqueous-organic-aqueous transport type systems and ISE's. In these receptors, the attached cytosine group was expected to provide nucleotide selectivity for GMP.[806] The proposed selectivity was based upon a combination of nucleobase pairing interactions and hydrogen bonding interactions at the pyrrole units. In the membrane studies, the expected

selectivity for GMP was observed for *meso*-linked receptor **799** but not for receptor **798**. The success of receptor **799** was attributed to the increased spacing and flexibility between the two recognition moieties of this receptor. When incorporated into ISE's, selectivity for CMP was observed, presumably due to the innate affinity of unsubstituted calixpyrrole **783** for CMP.

In another application-driven study, Sessler, Gale and co-workers reported that the ferrocene receptor **800** displayed an electrochemical response when exposed to anions (TBA salts) in acetonitrile/DMSO mixtures.[807] The strongest signal was observed for dihydrogenphosphate. Later studies by Radecka and co-workers in aqueous solution demonstrated that the anion recognition process was highly dependent on the nature of the matrix in which the receptor was immobilized.[808] When receptor **800** was incorporated into carbon paste electrodes, the largest shift was induced by dihydrogenphosphate at pH 4.0. ISE's containing this receptor in a liquid membrane displayed responses in line with the Hofmeister sensitivity sequence. As a result, the response for dihydrogenphosphate was actually the lowest among a series of other anions (bromide, chloride, and fluoride) at the same pH.

More elaborate analogues of receptor **800**, namely receptors **801**, were investigated by Cheng and co-workers.[809] These latter receptors featured an aminophenyl linkage between the calix[4]pyrrole and the ferrocene units. Electrochemical studies in acetonitrile demonstrated that addition of TBA dihydrogenphosphate resulted in the largest cathodic shift among the various anions tested (i.e. fluoride, chloride, bromide, acetate, and hydrogensulfate). On the other hand, based on ^{1}H NMR spectroscopic titrations carried out in CD_3CN, receptor **801** was observed to have a strong preference for the fluoride and acetate anions rather than dihydrogenphosphate. The disparity in these results was attributed to the fact that increased stabilizing interactions between the bound anion and the ferrocene subunits and phenyl groups to which these groups were connected were possible in the case of dihydrogenphosphate, as compared to fluoride and acetate. These enhanced interactions with dihydrogenphosphate were expected to give rise to a greater perturbation of the electrochemical signal.

Gale and co-workers have also investigated the anion binding behavior of various calixpyrrole derivatives. For example, receptor **802** containing a tribromo-pyrrole ring was found to have stronger affinity toward anions than receptor **783**.[810] In this case, ^{1}H NMR spectroscopic titrations carried out in dichloromethane led to the conclusion that the NH group of the tribromo-pyrrole interacted with the anions (TBA salts). This additional interaction (relative to that provided by **783**) was used to explain the observed selectivity towards carboxylate anions over most halide anions, as well as over hydrogensulfate and dihydrogenphosphate. Addition of fluoride led to peak broadening in the ^{1}H NMR spectrum, precluding the determination of a binding constant.

Recently, Anzenbacher and co-workers developed various anion receptors based on modified versions of octamethylcalix[4]pyrrole. These systems included receptors **803**, which contain electron-withdrawing dye moieties and which were designed to permit the naked-eye detection of fluoride, acetate, pyrophosphate and dihydrogenphosphate.[811] UV-Vis studies carried out in DMSO / 0.5% water, revealed a higher anion affinity for receptors **803** than receptor **783**. As a class, receptors **803** were found to bind fluoride, acetate, and pyrophosphate more strongly than chloride and dihydrogenphosphate (all anions studied as TBA salts). For example, association constants with receptor **803a** were found to be 5.8×10^5 M^{-1} for pyrophosphate and 5.2×10^3 M^{-1} for dihydrogenphosphate in this solvent mixture. These studies were expanded to include various 1,3-indane-based calixpyrroles (**804**).[812] A change in the color of the receptor solution was observed upon the addition of

fluoride, acetate and, to a lesser extent, dihydrogenphosphate. Additional UV-Vis experiments revealed similar binding trends as those observed with receptor **803**. An association constant for receptor **804d** with dihydrogenphosphate of 1.6×10^5 M^{-1} was determined in DMSO / 0.5% water.

Danil de Namor and co-workers have carried out thermodynamic studies involving several calix[4]pyrrole compounds and various phosphate anion guests. In preliminary ^{1}H NMR spectroscopic studies, these researchers reported a 1:1 binding stoichiometry between receptor **783** and dihydrogenphosphate or hydrogenpyrophosphate (TBA salts) in both acetonitrile and DMF.[813] Conductance measurements confirmed the stoichiometry of the dihydrogenphosphate complexes in acetonitrile. Hydrogenpyrophosphate, however, displayed a 2:1 host:guest binding stoichiometry in acetonitrile, as inferred from analogous studies. Extraction experiments involving a partitioning between water-DCM with receptor **783** and hydrogenpyrophosphate provided support for the proposed 2:1 host:guest binding stoichiometry. On the basis of separate ITC measurements, an enthalpy-entropy compensation effect was inferred to be operative upon changing the solvent from acetonitrile to DMF. Thus, these studies are broadly supportive of those of Sessler, Schmidtchen, and Gale that led to the conclusion that the binding behavior of calix[4]pyrrole **783** is strongly dependent on the conditions, including choice of solvent.

Danil de Namor and co-workers also analyzed the anion binding interactions of the isomeric receptors **805**.[814] Interestingly, a 1:2 host:guest binding stoichiometry was observed for these receptors in the case of dihydrogenphosphate (TBA salt) in acetonitrile. Isomer **805b** displayed a higher overall affinity for dihydrogenphosphate (log K = 9.46) than receptor **805a** (log K= 6.1), as inferred from ^{1}H NMR spectroscopic studies. On the other hand, fluoride displayed a 1:1 stoichiometry with receptor **805a** (log K = 3.08) but a 1:2 stoichiometry with receptor **805b** (log K = 9.72) in acetonitrile.

These researchers also studied receptor **806**. In general, this latter system gave rise to weaker anion interactions than receptor **783**.[815] The influence of the medium was also examined. While **806** displayed a preference for the fluoride anion in acetonitrile, in DMF dihydrogenphosphate was bound more strongly than F^- (log K = 4.81 *vs.* 4.23, respectively). This change in selectivity was attributed to solvation effects. All anions were studied as the TBA salts.

The effect of altering the calixarene core was analyzed by the Danil de Namor group by replacing one of the pyrrole units with a thiophene unit (**807**).[816] This receptor was found to form a 2:1 (host:anion) complex with dihydrogenphosphate (TBA salt) in acetonitrile. Different binding behavior, however, was observed using propylene carbonate (PC) as the solvent. In this case, a 1:1 binding stoichiometry was observed. The change in stoichiometry was attributed to enhanced solvation of the dihydrogenphosphate anion in PC. A log K value of 4.22 was measured through ITC studies.

A modified sulphonium calixpyrrole **808** was reported by Schmidtchen and co-workers.[817] This system was used to examine the effect of installing positive charges at the *meso*-positions of a calix[4]pyrrole core. Higher anion affinities were observed with this receptor, presumably due to a combination of hydrogen bonds and electrostatic effects. ITC titrations carried out in DMSO yielded a binding constant for the first interaction with dihydrogenphosphate (TBA salt) of 3.3×10^5 M^{-1}. A second binding constant, attributed to non-specific electrostatic interactions, was reported to be 6.5×10^3 M^{-1}. In addition, receptor **808** displayed a strong preference for dianions, such as 1,3-benzenedicarboxylate, over monoanions, such as benzoate (triethylammonium salts).

In a different modification of the core, Sessler and co-workers examined the anion binding ability of calix[4]pyrrole[2]carbazole receptor **809**.[818] It was proposed that replacing two of the four bridging methylene units of calixpyrrole **783** with carbazole groups would enhance ion affinity through increased hydrogen bonding interactions and through expansion of the macrocycle core. In addition, the carbazole unit was expected to serve as a fluorescence signaling moiety. Emission studies in dichloromethane led to the detection of strong 1:1 complexes of receptor **809** with acetate, benzoate, pyrophosphate, and dihydrogenphosphate. Significantly lower affinities were observed for the complexation of oxalate, succinate, and chloride while no appreciable binding was observed for the bromide, nitrate, and hydrogensulfate anions. All anions were studied as the TBA salts. Binding constants for dihydrogenphosphate and pyrophosphate were recorded at 7.2×10^4 M^{-1} and 6.4×10^4 M^{-1}, respectively; however, a preference was observed for carboxylate anions.

In 2005, Sessler and Lee prepared the expanded bipyrrole-containing calixpyrroles **810**.[819] In this case, ^{1}H NMR spectroscopic titrations carried out in acetonitrile revealed binding constants for dihydrogenphosphate (TBA salt) that varied considerably. For instance, a $K >$ 240 M^{-1} was recorded for receptor **810a**, and a $K > 10^4$ M^{-1} was found for receptor **810b**. These differences in binding affinity were attributed in large measure to the different macrocycle geometries resulting from the greater bipyrrole-bipyrrole distance caused by the thiophene linker in **810** relative to **810a**. The highest affinity, however, was observed with carboxylate anions.

Sessler and co-workers have also explored the behavior of calix[n]bispyrrolylbenzenes (**811**) as anion receptors.[820] While these receptors exhibited a preference for halide anions, association constants for dihydrogenphosphate were determined by ^{1}H NMR spectroscopic methods and found to be 6.3×10^3 M^{-1} and 1.7×10^3 M^{-1} in dichloromethane-d_2 for **811a** and **811b**, respectively (all anions studied as their TBA salts). The improved affinities as compared to receptor **783** ($K = 97$ M^{-1} in CD_2Cl_2, see above) were attributed to the increased donor ability of the bispyrrolylbenzene subunit in **811** as compared to the dimethyldipyrromethane moiety present in **783**. Geometrical effects may also play a role in accounting for these observed differences in affinity.

The anion binding behavior of *N*-confused calix[4]pyrroles (NCCPs) has also been explored. In these calixpyrrole isomers, the inverted nature of one of the pyrroles precludes formation of an ideal cone conformation.[821] On the other hand, these species do bind anions. The binding of negatively charged analytes generally occurs as the result of interactions involving the β-CH of the inverted pyrrole group as well as the three N-H's of the non-inverted pyrroles. An extensive study of N-confused calix[4]pyrroles (**812**) as anion hosts was conducted by Anzenbacher and co-workers.[822] NMR spectroscopic titrations carried out in DMSO-d_6 with TBA anion salts led to the conclusion that receptor **812** bound spherical anions more weakly than calixpyrrole **783**. At the same time, the chromogenic receptors **812b** and **812c** displayed a high inherent affinity for hydrogenpyrophosphate (5.6×10^3 M^{-1} for receptor **812b**) and dihydrogenphosphate (8.1×10^5 M^{-1} for receptor **812c**), as inferred from UV-Vis titrations. A preference for acetate was also observed for both receptors.

Other examples of *N*-confused calix[4]pyrroles (e.g., **813**) were reported by Dehaen and co-workers.[823] UV-Vis studies of receptors **813**, which include a chromophoric azo group, were conducted in acetonitrile with TBA anion salts. No clear trend in binding affinity was observed among the different substituents, with log β values ranging from 3.32 to 3.87 for dihydrogenphosphate. The most stable dihydrogenphosphate complex was found with receptor **813e** (log β = 3.87). When similar studies were performed in dichloromethane, however, the substituents were observed to influence the measured binding affinities. The binding affinity increased as a function of the electron-withdrawing nature of the azo group

(log β = 3.10 for receptor **813d** and log β = 4.45 for receptor **813e**). In a further extension of this theme, Dehaen and Gale reported the synthesis and binding behavior of receptors **814**.[824] Here, ^{1}H NMR spectroscopic titrations carried out in DMSO-d_6 / 0.5% water with TBA salts led to the suggestion that receptors **814** are selective for carboxylate anions. However, a relatively low affinity for dihydrogenphosphate (38-197 M^{-1}) was also observed. This selectivity was rationalized in terms of the basicity of the analytes.

Several phosphate receptors based on porphyrin macrocycles have also been developed.[825] While free base porphyrins are generally poor anion receptors, the incorporation of peripheral substituents can be used to improve their anion recognition capacity and, in many cases, allowed for the selective complexation of anionic guests. For example, porphyrin **815a**, prepared by Aoyama and Ogoshi, contained protonated pyridyl substituents as well as a protonated porphyrin core.[826] It was predicted that this receptor would interact with anions through a combination of hydrogen bonds and electrostatic interactions. Indeed, discrimination between monoalkylphosphate and dialkylphosphate anions was observed via ^{1}H and ^{31}P NMR spectroscopic analyses carried out in chloroform. The phenyl-substituted porphyrin **815b** displayed a lower anion affinity, presumably due to the loss of the pyridinium-anion interactions.

Král and Schmidtchen investigated a different charged receptor unit, namely the tetraguanidinium porphyrin **816**.[827] These compounds were found to form ordered aggregates in water. Circular dichroism (CD) studies led to the conclusion that receptor **816** bound dihydrogenphosphate, which disturbed the aggregates. The largest spectroscopic change was observed for acetate. Král and co-workers also prepared the tetrabrucin-porphyrin **817**.[828] UV-Vis titrations, carried out in MeOH/HEPES buffer (1:1), revealed that receptor **817** interacts more strongly with ATP ($K = 6.4 \times 10^{4}$ M^{-1}) than with ADP or AMP. The binding interactions were predicted to originate from electrostatic interactions between the ammonium groups and the phosphate moieties, as well as π-surface interactions involving the aromatic elements of both compounds.

The neutral β-functionalized porphyrin **818** with an appended disulfonamine chromophore was developed by Starnes and co-workers.[829] In this case, UV-Vis-based binding studies carried out in dichloromethane with TBA anion salts led to the conclusion that the anion selectivity of receptor **818** correlated with the basicity of the analytes. The strongest interactions were observed for fluoride, followed by dihydrogenphosphate ($K = 7 \times 10^{-4}$ M^{-1}).

Another strategy pursued by several groups has involved the incorporation of metal into the porphyrin structure. The presence of a metal center allows the resulting porphyrin complexes to act as anion sensors via axial ligation. In early work by Pasternack, Gibbs, and Gaudemer, the interaction of pyridyl porphyrin **819** and its metal complexes with nucleosides and nucleotides was reported.[830] In this case, ^{1}H NMR spectroscopic studies carried out in neutral aqueous solution provided support for the notion that more stable complexes are formed with purine nucleotides than the corresponding pyrimidine derivatives. Binding constants were determined to be on the order of 10^{3} M^{-1}. Detailed spectrophotometric experiments established that only metal complexes with zero or one axial ligand (such as the Cu(II) or Zn(II) complexes) interacted appreciably with the nucleotides. These results led to the suggestion that an open axial position was necessary to obtain a measurable binding affinity. Indeed, species with two axial ligands (such as the complexes Mn(III)) displayed no spectroscopic changes upon nucleotide addition.

The cobalt(III) porphyrin complex **820** was prepared by Jyo and co-workers.[831] This metalloporphyrin was then incorporated into the membrane of an ion-selective electrode.

While it was demonstrated that receptor **820** interacts with dihydrogenphosphate, the highest affinity was observed with SCN^-. Later, Kuroda and Ogoshi studied the binding behavior of porphyrin **821**, a Rh(III) complex that bears two ammonium "tails" off the macroyclic periphery.[832] In this case, UV-Vis studies carried out in aqueous solution demonstrated that receptor **821** binds most nucleotides well (i.e., K(dAMP)) = 860 M^{-1}), except for UMP. Further studies involved analysis of dicationic metal porphyrin **822**.[833] This receptor was used to extract AMP from aqueous solution into a chloroform phase. Under these conditions, receptor **822** displayed selectivity for AMP over other nucleotide monophosphates, such as UMP, GMP, and CMP.

A tetracationic cobaltocenium porphyrin receptor (**823**) was prepared by Beer and co-workers.[834] This system, which relies on peripheral metal substitution, was found to interact with anionic guests (TBA salts) in acetonitrile. Both hydrogen bonds with the amide groups and electrostatic interactions with the positively charged cobalt centers were thought to account for the observed binding. As such, an electrochemical response was expected in the presence of anions. In fact, as determined via CV analyses, the largest cathodic shift was seen upon the addition of dihydrogenphosphate. Other amide systems developed by Beer and co-workers are the zinc porphyrins **824a** and **824b**.[835] UV-Vis titrations revealed that both receptors displayed a preference for tetrahedral anions (TBA salts). Receptor **824b** displayed selectivity for hydrogensulfate over dihydrogenphosphate (log K = 4.8) in DMSO. In contrast, dihydrogenphosphate (log K = 5.4) proved to be the anion bound by receptor **824a** in acetonitrile. Square-wave and cyclic voltammetry analyses provided further support for these conclusions. Notably, receptor **824a** displayed the most dramatic cathodic shift in the presence of dihydrogenphosphate relative to hydrogensulfate, chloride, nitrate, and perchlorate in acetonitrile. Beer and co-workers also analyzed the binding behavior of other amide-functionalized metalloporphyrins of general structure **825**.[836] In this case, UV-Vis titrations in different solvents with TBA anion salts led to the conclusion that the identity of the metal influenced both the affinity and the selectivity of the anion recognition process. For instance, in DMSO receptors **825f** and **825g** were found to interact strongly with dihydrogenphosphate, displaying log K values of 4.4 and 3.6, respectively. In general, halide anions were found to interact more strongly than dihydrogenphosphate with these cadmium(II) and mercury(II) receptors. However, the other metal receptors did not bind any of the tested anions in this competitive solvent.

Metal and free-base complexes of the tetraurea porphyrins (**826**) were studied by Burns and Scheidt.[837] As inferred from ^{1}H NMR spectroscopic studies carried out in DMSO-d_6, these receptors display a strong preference for halide anions over dihydrogenphosphate. A binding constant of 1.4×10^3 M^{-1} was measured for the interaction of dihydrogenphosphate with receptor **826c**, while an association constant of greater than 10^5 M^{-1} was estimated for chloride in this solvent. In the case of dihydrogenphosphate, the binding affinity was found to increase when more electron-withdrawing substituents were present on the receptor. The metal complexes of receptor **826** displayed weaker anion binding affinity than the free base forms. In later studies, Burns reported that receptor **826c** interacts more strongly with dihydrogenphosphate than chloride in dichloromethane.[838,839] This result stands in contrast to what was observed in DMSO. On the other hand, in the case of the diurea receptor **827** similar studies revealed a higher selectivity for dihydrogenphosphate in both solvents. The halide preference found for receptor **826c** in DMSO was attributed to the presence of a single solvent molecule within the receptor-anion complex. It was proposed that this solvent molecule provided an extra charge-dipole interaction that helped stabilize the complex. Such ancillary solvent effects were not thought to operative in the case of dihydrogenphosphate and receptor **826c**, or across the board for the diurea receptor **827**. These findings were supported by single crystal X-ray diffraction analyses (**826c**:dihydrogenphosphate complex shown in Figure 10). All studies relied on the use of TBA anion salts.

Hong and co-workers later investigated the anion binding behavior of the free-base and zinc(II) complexes of the functionalized tetrabenzylurea porphyrins **828**.[840] Anion interactions were analyzed using UV-Vis and ^{1}H NMR spectroscopic titrations carried out in DMSO. Receptors **828a** and **828b**, which contain phenol groups as substituents, were found to display a preference for acetate and dihydrogenphosphate over other anions. The association constants corresponding to the binding of dihydrogenphosphate (TBA salt) to receptors **828a** and **828b** were reported to be 4.5×10^4 and 2.0×10^4 M^{-1}, respectively. The incorporation of colorimetric units, giving analogues **828c** and **828d**, permitted the naked-eye detection of anions. UV-Vis studies with receptors **828c** and **828d** revealed interactions with fluoride, acetate, and dihydrogenphosphate. The anion selectivity was found to correlate with basicity.

Recently, Li and co-workers prepared the double strapped porphyrin **829**.[841] While the free-base form (porphyrin **829a**) did not display significant anion binding affinities through UV-Vis titrations carried out in chloroform:DMSO (50:1), the corresponding zinc complex, **829b**, bound dihydrogenphosphate (TBA salt) under these conditions. A binding affinity of 8.43×10^5 M^{-1} for dihydrogenphosphate and **829b** was reported. A preference for this latter anion over other test anions, such as fluoride, acetate, chloride, bromide, iodide, nitrate, and perchlorate, was reported. This strong affinity and selectivity for $H_2PO_4^-$ was attributed to a combination of hydrogen bond interactions involving the urea groups and coordination to the Lewis acidic metal center.

Boronic acid substituted porphyrins of general structure **830** were introduced by Shinkai and co-workers as glucose phosphate receptors.[842,843] It was found that the zinc complex, receptor **830b**, could discriminate between glucose-1-phosphate (dipotassium salt) and glucose-6-phosphate (disodium salt) as inferred from ^{31}P NMR spectroscopic analyses carried out in DMSO-d_6. Proton NMR spectroscopic titrations led to measured binding constants of 250 M^{-1} and 1500 M^{-1} for glucose-1-phosphate and glucose-6-phophate, respectively. CD studies also supported this selectivity. For instance, a strong Cotton effect was observed upon the addition of glucose-6-phosphate to receptor **830b**. In contrast, a weak Cotton effect was observed upon the addition of glucose-1-phosphate. The stronger interaction with glucose-6-phosphate was attributed to a judicious combination of interactions between the boronic acid and the 1,2-diol and complexation of the phosphate group to the centrally bound zinc center. This study was expanded to include other analytes, such as 3,4-dihydroxyphenylalanine (DOPA) and its derivatives.

A large number of so-called expanded porphyrins have been reported to date. These larger analogues of porphyrins have often been found to bind anions in their protonated forms. Sapphyrins are the oldest and the best studied of the expanded porphyrins in terms of their phosphate recognition properties. In contrast to porphyrins, sapphyrins possess a large and basic inner cavity.[844,845] This cavity, when protonated, has been found to be well suited for interacting with phosphate anions. Sapphyrin and many of its derivatives are positively charged at neutral pH. Further, these macrocycles have been found to interact well with phosphate anions via a combination of electrostatic interactions and hydrogen bonds. This was established *inter alia* by X-ray diffraction analyses. For example, single crystal X-ray diffraction analysis revealed that receptor **837a** (discussed in detail below) formed 1:1 complexes with hydrogenphosphate and dihydrogenphosphate in the solid phase (Figure 11).[846] Interestingly, the oxygen atoms in dihydrogenphosphate were observed to be bound by five nitrogen-based hydrogen bonds, with N-O bond distances in the range of 2.8-2.9 Å. On the other hand, complexes of 2:1 guest:host stoichiometry were found with diphenylphosphate and monobasic phenylphosphate. With these anions, the molecule "above" and the molecule "below" the macrocyclic plane were held in place by three and two hydrogen bonds, respectively, with N-O bond lengths in the range of 2.8-3.0 Å. In

addition, the phenyl group of monobasic phenylphosphate was found to lie in the plane of the macrocycle, at distances (3.5-3.6 Å) in the range of van der Waals contact interactions.

Early studies by Sessler and co-workers demonstrated that sapphyrin **831** could serve as a carrier in the transport of nucleotides and nucleotide analogues using a U-tube membrane model.[847] This model consisted of two aqueous phases (Aq. 1 and Aq. 2) separated by an organic layer. Transport was observed when the protonated sapphyrin was dissolved in CH_2Cl_2 and GMP was dissolved in the first aqueous phase (Aq. 1) at pH 2.5. GMP was rather quickly thereafter observed to build up in Aq. 2. The attachment of cytosine units onto the periphery of this expanded porphyrin (receptor **832a**) allowed for the transport of nucleotides at, or near, neutral pH.[848] Receptor **832a** displayed selectivity for GMP over AMP and CMP. However, the incorporation of an additional cytosine unit (receptor **833a**) led to a loss in selectivity, perhaps as the result of permitting other, less selective, hydrogen bonding interactions. Further studies were carried out to confirm that the transport selectivity was indeed determined by the interaction with the complementary nucleobase.[849,850] For this purpose, receptors **832b** and **833b** bearing guanine units were prepared. As predicted, higher selectivities were found toward cytidine derivatives.

In a different study, sapphyrin **831** was included in a PVC liquid membrane and used to create an ion-selective electrode.[173] This ISE was found to give a response toward AMP that was slightly more intense than that for GMP at pH 6.6. This selectivity was attributed to increased π-surface interactions with the adenine base.

The anion recognition features of sapphyrins were further studied by Sessler and co-workers in the context of other possible applications. For example, the silica-bound sapphyrin **832c** was prepared and used as a solid support for chromatographs. It was found to allow the HPLC-based separation of monomeric and small oligomeric nucleotides at pH 7.[851] Separation was observed between mono-, di-, and triphosphates. Separation between nucleobases, however, was not achieved with this receptor-modified surface. By incorporating a cytosine group within the support system, producing functionalized **833c**, separation of nucleotide monophosphates could be achieved.[852] On the other hand, peak broadening was seen when this support was used for mixtures of di- and triphosphates. This broadening was attributed to kinetically slow associations and/or dissociations for these anions resulting from relatively enhanced binding affinities. Sapphyrin **832c** also interacted with inorganic anions, such as arsenate, phosphate, sulfate, nitrate, chloride, bromide, and iodide, as inferred from the fact that decreased elution times for AMP were observed in the presence of these anions.[853] The greatest inhibition was observed with arsenate and phosphate anions. On this basis, it was inferred that these tetrahedral anions interacted most strongly with sapphyrin under the conditions of the experiment (aqueous media, pH 6-8).

Sessler and co-workers also reported that oligosapphyrins **834-836** could serve as carriers for nucleotide di- and triphosphate anions at neutral pH.[854] Specifically, sapphyrin trimers **834** and **835** were found to be efficient carriers for nucleotide diphosphates. The tetramer **836** was reported to allow for the transport of all the phosphorylated species included in the study. However, it did display a preference for adenosine-derived nucleotides.

The phosphate binding behavior of more water-soluble sapphyrins has also been analyzed. For example, Sessler and co-workers examined interactions between sapphyrins **837a-b** and inorganic phosphate using NMR spectroscopy and UV-Vis analyses carried out in highly competitive media.[846,855] Association constants on the order of 10^4 M^{-1} were measured in methanol. Likewise, association constants on the order of 10^2 M^{-1} were recorded in 10 mM aqueous bis-Tris at pH 6.1. Sessler and co-workers also exploited the aggregation tendencies of certain sapphyrins, such as **838**, to effect phosphate detection in aqueous media.[856]

Interactions were analyzed by monitoring changes in the absorbance and fluorescence spectra as a function of phosphate anion concentration in aqueous media (25 mM PIPES buffer, pH = 7.0). It was found that phosphate helped stabilize the monomeric form of sapphyrins **838**. Since the monomeric form was far more fluorescent than various aggregated forms, phosphate addition led to an increase in fluorescence intensity. Effective association constants ranging from approximately 6 to 19 M^{-1} were reported. In later studies, Sessler and co-workers prepared receptors **837c-f** and **839**.[857] These water solubilized sapphyrins were found to act as catalysts for the hydrolysis of bis(4-nitrophenyl)phosphate (BNPP) near neutral pH. The interactions between receptor **837d** and BNPP were studied using UV-Vis spectroscopy. On the basis of these analyses, a binding constant of 400 M^{-1} was determined at pH 7.5 (0.01 M HEPES/0.1 M $NaNO_3$).

Charvatova and co-workers prepared capillaries coated with sapphyrin **837a** for use in open-tubular capillary electrochromatography (CE).[858,859] These capillaries were found to separate nucleotide monophosphates selectively as a function of the nucleobase. Furthermore, significant separation was observed between mono-, di-, and triphosphates. Moderate resolution was observed in the case of di- and triphosphates and was found to be correlated with the nature of the nucleobase. In the course of the study it was found that phosphoserine and phosphothreonine (added as the phenylthiohydantoin derivatives) could not be eluted readily from the column. Presumably, this reflects a strong interaction with the sapphyrin-coated capillary. These researchers suggested that the nature of the organic moiety present in the analytes had a significant effect on the nature and strength of the interactions with the solid phase.

Other expanded porphyrins have also been employed as phosphate receptors. For example, Sessler and co-workers analyzed the interactions between rubyrin **840**, a hexapyrrolic expanded porphyrin and several nucleotides. This was done using a triphasic U-tube transport system operating at neutral pH, in analogy to what was used in the case of sapphyrins.[860] Upon the addition of triisopropylsilyl-protected cytidine (C-Tips), the transport of GMP was found to be 30-fold faster in the case of rubyrin than with sapphyrin. This increase was attributed to the formation of a cooperative supramolecular structure. Separate from this, the binding behavior of *meso*-functionalized smaragdyrin **841** was analyzed by Chandrashekar and co-workers.[861] In this case, UV-Vis studies conducted in methanol led to the conclusion that receptor **841** bound AMP with a binding constant of 2.6×10^5 M^{-1}. However, the affinity for fluoride ($K = 7.6 \times 10^5$ M^{-1}) was found to be higher than that for AMP.

Expanded pyrrolic systems containing diamidodipyrromethane units were developed by Sessler and Ustynyuk and tested as anion receptors.[862] One of these, receptor **842**, displayed a preference for tetrahedral anions as inferred from UV-Vis titrations in acetonitrile (anions studied as TBA salts).[863] The strongest anion interaction was observed for dihydrogenphosphate. A 2:1 (guest:host) binding stoichiometry was inferred on the basis of Job plots and fits to the binding curves. These latter analyses allowed first and second association constants of 3.4×10^5 and 2.6×10^4 M^{-1}, respectively, to be calculated. Further studies demonstrated that relatively simple structural modifications could give rise to different anion binding selectivities. For example, receptor **843** was found to interact strongly with hydrogensulfate, ($K = 1.1 \times 10^5$ M^{-1}), while receptor **844** proved selective for chloride ($K = 1.2 \times 10^5$ M^{-1}) in acetonitrile. The association constants for dihydrogenphosphate were measured to be 2.9×10^4 and 1.5×10^4 M^{-1}, respectively, in this same solvent.

Following these studies, several additional macrocycles were prepared via anion templation. After removal of the anion, compounds **845-847** were obtained.[864,865] All three systems

were found to act as anion receptors, displaying selectivity toward dihydrogenphosphate and hydrogensulfate (TBA salts) as inferred from UV-Vis titrations carried out in acetonitrile. Receptor **847** displayed a 3:1 guest:host stoichiometry with dihydrogenphosphate, and the first binding constant was found to be log $K = 6.7$.

Recently, a new series of pyrrolic macrocycles with amido-imine functionalities, namely receptors **848-850**, was developed by Sessler and Katayev.[866] The seemingly minor changes within this set were reflected in demonstrably different anion binding properties. For example, in acetonitrile, receptor **848** was found to interact strongly with the hydrogensulfate anion (TBA salt). In contrast, the acetate anion was bound in preference over other anions by receptor **849**. Furthermore, the more rigid receptor **850** displayed a high affinity toward chloride. In general these receptors also displayed a strong affinity toward dihydrogenphosphate. Binding constants on the order of 10^5 M^{-1} were reported for this latter anion in acetonitrile. In related studies, the bipyrrole-derived macrocycles **851-854** were synthesized by Katayev and co-workers.[867] Competitive ^{1}H NMR spectroscopic titrations were performed in the presence of acetate in DMSO / 0.5% water. These experiments revealed that receptor **852** displayed selectivity toward dihydrogenphosphate (TBA salt). An association constant of greater than 10^4 M^{-1} was inferred from these studies.

Sessler and Furuta developed the first example of an expanded pyrrole macrocyclic containing dipyrrolylquinoxaline units.[868] This system, receptor **855**, displayed a high affinity toward fluoride and dihydrogenphosphate (TBA salts) in dichloromethane as determined from UV-Vis spectroscopic titrations. A log K of 3.8 was reported for the binding of dihydrogenphosphate. The presence of the quinoxaline subunits allowed for the naked-eye detection of both anions in this apolar solvent.

Perhaps the most complicated pyrrolic anion receptor reported to date is the bipyrrole-based [2]catenane **856** prepared by Sessler, Vögtle and co-workers.[869] In this case, ^{1}H NMR spectroscopic titrations carried out in 1,1,2,2-tetrachloroethane-d_2 confirmed that this multi-dimensional, catenated system interacts strongly with several anions (TBA salts). A high selectivity toward dihydrogenphosphate was reported. The binding constant for this anion was found to be greater than 10^7 M^{-1}. It was proposed that receptor **856** contained a tetrahedral cavity between the rings. This geometry would be expected to result in a strong and selective receptor for dihydrogenphosphate.

5. Conclusions

The goal of obtaining strong and selective binding agents for phosphate and phosphorylated compounds has attracted the attention of many research groups, each with a slightly different approach. This has resulted in what is now an extensive set of artificial receptors. While many of the determinants and underlying binding affinities and selectivities are common to all supramolecular interactions, several factors are particularly important in phosphate binding. For example, phosphate selectivity is often determined by the geometry of the receptor binding cavities. Further, the arrangement of various hydrogen bond donors and acceptors can be used to maximize interactions with the shape and polarity of this tetrahedral anion. These hydrogen bonding interactions play an important role in nearly all the successful binding systems reported in this review, including those wherein electrostatic interactions appear to dominate the binding affinity. As a general rule, receptors with C_{3v} symmetry appear to maximize these various stabilizing interactions, although favorable orientations have also been achieved through the use of well-designed macrocyclic and cleft receptors. While challenging to design and prepare, appropriately sized receptors with an ability to form inclusion complexes with phosphate anions have also proven to be highly effective.

Polyammonium-based compounds, with which this review began, bind anions mainly via electrostatic interactions. These systems provide some of the strongest phosphate interactions in aqueous media at neutral pH. These interactions depend strongly on the distribution of the charge within the receptor, both in terms of charge density and size/shape complementarity. Increasing the charge density of alkyl polyammonium receptors requires a careful balance of the spacing between the nitrogen atoms, the size of the macrocyclic ring and the methylation pattern. At the same time, hydrogen bonding interactions, even in the simplest polyammonium systems, can be critical contributors to the phosphate binding affinity. However, the overwhelming influence of electrostatic interactions in polyammonium systems can "swamp" the more subtle selectivities that would be expected to be provided by other recognition motifs.

Guanidinium receptors are also positively charged near neutral pH. As a general rule, these systems give rise to phosphate complexes that are less stable than those produced with polyammonium systems. Presumably, this reflects the lower charge density of the guanidinium group in water. The high pK_a of these units, however, allows guanidinium-based receptors to stabilize electrostatic interactions with phosphate anions over a much wider pH range than ammonium receptors. The use of guanidiniums can also allow for the placing of charged groups in much closer proximity to the targeted anions than do ammonium motifs. Binding studies via ITC are also simplified, as protonation and deprotonation events do not need to be accounted for in the data analysis. Perhaps the most attractive feature of guanidinium subunits is that these units stabilize complexes characterized by the presence of two parallel hydrogen bonds. This geometry generally imparts a strong preference for oxoanions over other anions of like charge. As a consequence, guanidinium units are found in some of the most successful phosphate receptors produced to date.

The large number of neutral receptors based on hydrogen bond donor subunits has allowed insight into the design criteria needed to achieve selective phosphate recognition. In general the most effective hydrogen bond donors for phosphate recognition are thiourea motifs that are both relatively acidic and which stabilize parallel hydrogen bonding geometries. With these and other motifs, factors that increase the effective acidity serve to increase the hydrogen bond donating ability. As a given rule, this enhances binding. However, a balance must be maintained in order to avoid deprotonation by the anion of interest. The use of receptors with multi-point binding moieties preorganized for interactions with tetrahedral anions can overcome the innate selectivity arising from differences in anion basicity, something that often favors fluoride and acetate over dihydrogenphosphate. This approach has been exploited successfully through the design of clefts that are too large for other competing species or inaccessible to spherical anions.

Another caveat that must be considered is that certain phosphate anions have a propensity to dimerize in less polar media. This can complicate binding studies carried out in organic solvents. On the other hand, this phenomenon may be exploited to produce highly selective receptors that target the dimeric form. The organic solubility of many neutral hydrogen-bond receptors has been used to create effective phosphate transport systems. At the same time, other applications may require the use of systems that function in aqueous environments.

The incorporation of metal cations into appropriately designed frameworks can give highly effective phosphate anion receptors. For example, metal complexes of polyammonium macrocycles were found to bind phosphate anions two to three-fold more strongly than their diprotonated counterparts. This is thought to reflect the presence of favorable Lewis acid-base interactions. Metal coordination has also been used to preorganize the surrounding ligand structure and to generate cavities optimized for phosphate binding. Separate from

this, combining metallic units with electrochemical or luminescent properties with other types of receptor motifs can provide for useful signaling. This approach can also be used to introduce ancillary electrostatic binding interactions. Metal coordination-based strategies also offer advantages for phosphate binding and sensing in aqueous media, including increased solubility of the receptor and a source of consistent Lewis acid-base interactions.

Finally, macrocyclic receptors, in particular pyrrolic receptors, have been extensively studied as phosphate anion receptors. In many cases, high anion binding constants were observed, which is ascribed to the inherent structural preorganization. In the case of sapphyrins, systems that are charged at neutral pH, significant selectivity for nucleotides was observed based in the case of systems bearing complementary nucleobase functionality. In the case of neutral, non-aromatic pyrrole macrocycles, several specific geometries were noted that gave rise to high phosphate anion selectivities. However, this optimization remains difficult to achieve and, in fact, low phosphate selectivities are generally observed with most simple pyrrolic receptors, including the easily synthesized calixpyrroles.

As noted throughout this review, considerable progress has been made in the area of phosphate and phosphorylated substrate recognition. On the other hand, there is clearly room for improvement. Although many receptors have been reported, few display appreciable selectivity for phosphate over other anions. Discrimination between phosphate guests has been even more rarely achieved. Achieving this selectivity in a routine, predictable manner is thus a clear challenge for the future. Another challenge is generating receptors, particularly neutral systems, that function well in aqueous media. While much has been learned through the study of binding interactions in organic media, applications involving biological phosphate targets will require the use of selective receptors whose function is not impaired when exposed to water. While the use of highly charged systems, such as those based on guanidinium subunits, is one way to address this problem, many of the most attractive applications, including those associated with through-membrane transport and selective phosphorylated substrate extraction, will likely require the use of neutral receptor systems or those of low charge density that function in the presence of water and salts. The need to produce such systems, as well as a desire to understand non-first order effects, such as ion pairing, aggregation, and solvation, underscores the benefits that could accrue from further work in the phosphate recognition area. It is hoped that by summarizing the current state-of-the-art, this review will help advance efforts in this all-important area and facilitate the development of receptors that can be exploited for use in various real-world applications.

Acknowledgments

The authors gratefully acknowledge the contributions of Ms. Taylor Beaver, Professor Marco Bonizzoni, Dr. Chance Rainwater, Mr. Vladimir Roznyatovskiy, Dr. Natalie Barkey, and Dr. Sung Ok Kang. Professor Sessler thanks the National Institutes of Health (GM 58907) and the Robert A. Welch Foundation (F-1018). Support under the WCU (World Class University) program (R32-2010-10217-0) is also acknowledged. Professor Anslyn thanks the NIH, NSF, and Welch Foundation for support over his 22 year career.

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Biographies

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Eric V. Anslyn received his Ph.D. in Chemistry from the California Institute of Technology in 1987 under the direction of Robert H. Grubbs. After two years at Columbia University as a National Science Foundation Post-Doctoral Fellow with Ronald Breslow, he joined the chemistry faculty at the University of Texas at Austin in 1989. He was named a Sloan Scholar, Dreyfus Teacher Scholar, Searle Scholar, and Presidential Young Investigator and then promoted to Associate Professor in 1994. In 1997 he was named Full Professor, and in 2004 he was awarded the Norman Hackerman Professorship in Chemistry and Biochemistry. He is also a 2005 Cope Scholar Awardee. In 2006 Professor Anslyn was elected as a fellow to the American Association for the Advancement of Science and in 2007 was inducted as an Honorary Professor at East China University of Science and Technology. Currently, he sits as an Associate Editor for the Journal of the American Chemical Society and serves on the editorial board of Supramolecular Chemistry. His research encompasses physical organic and bioorganic chemistry and generally explores the use of synthetic receptors for sensing and catalysis applications.

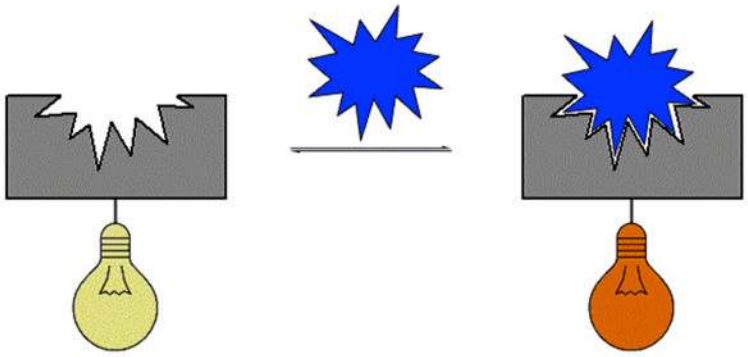


Figure 1.
Covalently attached reporter-receptor system. The reporter unit is represented by the light bulb graphic with the receptor in gray and analyte in blue.

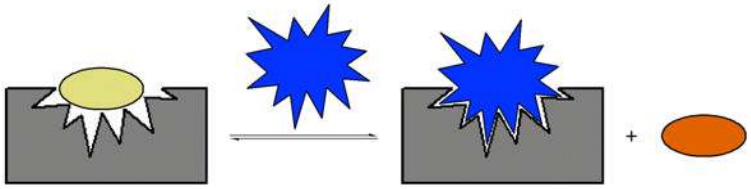


Figure 2.
Indicator displacement assay (IDA). The indicator is represented by the oval, while the receptor unit is gray and the analyte blue.

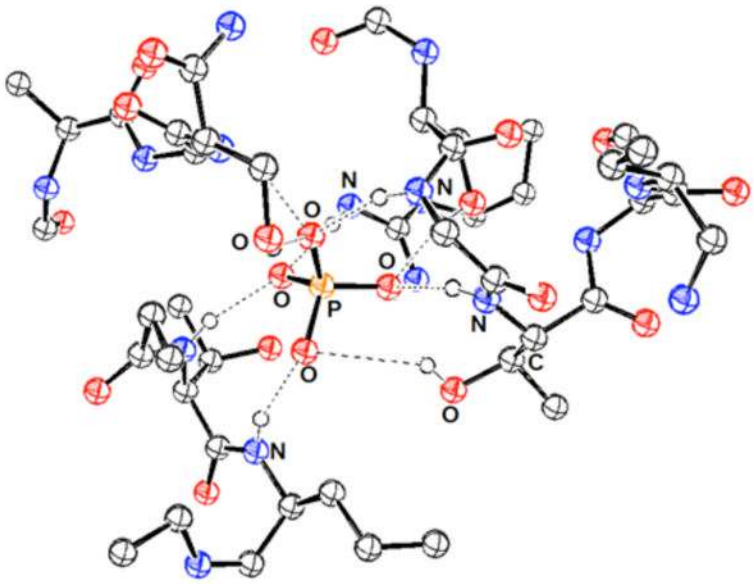


Figure 3.
View of the phosphate binding site of the phosphate binding protein. Drawing generated from X-ray diffraction data obtained from the RCSB Protein Data Bank and originally published in reference 115.

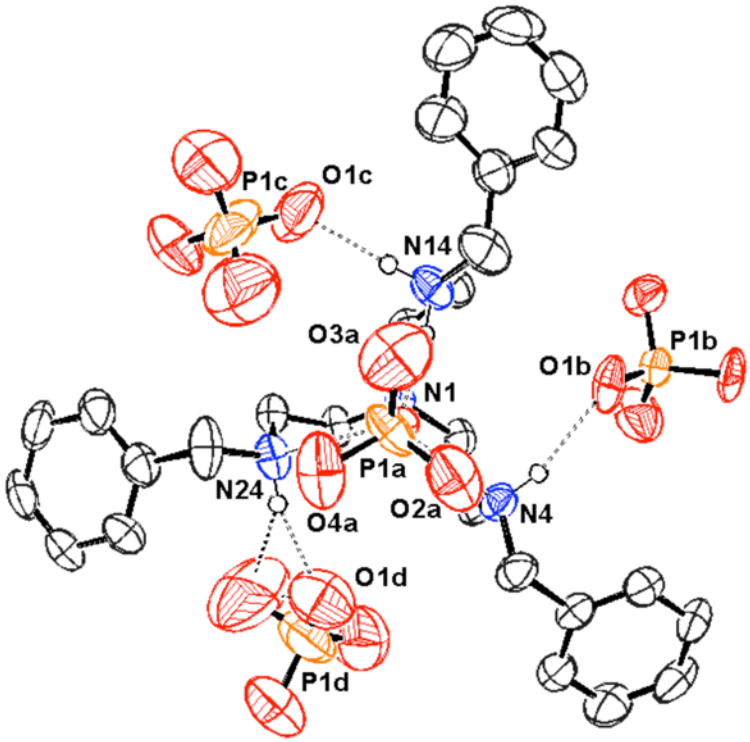


Figure 4.
View of the H_3(**77**):$(H_2PO_4)_3$•H_3PO_4 complex. Drawing generated from X-ray diffraction data originally published in reference 189. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

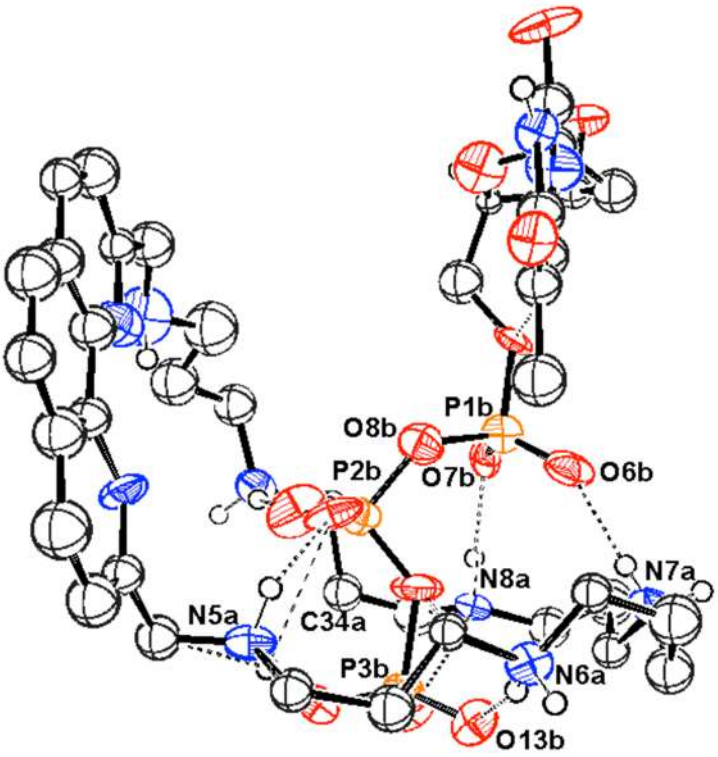


Figure 5.
View of the **100**:TTP complex. Drawing generated from X-ray diffraction data originally published in reference 209. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

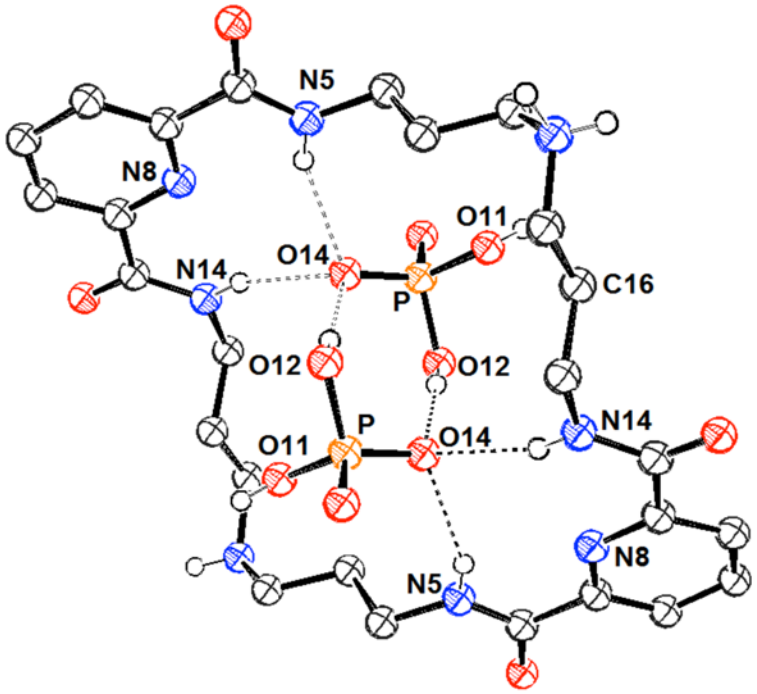


Figure 6.
View of the H_2(**284b**):(H_2PO_4)$_2$ complex. Drawing generated from X-ray diffraction data originally published in reference 365. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

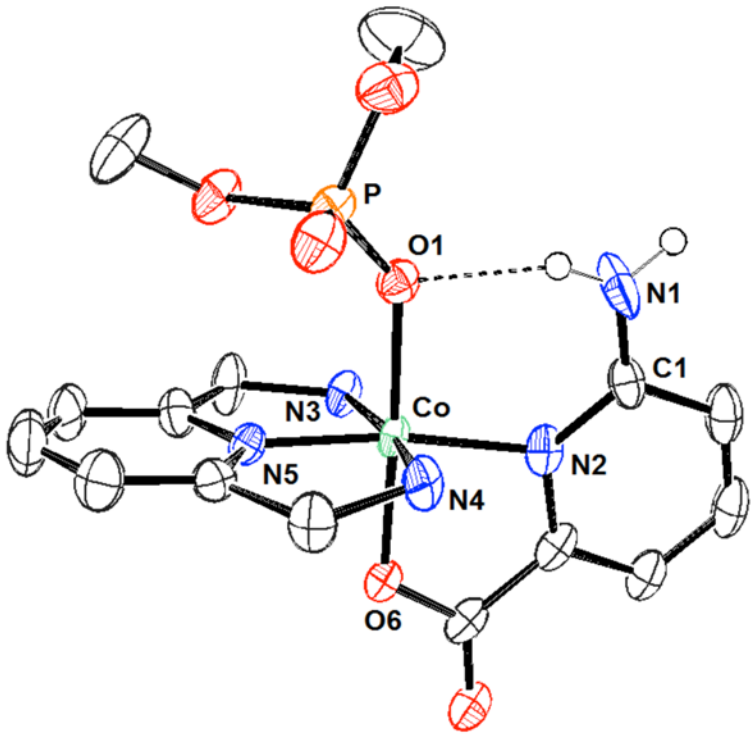


Figure 7.
View of the **497a**:dimethylphosphate complex. Drawing generated from X-ray diffraction data originally published in reference 550. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

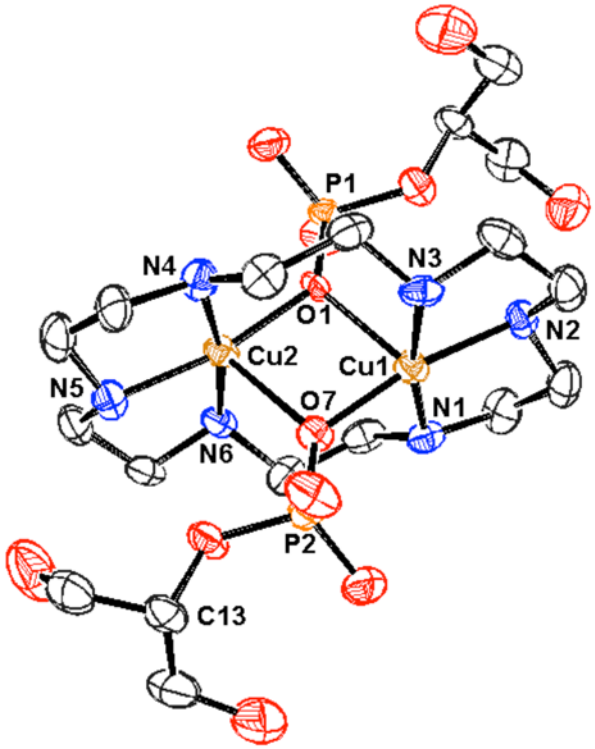


Figure 8.
View of the **511**:glycerol 2-phosphate complex. Drawing generated from X-ray diffraction data originally published in reference 562. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

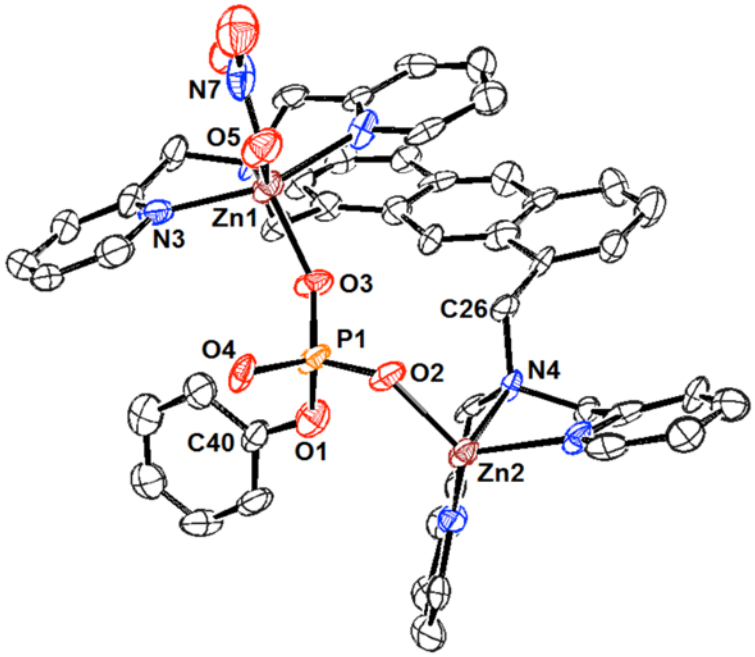


Figure 9.
View of the **546**:phenylphosphate complex. Drawing generated from X-ray diffraction data originally published in reference 594. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

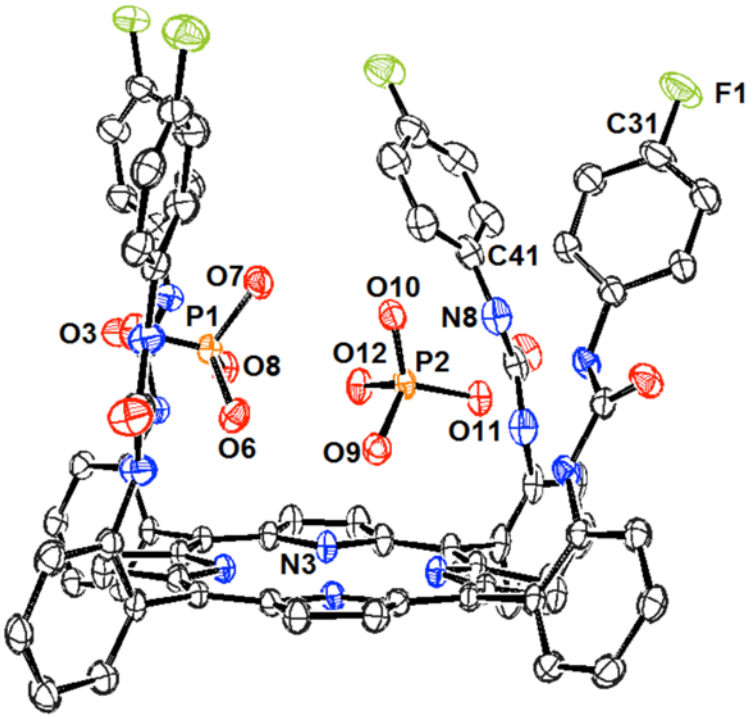


Figure 10.
View of the **826c**:$(H_2PO_4)_2$ complex. Drawing generated from X-ray diffraction data originally published in reference 836. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.

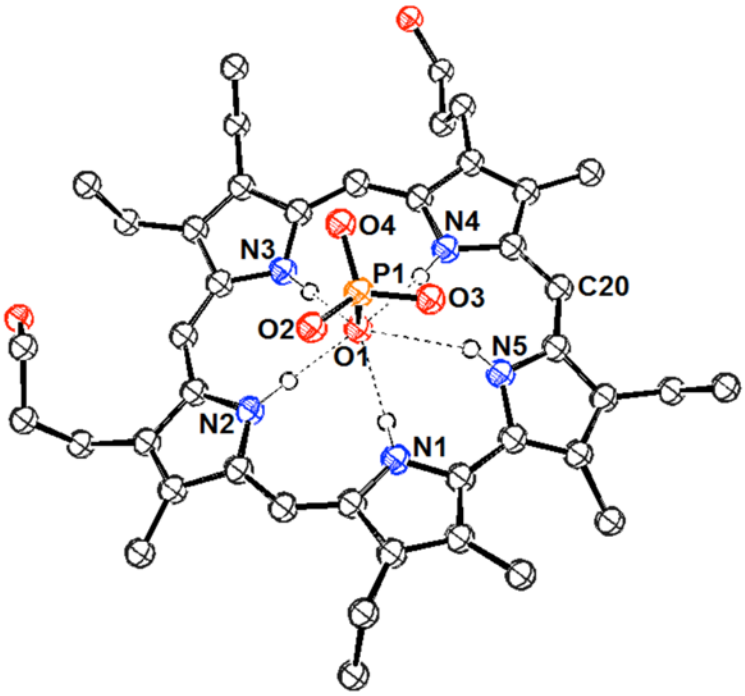
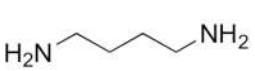


Figure 11.
View of the **837a**:dihydrogenphosphate complex. Drawing generated from X-ray diffraction data originally published in reference 843. In this representation, solvent molecules and most hydrogen atoms have been omitted for clarity.



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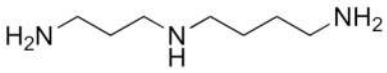

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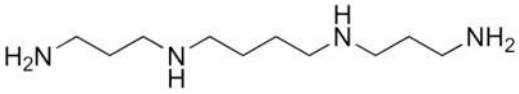

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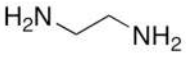

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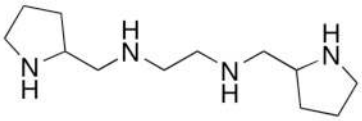

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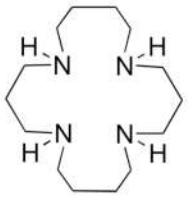

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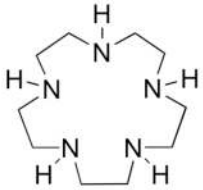

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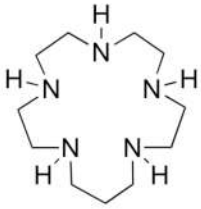

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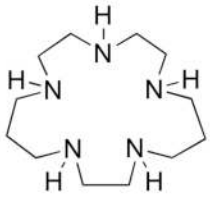

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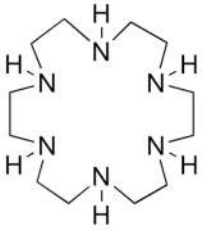

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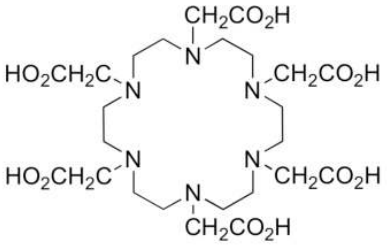

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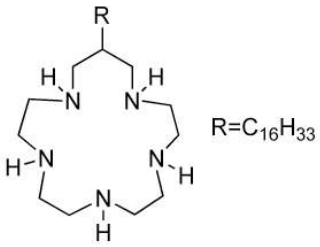

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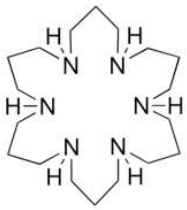

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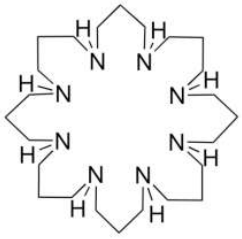

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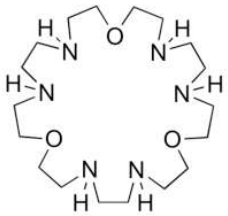

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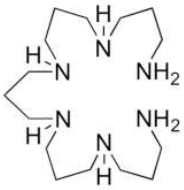

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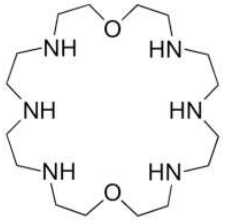

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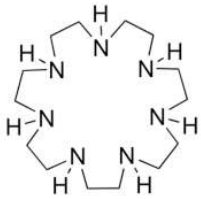

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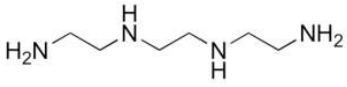

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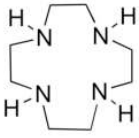

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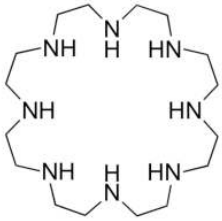

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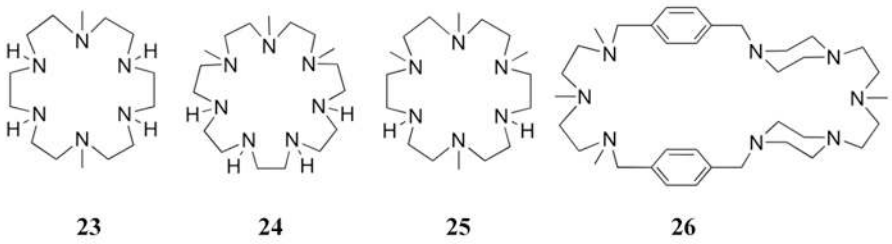

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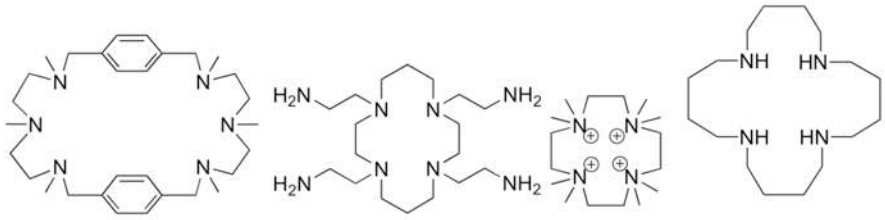

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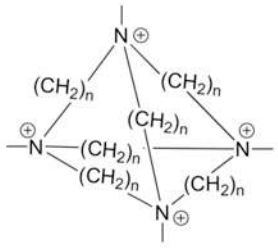

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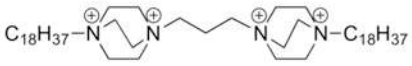

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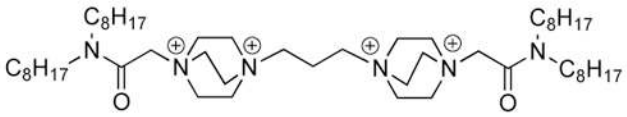

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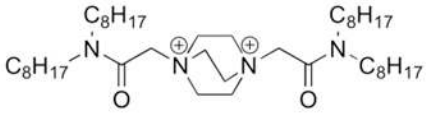

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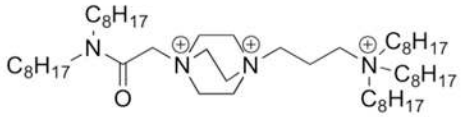

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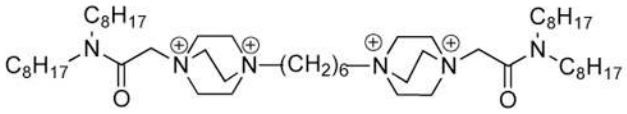

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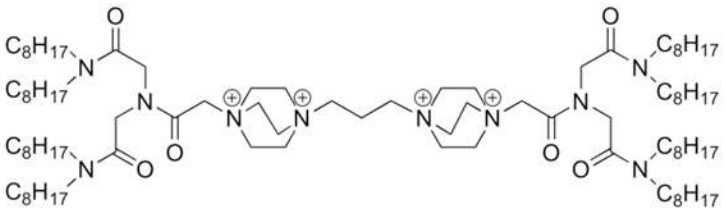

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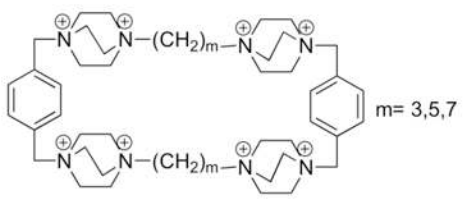

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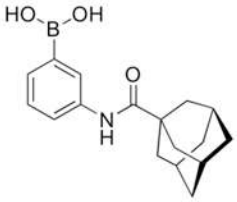

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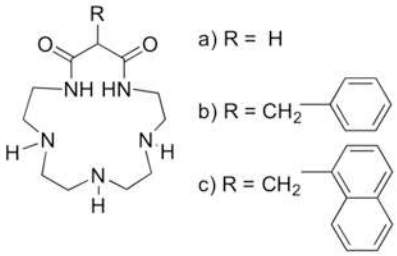

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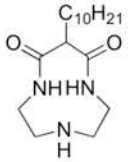

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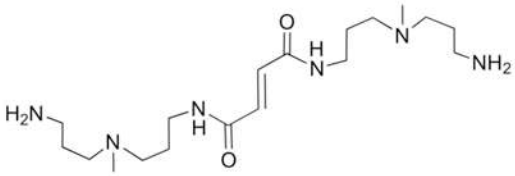

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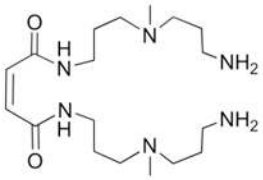

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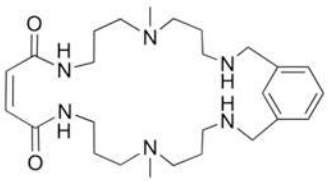

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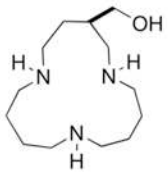

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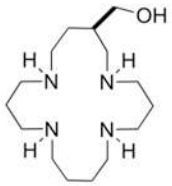

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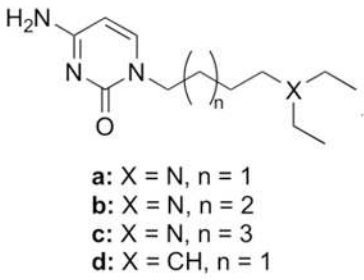

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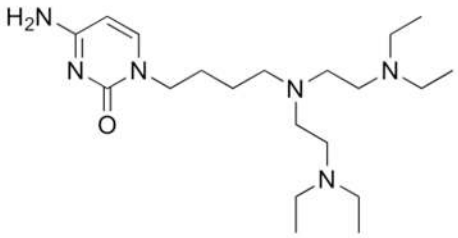

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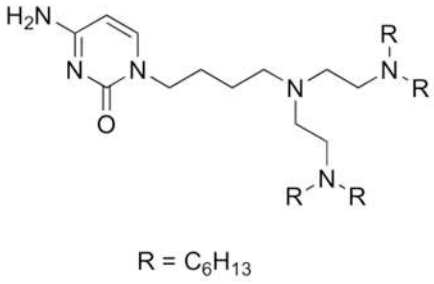

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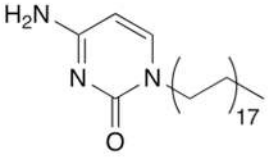

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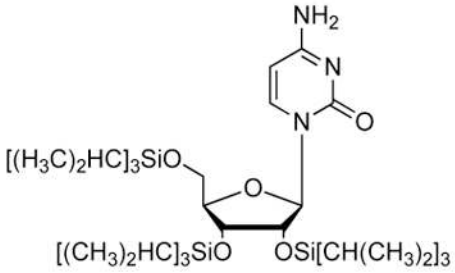

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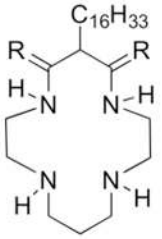

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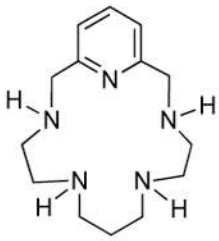

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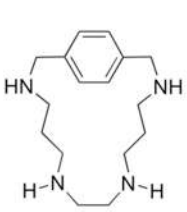

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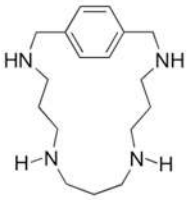

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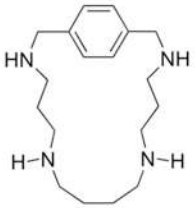

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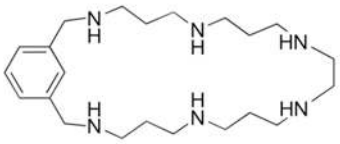

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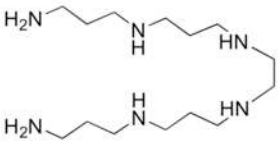

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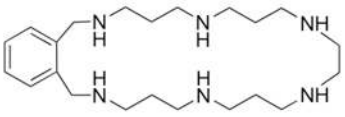

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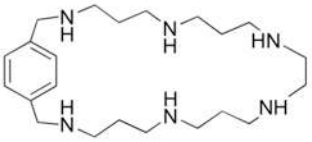

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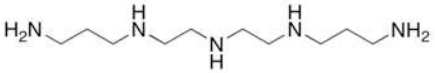

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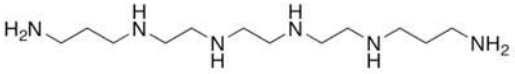

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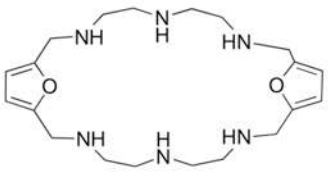

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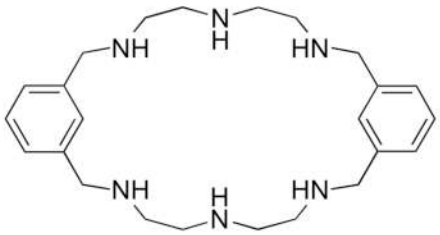

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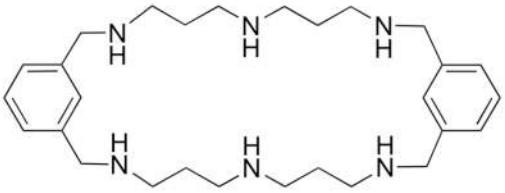

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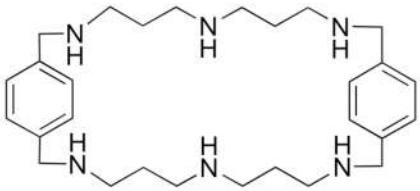

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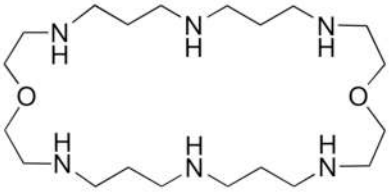

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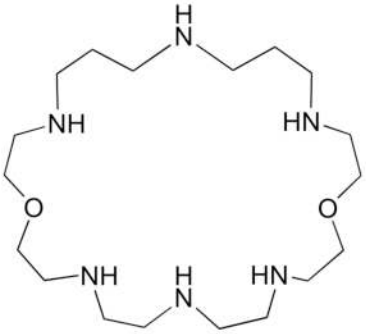

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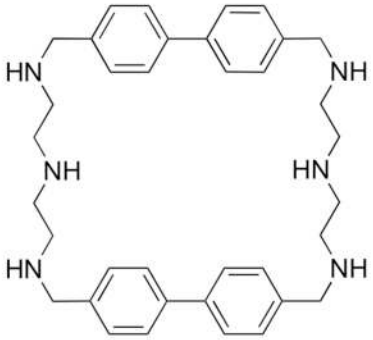

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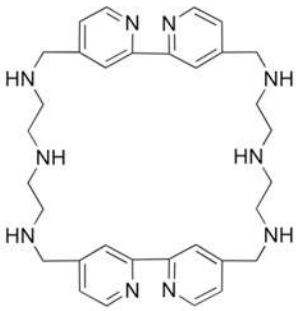

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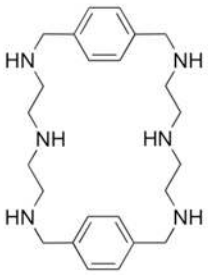

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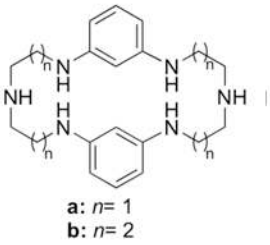

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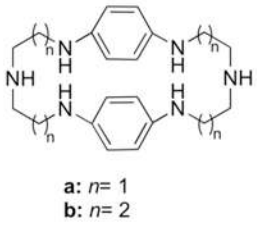

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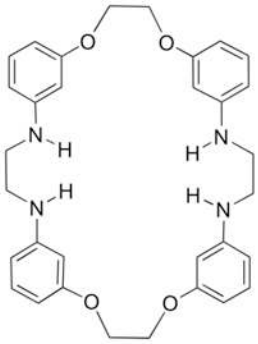

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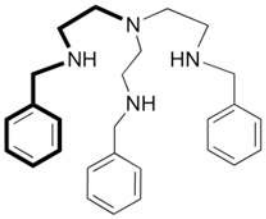

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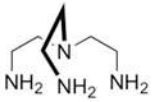

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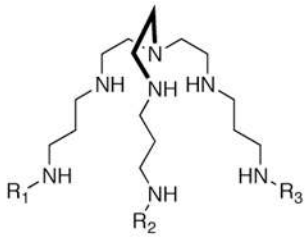

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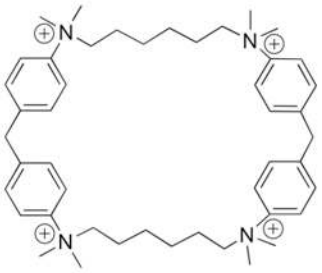

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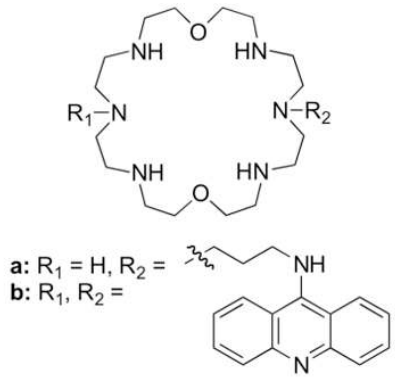
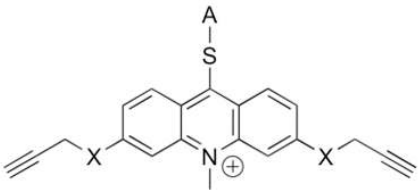


a: R_1 = H, R_2 =
b: R_1, R_2 =

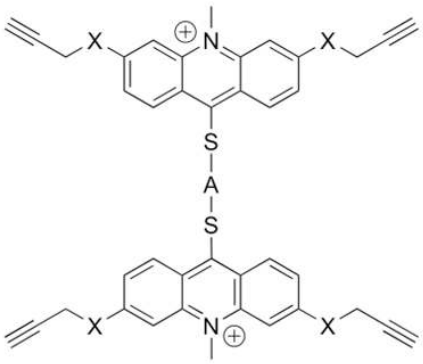
82



a: X = O, A = $-CH_2CH_2CH_3$

b: X = NH, A = $-CH_2CH_2CH_3$

83



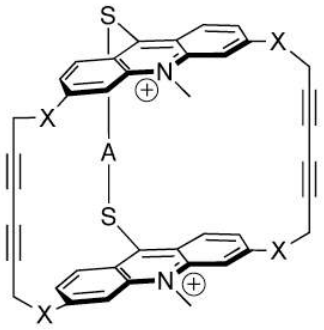
a: X = O, A = $-(CH_2)_6-$

b: X = O, A = $-CH_2CH_2OCH_2CH_2-$

c: X = O, A = $-CH_2CH_2OCH_2CH_2OCH_2CH_2-$

d: X = NH, A = $-CH_2CH_2OCH_2CH_2-$

84



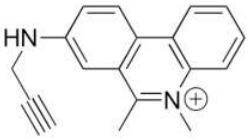
a: X = O, A = —$(CH_2)_6$—

b: X = O, A = —$CH_2CH_2OCH_2CH_2$—

c: X = O, A = —$CH_2CH_2OCH_2CH_2OCH_2CH_2$—

d: X = NH, A = —$CH_2CH_2OCH_2CH_2$—

85



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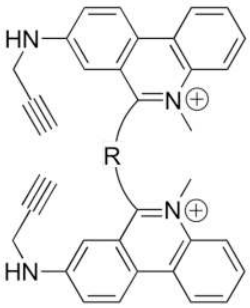
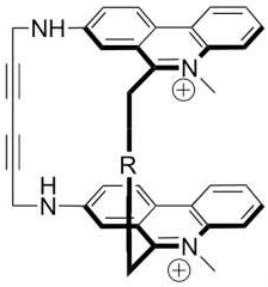


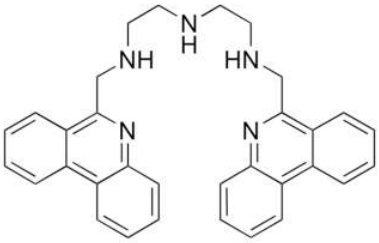
R = $(CH_2)_6$

87



a: R = $(CH_2)_4$
b: R = $(CH_2)_6$
c: R = *p*-C_6H_4

88



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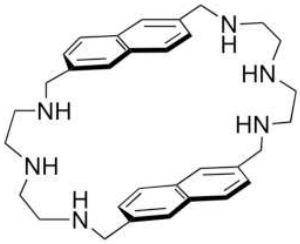

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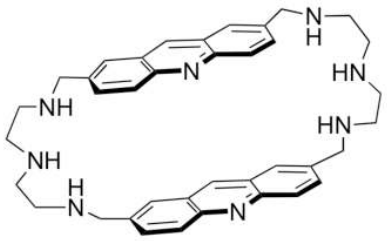

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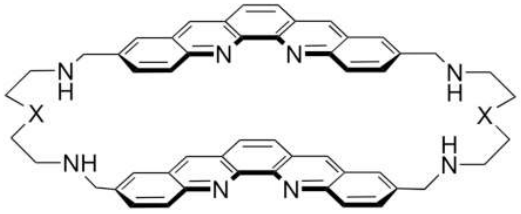
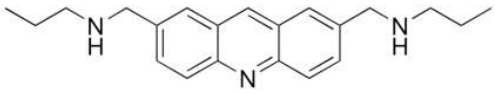


a: X = NH
b: X = O
c: X = $CH_2N(CH_3)CH_2$

92



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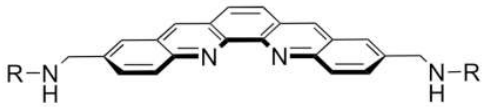
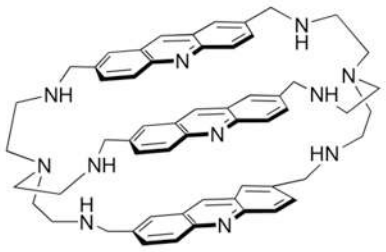


a: R = *n*-Pr
b: R = $CH_2N(CH_3)CH_2$

94



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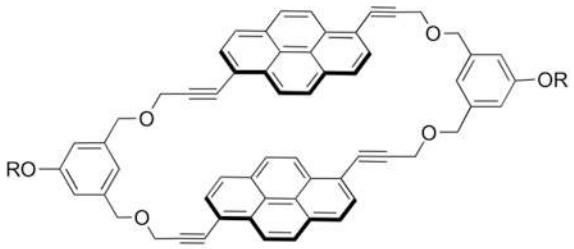
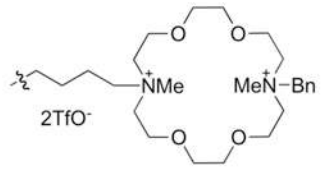


a: R = $(CH_2)_3N(CH_3)_3^+$, TfO^-

b: R =



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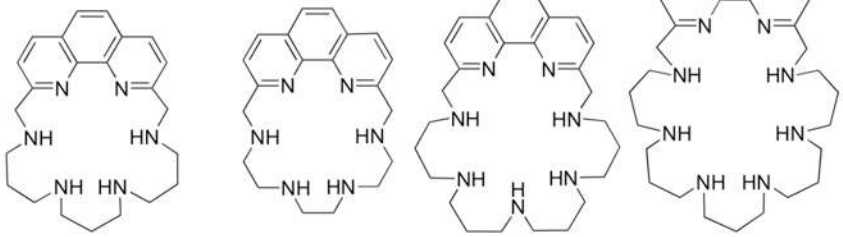
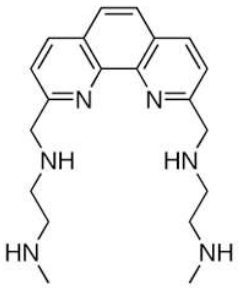


97 **98** **99** **100**



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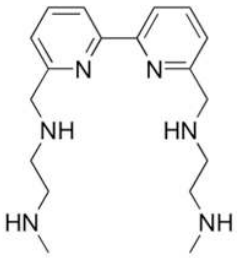
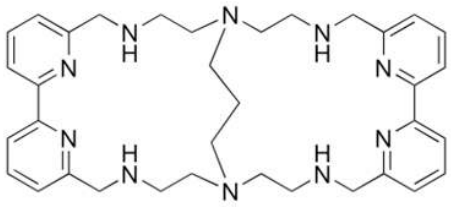


102



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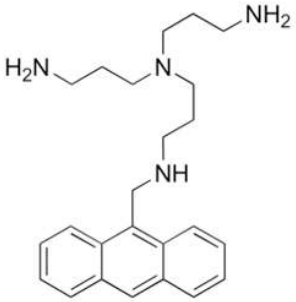

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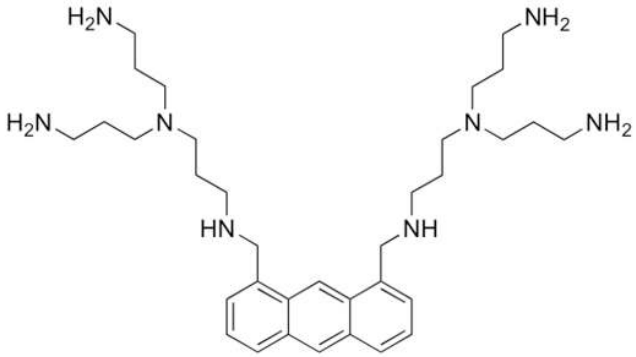

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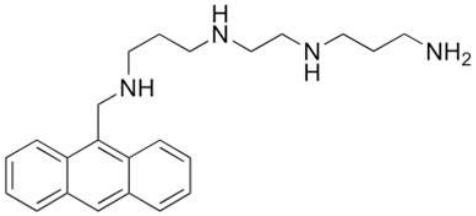

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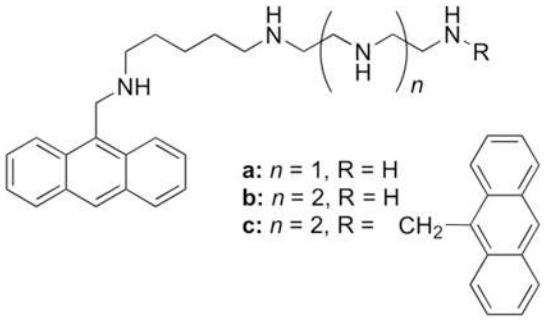

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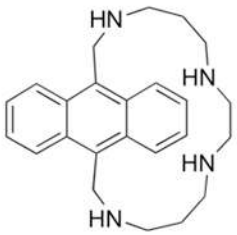

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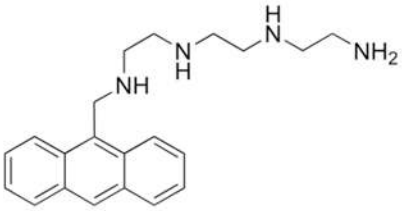

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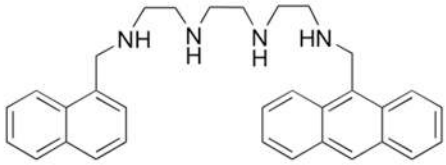

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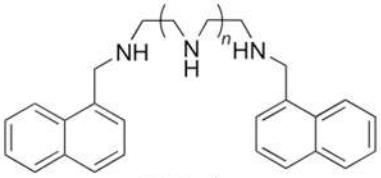

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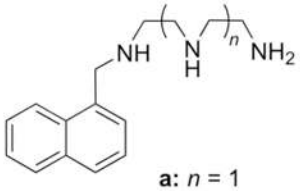

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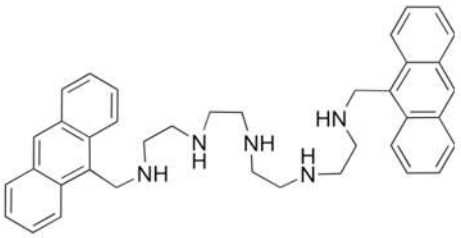

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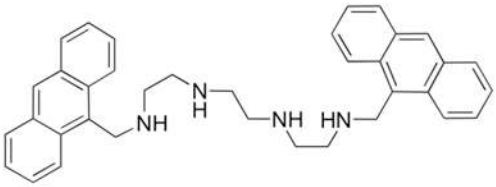

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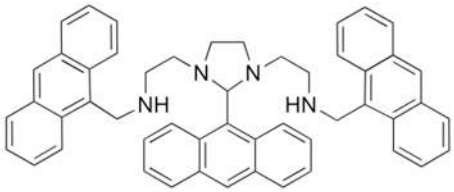

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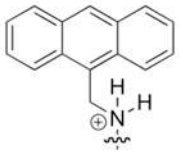
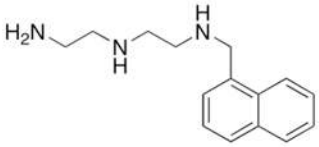


117



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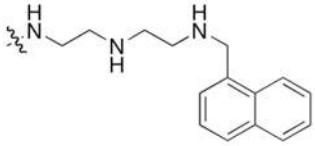

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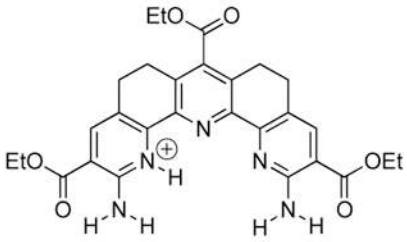

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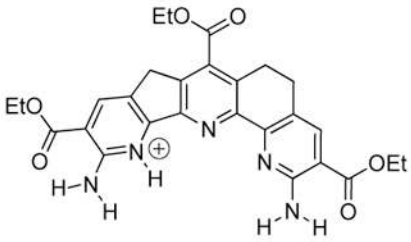
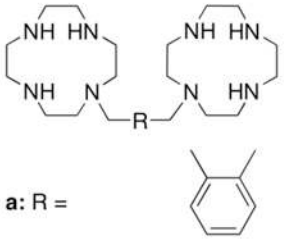
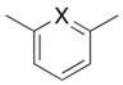


121

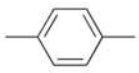


a: R =

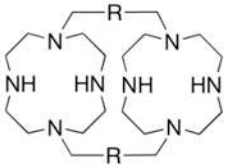
b: X = CH, R =
c: X = N, R =



d: R =

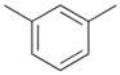


123

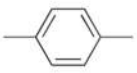


a: R =

b: R =



d: R =



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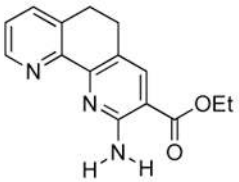

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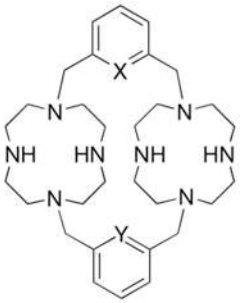
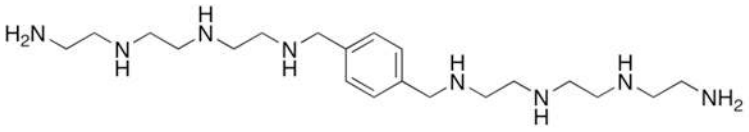
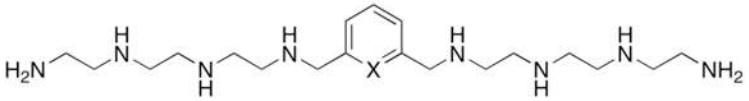


a: X = CH, Y = N
b: X = Y = N

125

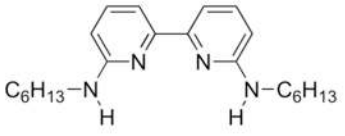


126



a: X = CH
b: X = N

127



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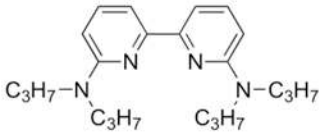

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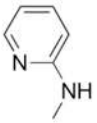

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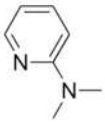

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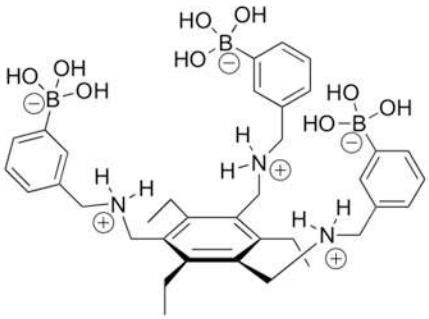

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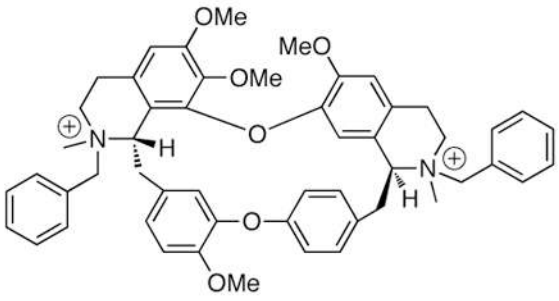

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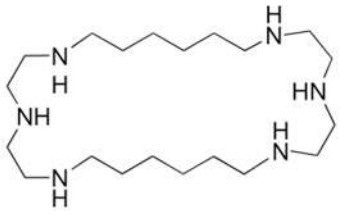
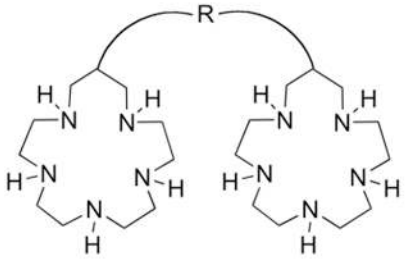
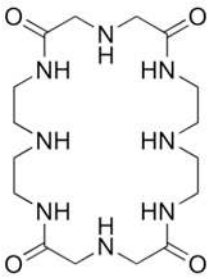


134



a: R = $(CH_2)_2O(CH_2)_2O(CH_2)_2$
b: R = $(CH_2)_3$

135



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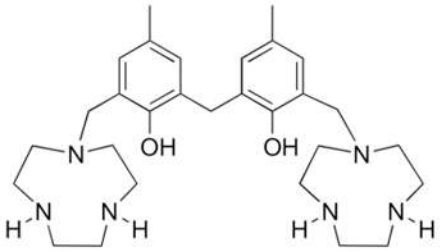
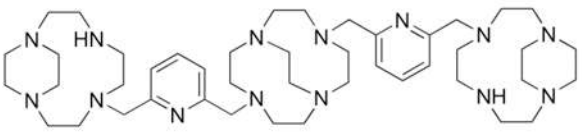
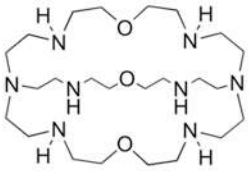


137



138



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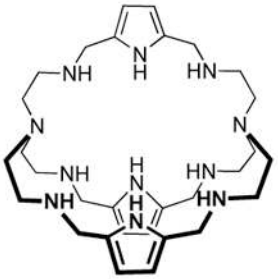

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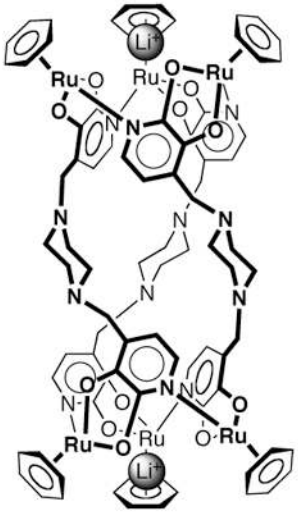
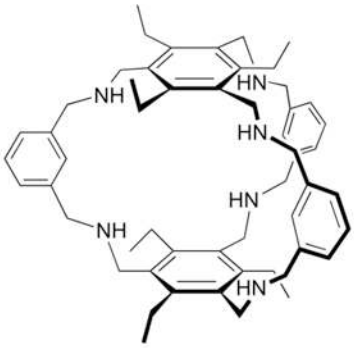


141



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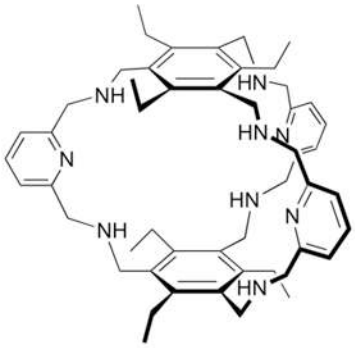

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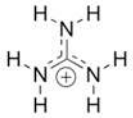

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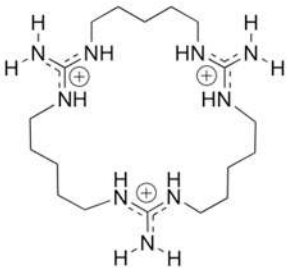
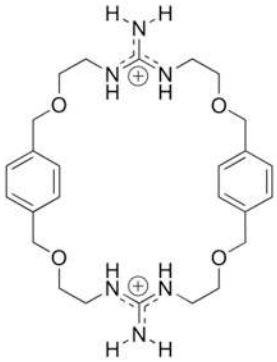


145



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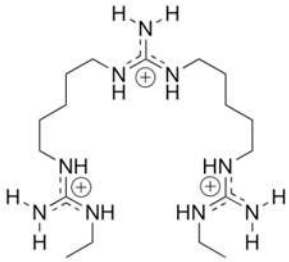
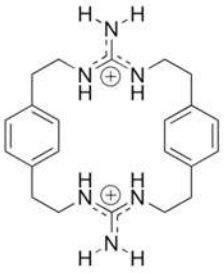


147



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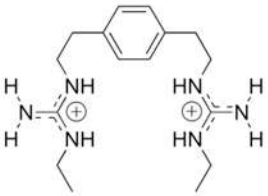

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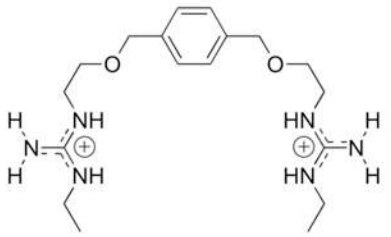

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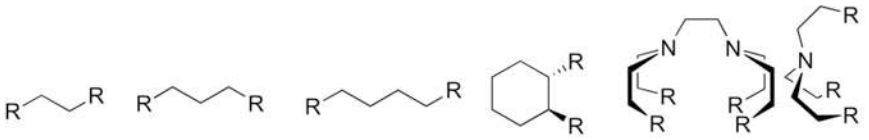
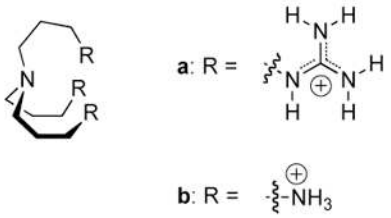


151 **152** **153** **154** **155** **156**



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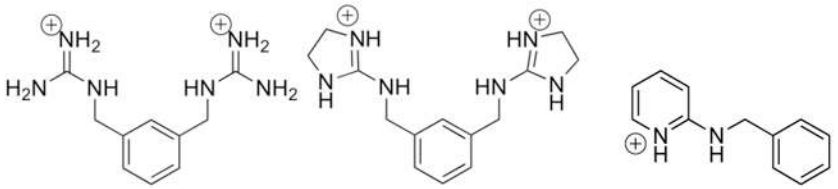
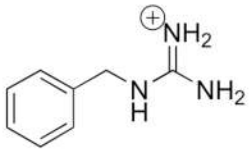


158 **159** **160**



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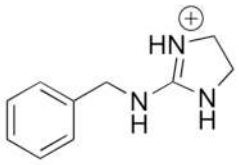

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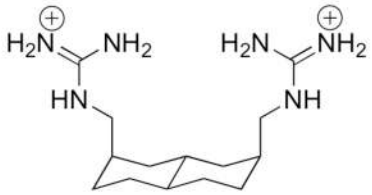

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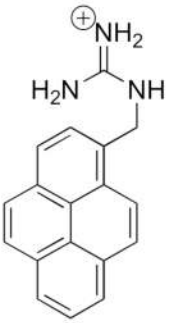

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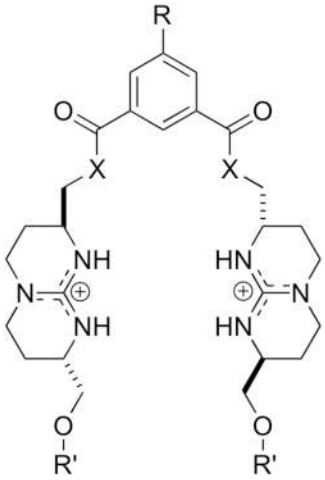
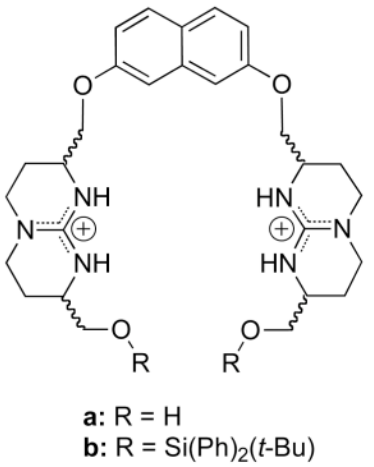


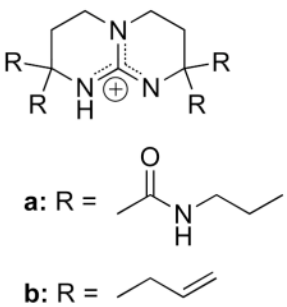
a: X = NH, R = H, R' = $Si(Ph)_2(t\text{-}Bu)$
b: X = NH, R = R' = H
c: X = NH, R = $CH_2SB_{12}H_{112}$-2, R' = $Si(Ph)_2(t\text{-}Bu)$
d: X = NH, R = OH, R' = $Si(Ph)_2(t\text{-}Bu)$
e: X = NH, R = $OCH_2C_6H_5$, R' = $Si(Ph)_2(t\text{-}Bu)$
f: X = NH, R = $OC_{16}H_{33}$, R' = $Si(Ph)_2(t\text{-}Bu)$
g: X = O, R = $OC_{16}H_{33}$, R' = $Si(Ph)_2(t\text{-}Bu)$

165



a: R = H
b: R = Si(Ph)$_2$(*t*-Bu)

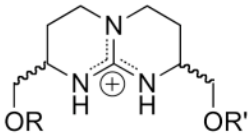
166



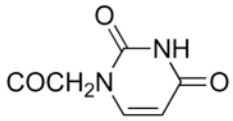
a: R =

b: R =

167



a: R = R' = 2-naphthyoyl
b: R = R' = palmitoyl
c: R = 2-naphthyoyl,R' =



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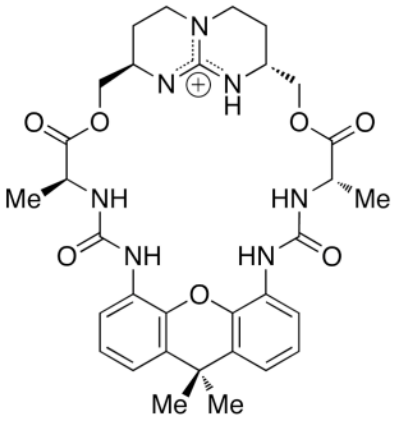

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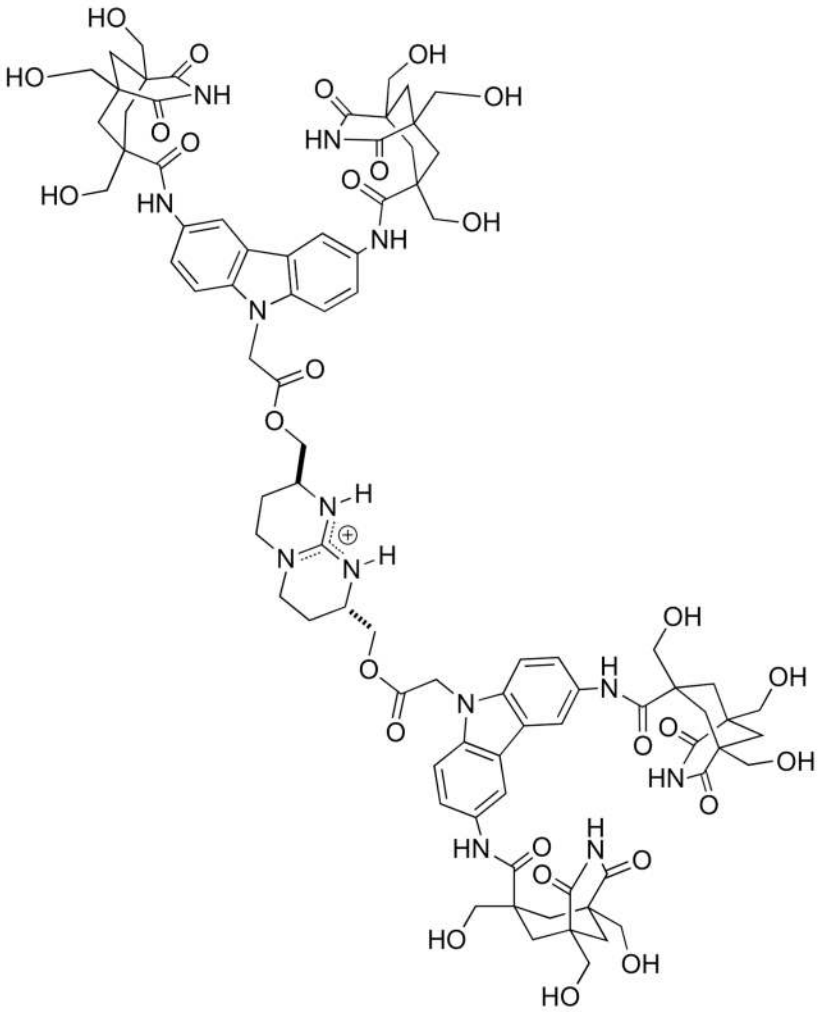

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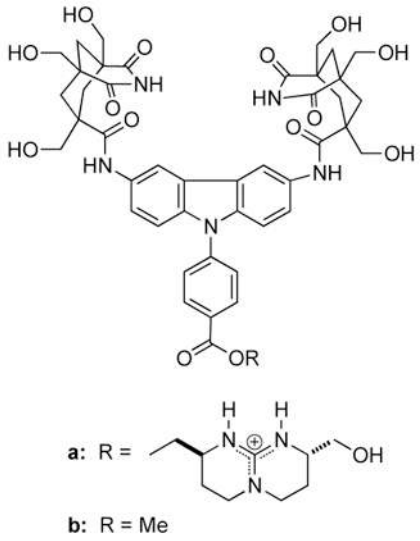

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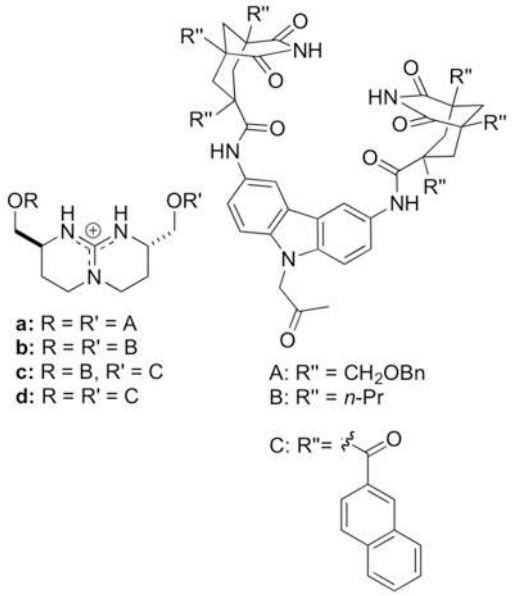

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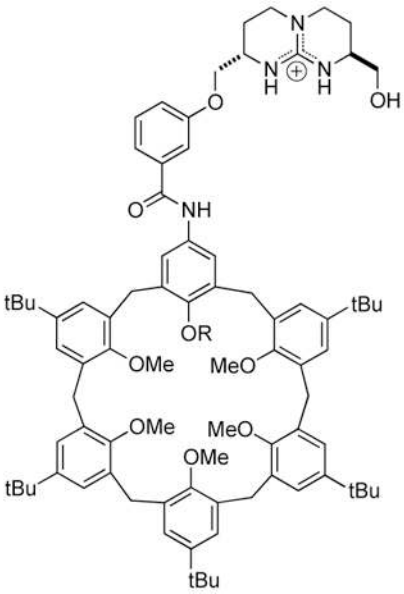

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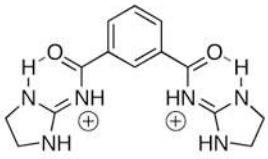

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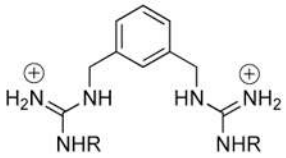

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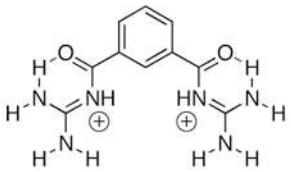

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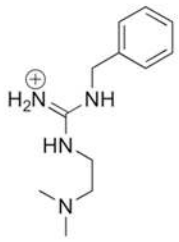

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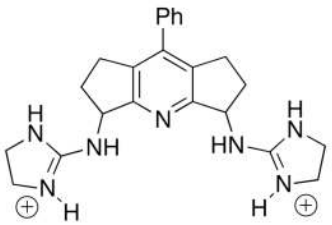

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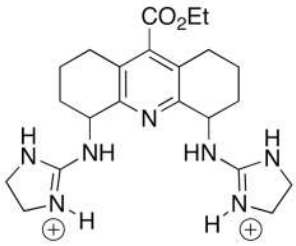

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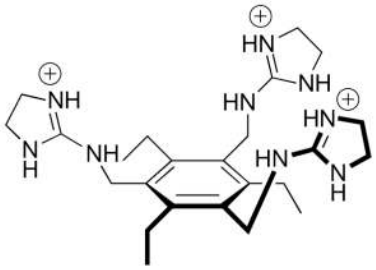

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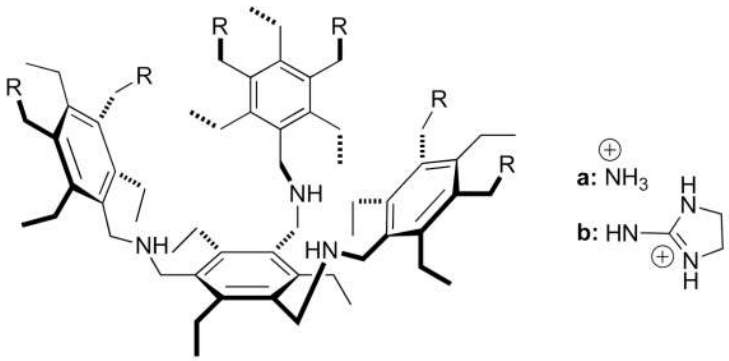

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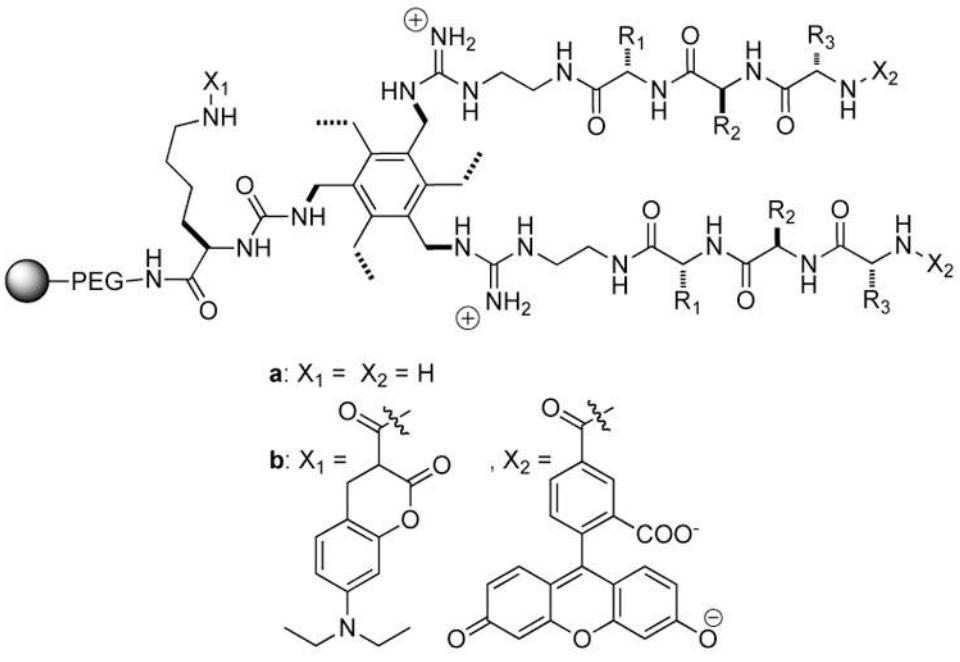

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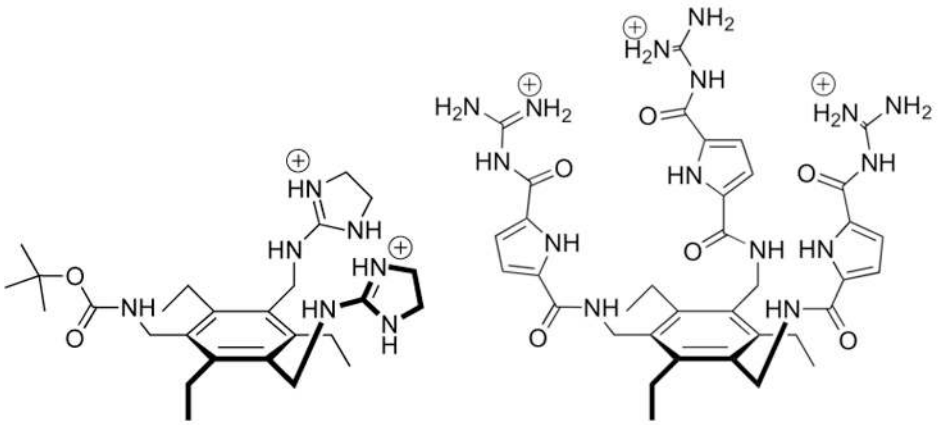

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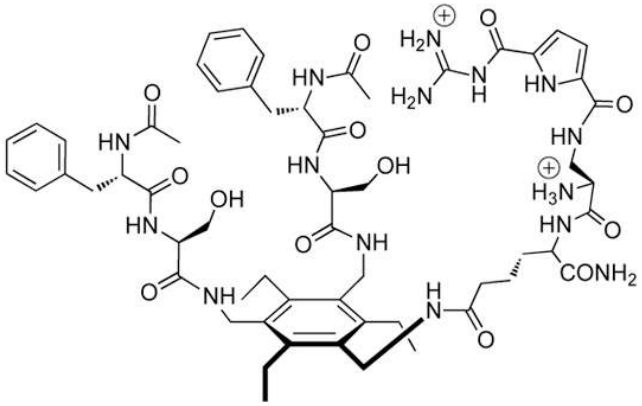

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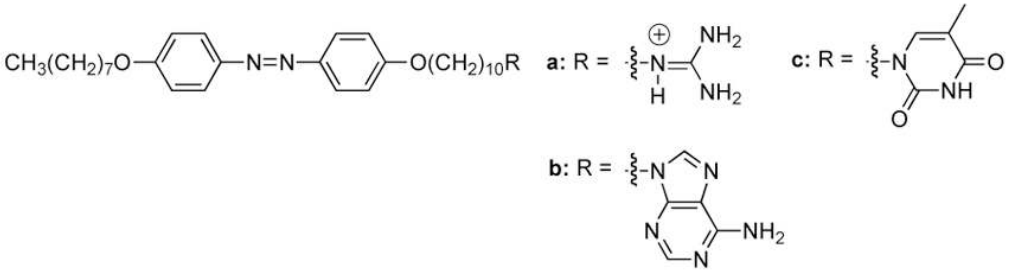

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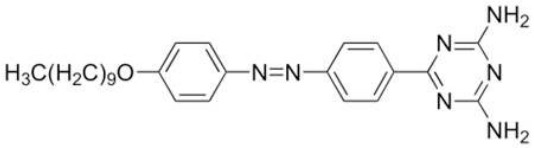

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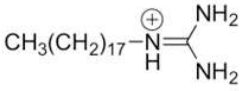

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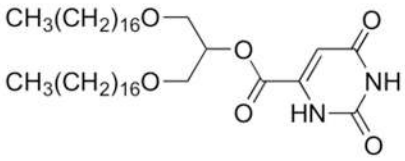

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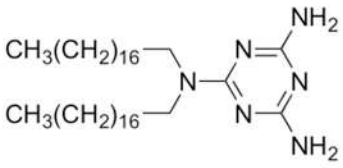

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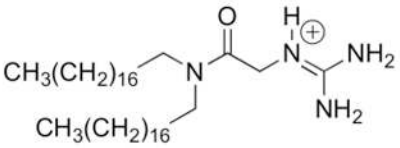

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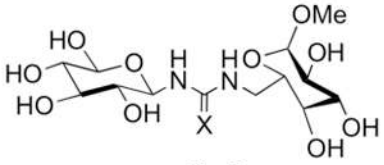

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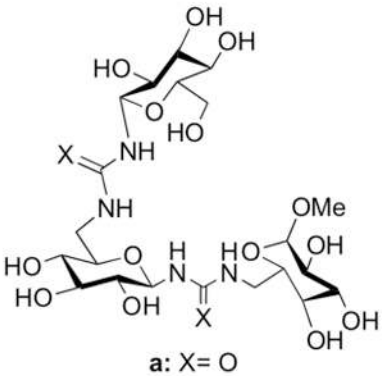

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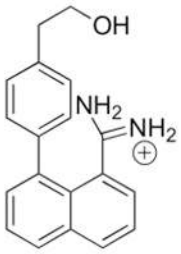

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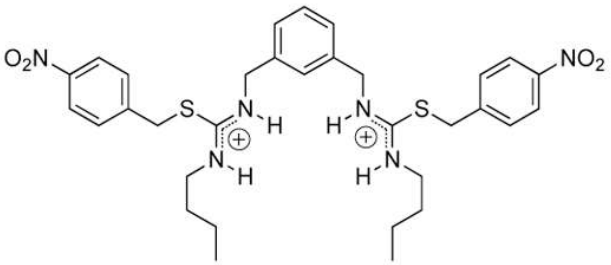

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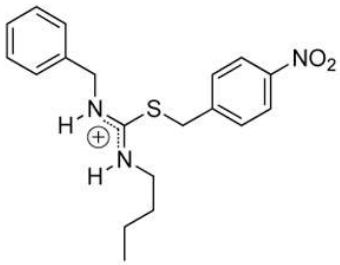

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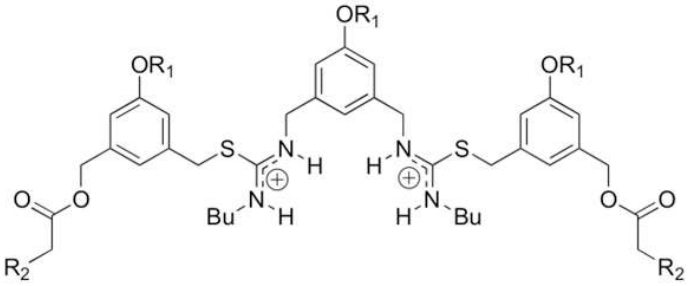
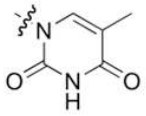


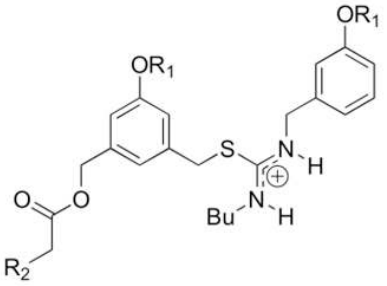
R_1 = dodecyl

a: R_2 =



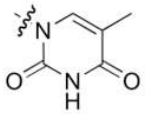
b: R_2 = H

197



R_1 = dodecyl

a: R_2 =



b: R_2 = H

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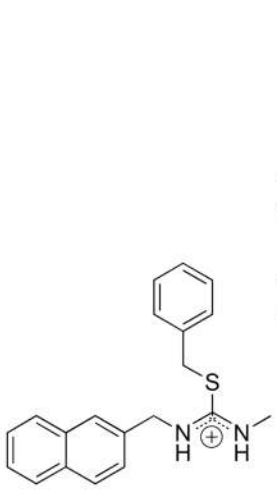

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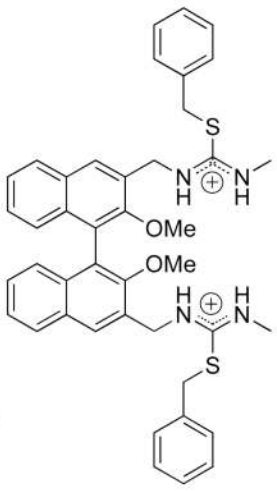

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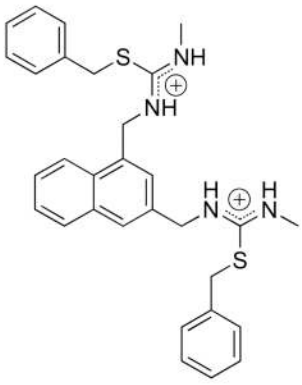

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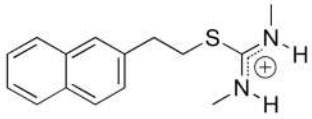

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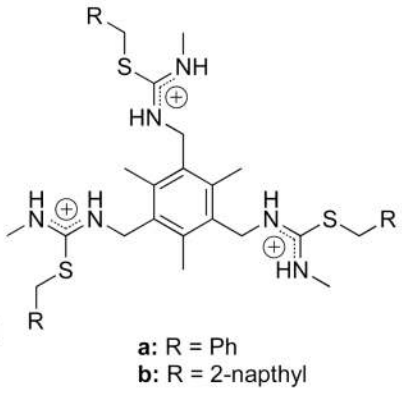

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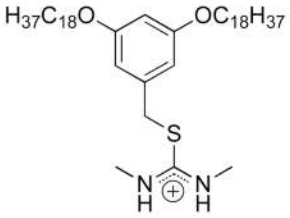

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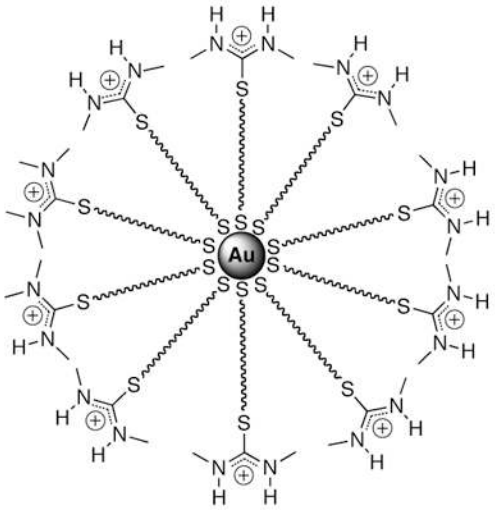

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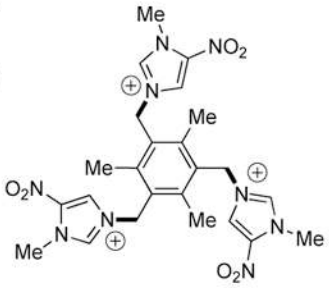

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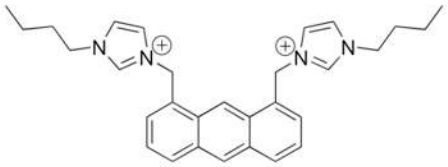

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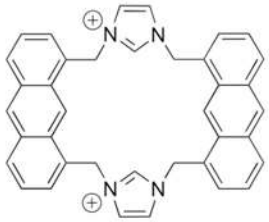

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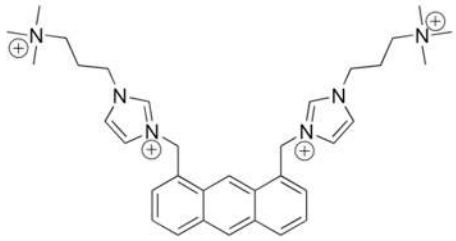

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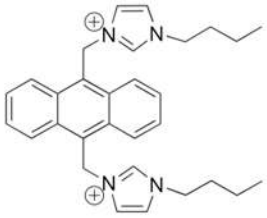

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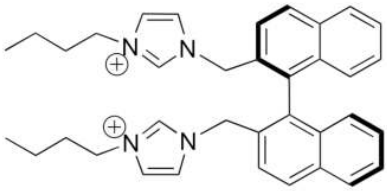

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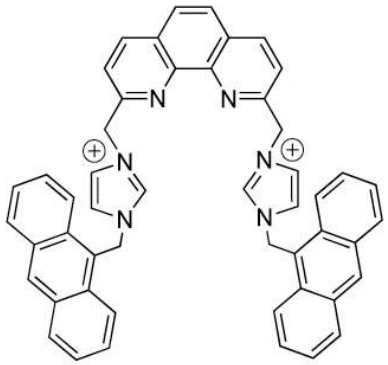

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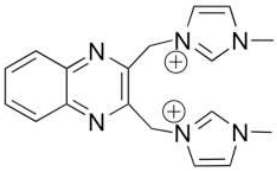

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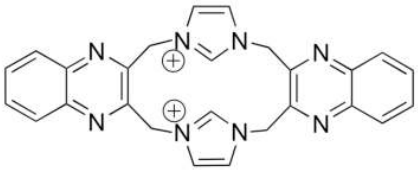

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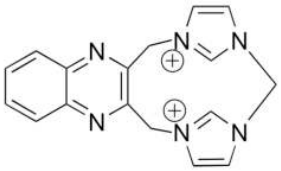

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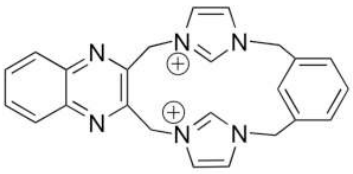

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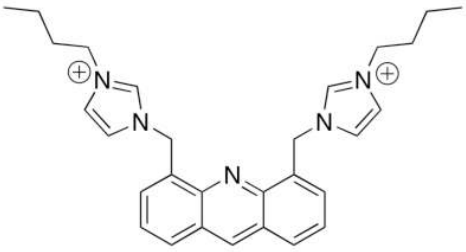

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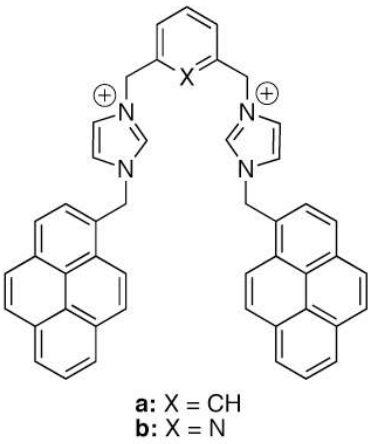

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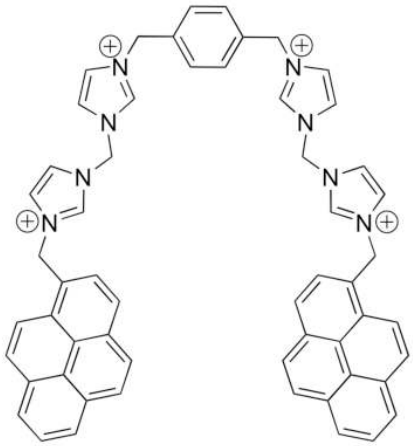

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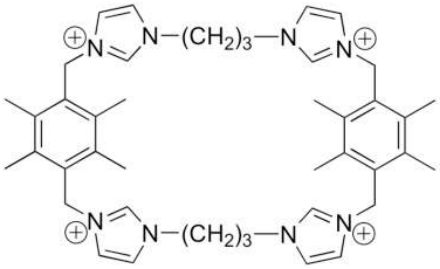

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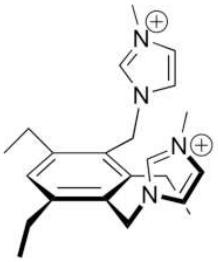

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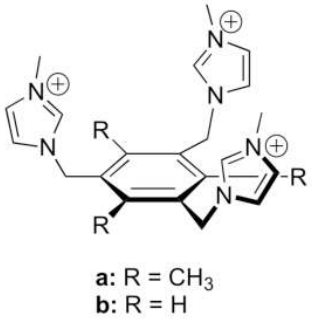

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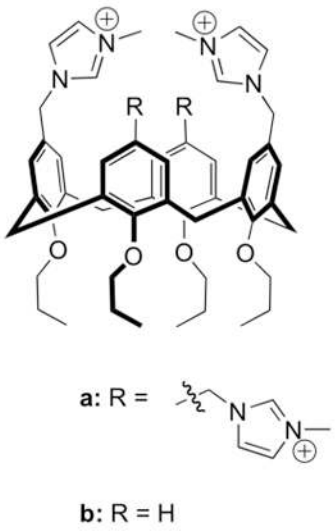

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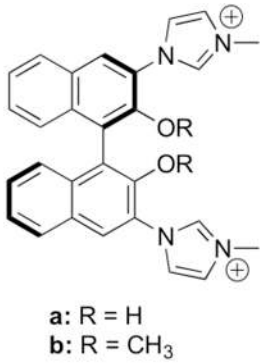

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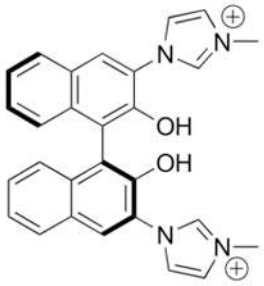

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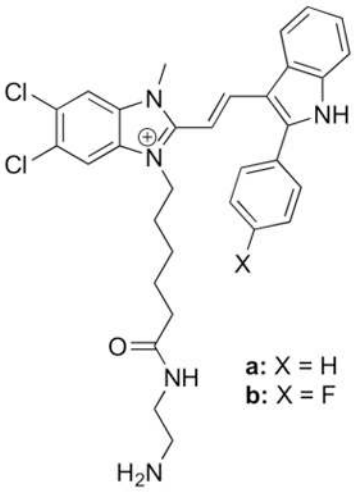

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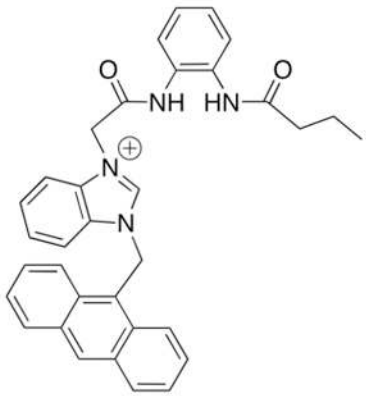

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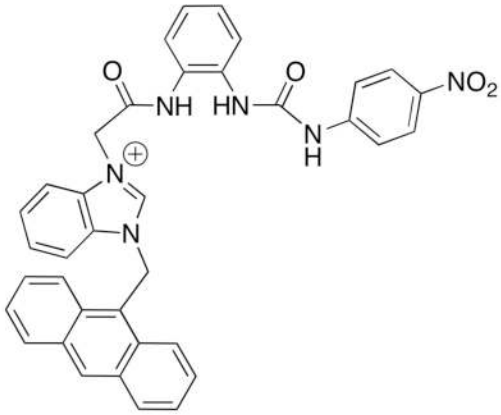

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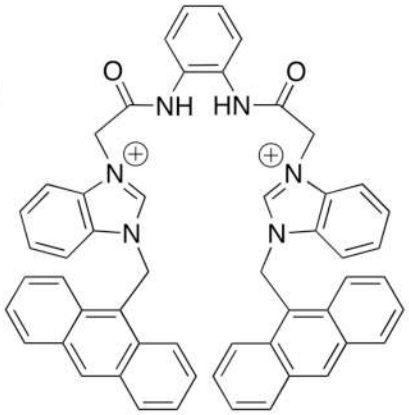

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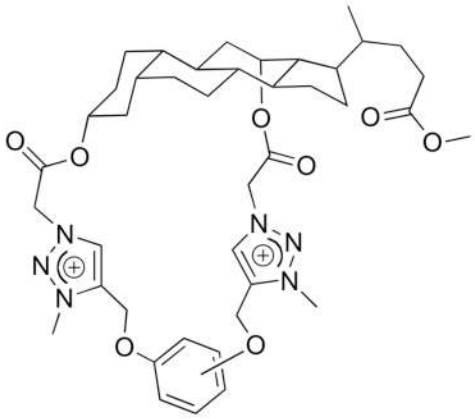

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b: *para*

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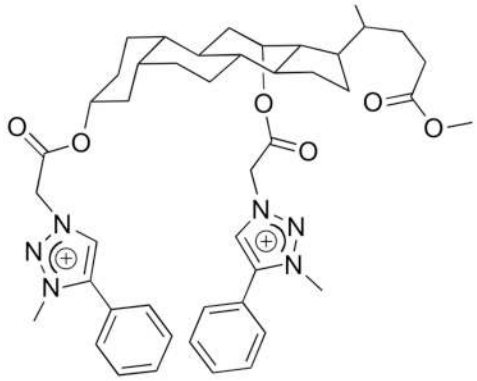

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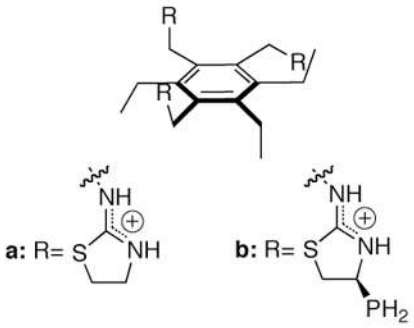

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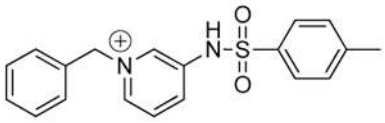

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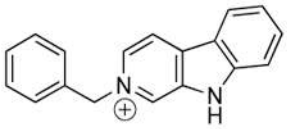

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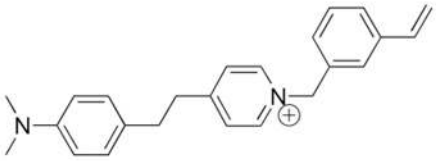

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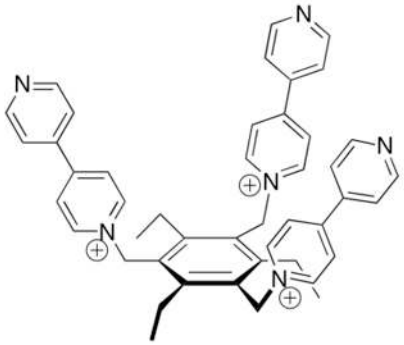

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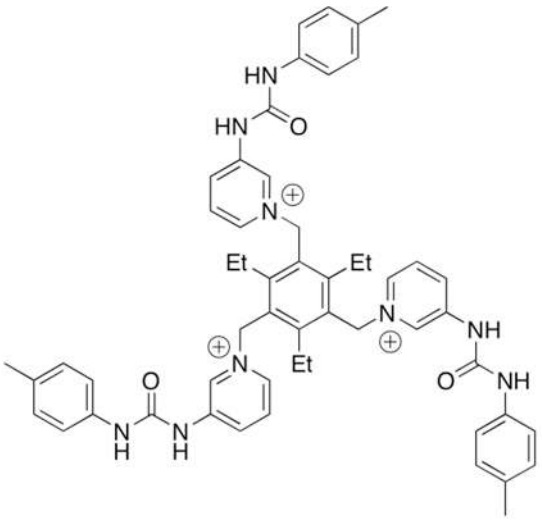

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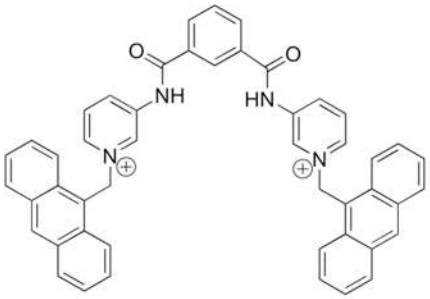

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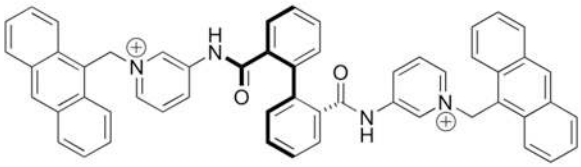

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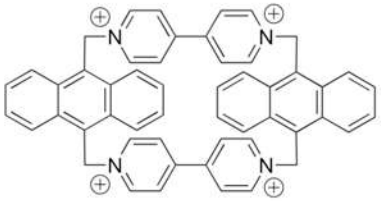

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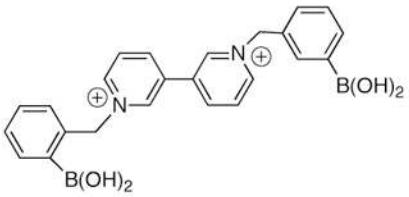

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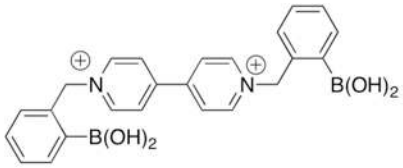

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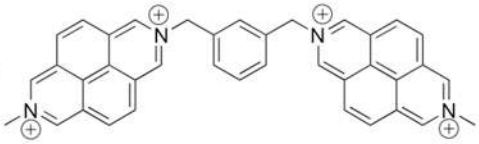

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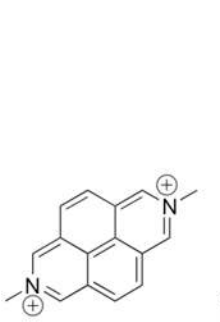

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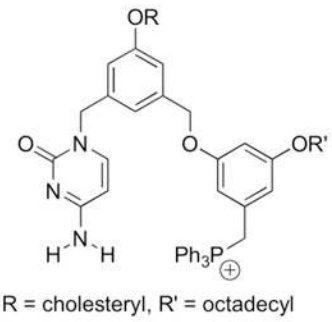
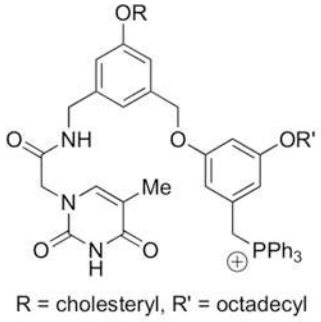


R = cholesteryl, R' = octadecyl

245



R = cholesteryl, R' = octadecyl

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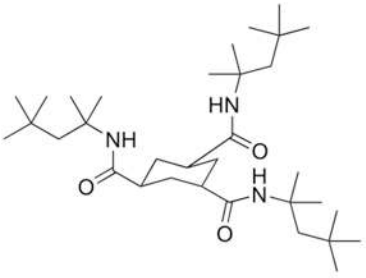

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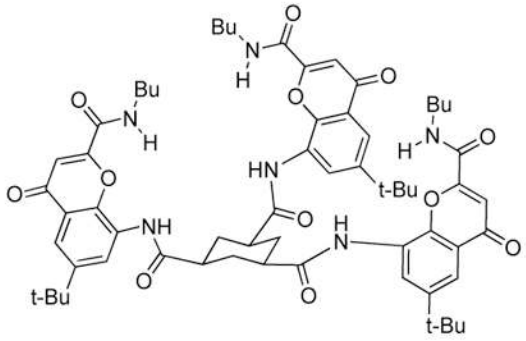

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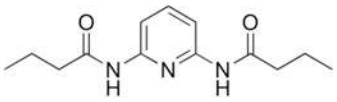

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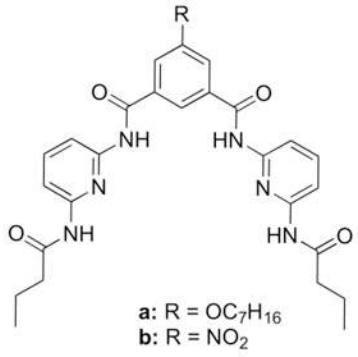

a: R = OC_7H_{16}
b: R = NO_2

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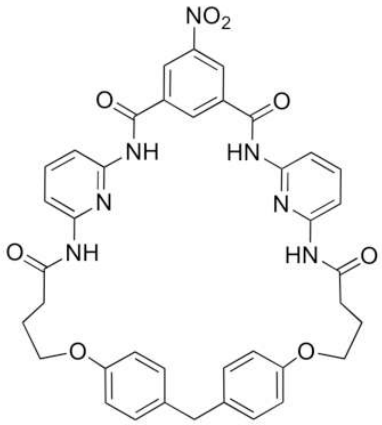

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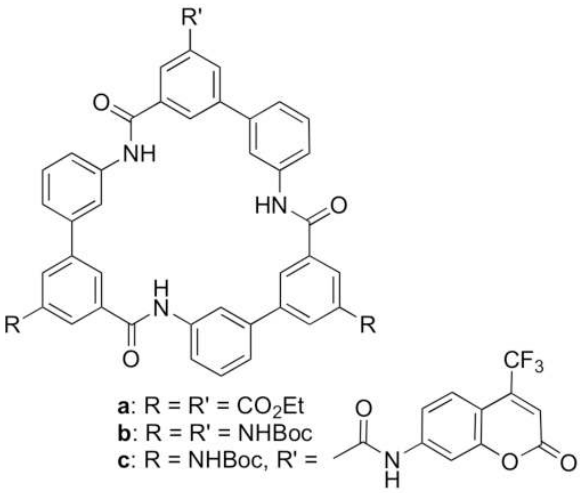

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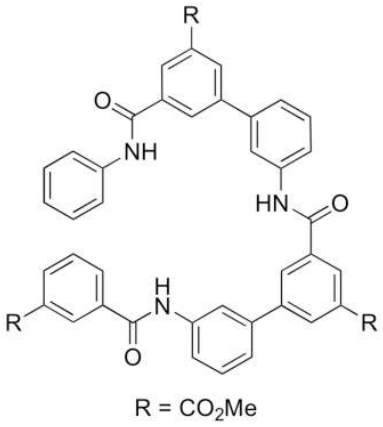
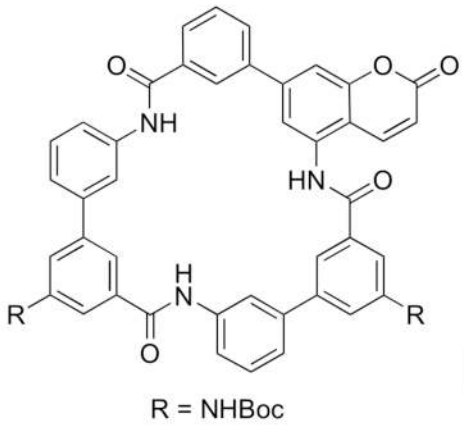
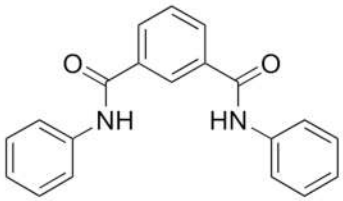


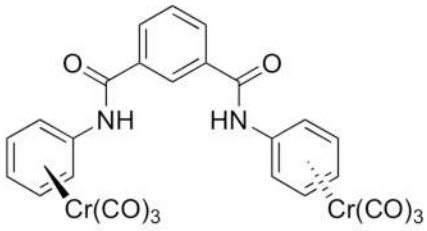
253



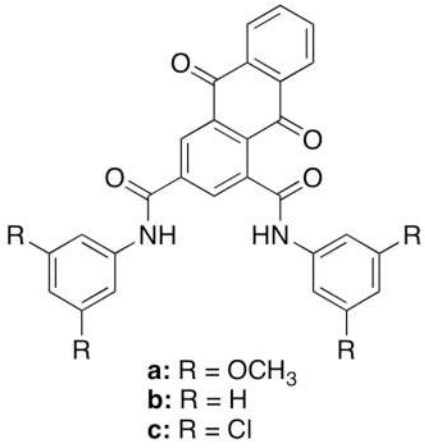
254



255



256



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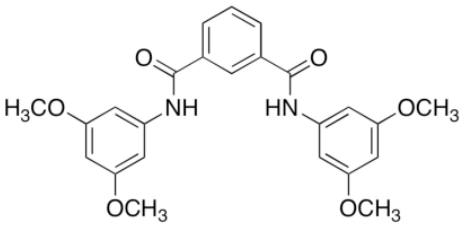

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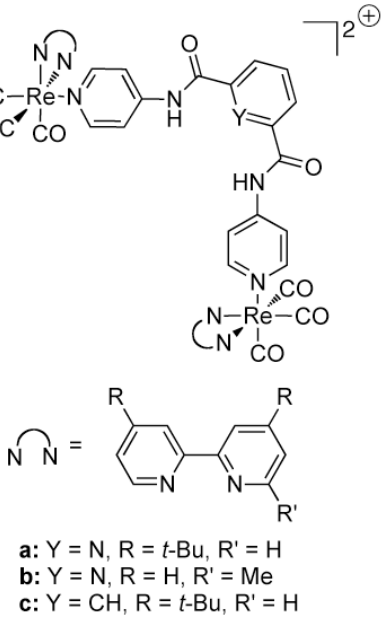

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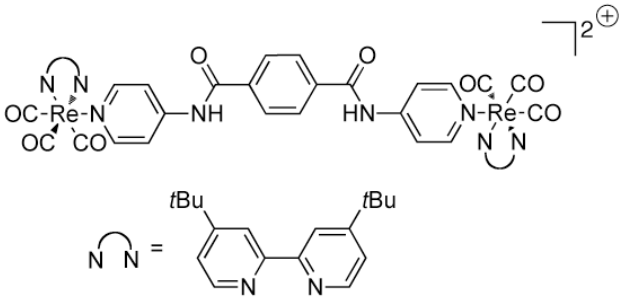

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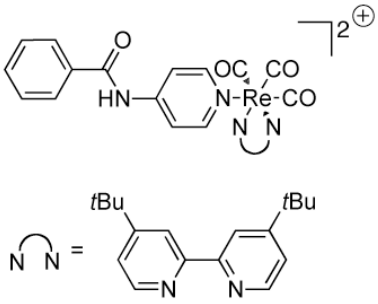

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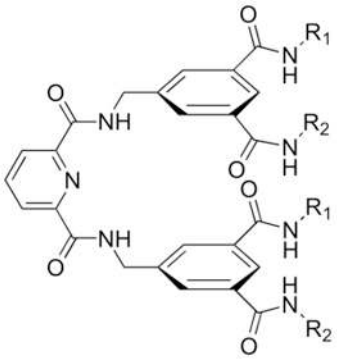


a: $R_1 = R_2$ = cyclohexyl

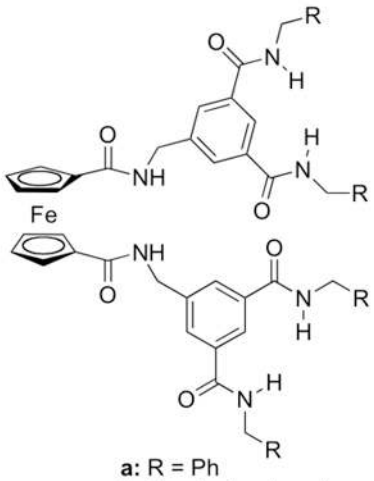
b: $R_1 = R_2$ = 1-methylpyrenyl

c: $R_1 = R_2 = C_{12}H_{25}$

d: R_1 = 1-methylpyrenyl, $R_2 = C_{12}H_{25}$

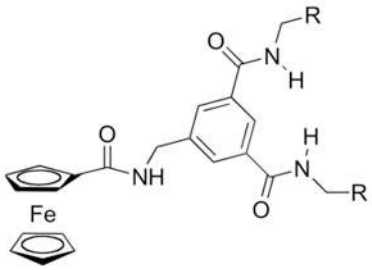
e: $R_1 = R_2 = CH_3$

262



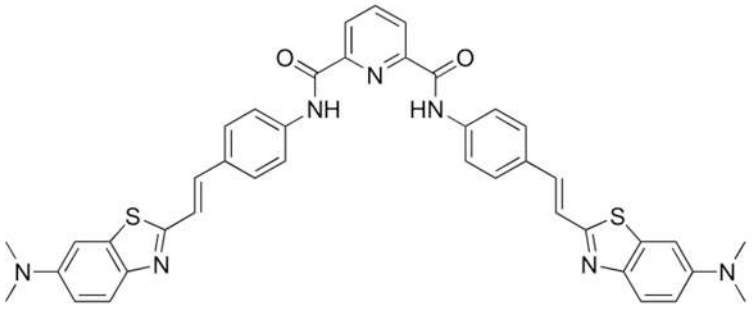
a: R = Ph
b: R = 4-nitrophenyl
c: R = 1-pyrenyl

263



a: R = Ph
b: R = 4-nitrophenyl
c: R = 1-pyrenyl

264



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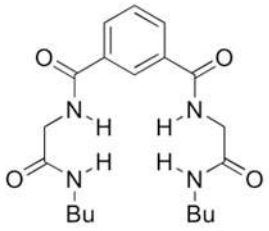

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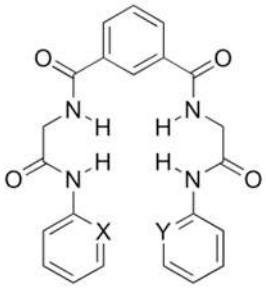
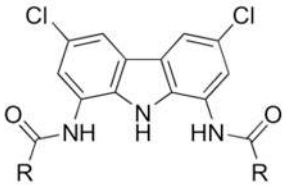


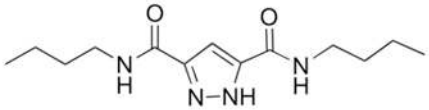
a: X = Y = CH
b: X = N, Y = CH
c: X = Y = N

267



a: R = Ph
b: R = Pr

268



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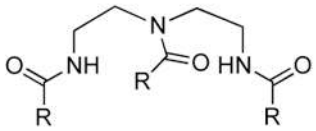
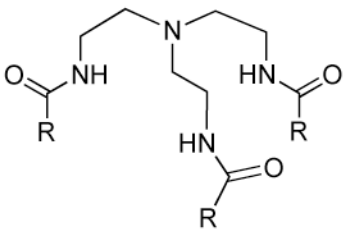


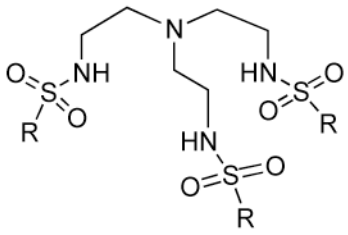
a: R = CH_2Cl
b: R = $(CH_2)_4CH_3$

270



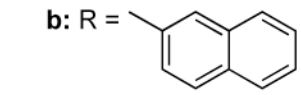
a: R = CH_2Cl
b: R = $(CH_2)_4CH_3$
c: R = C_6H_5
d: R = 4-$MeOC_6H_4$

271



a: R = 4-MeC_6H_4

b: R =



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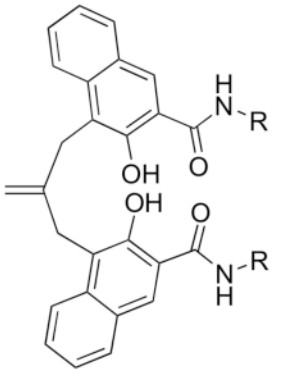
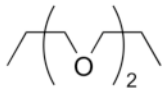
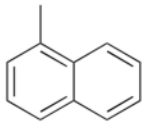


a: R =



b: R =



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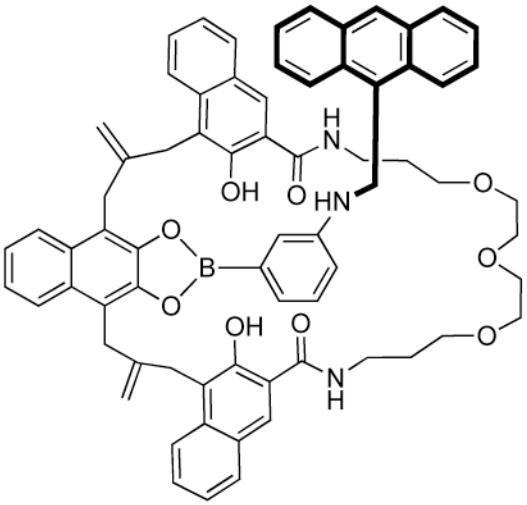

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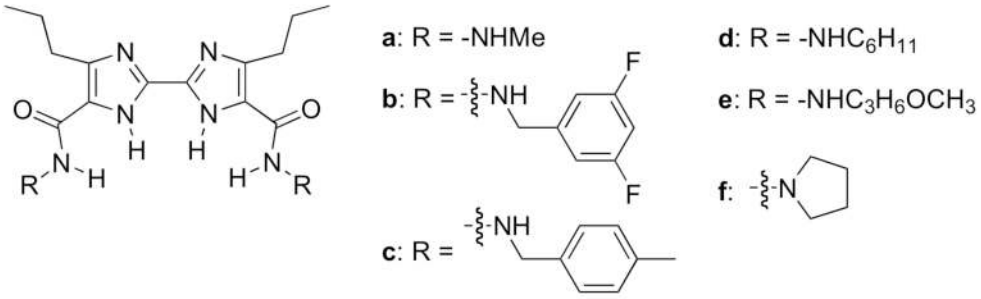

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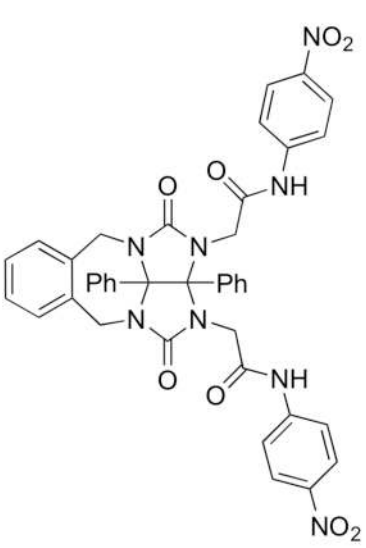

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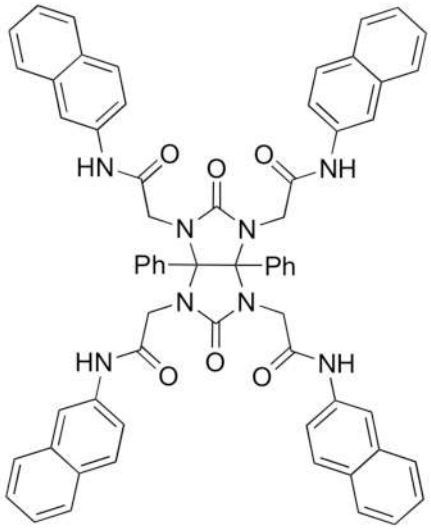

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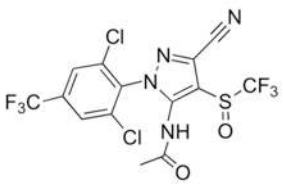

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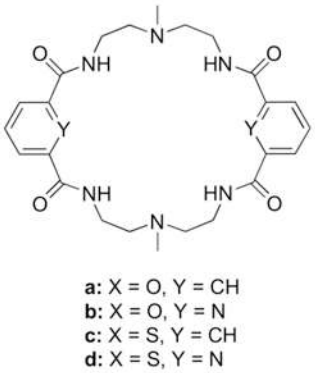

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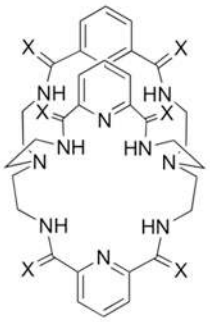

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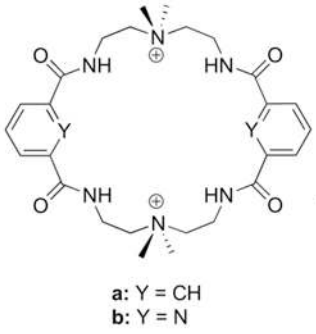

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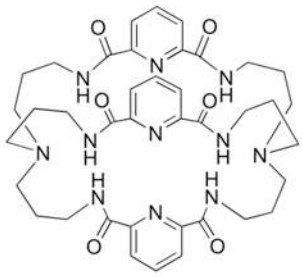

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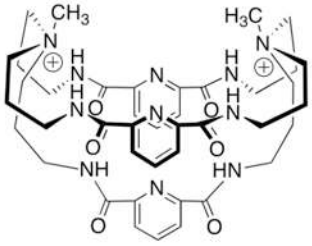

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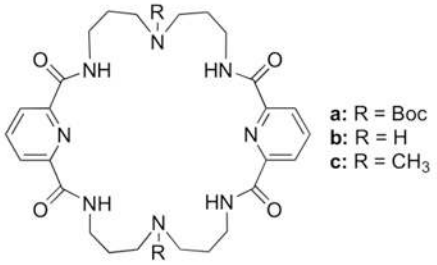

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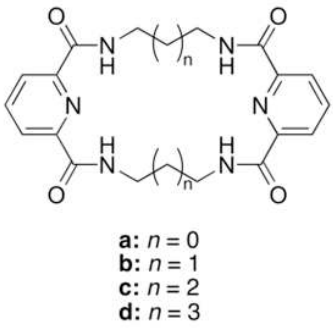

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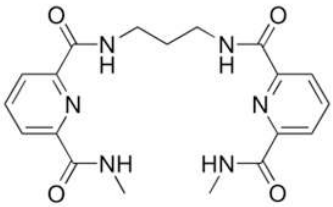

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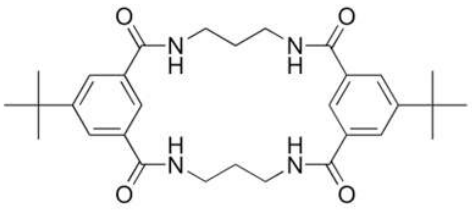

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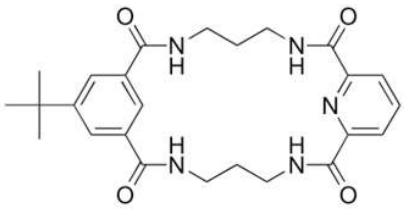

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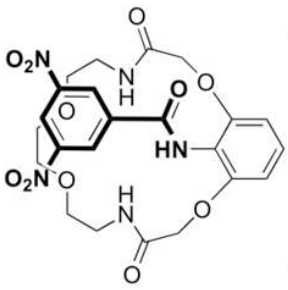

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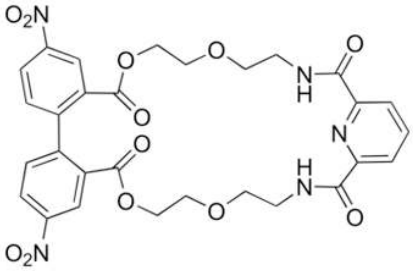

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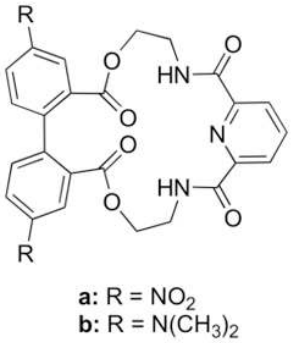

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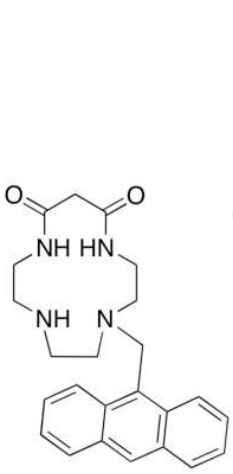

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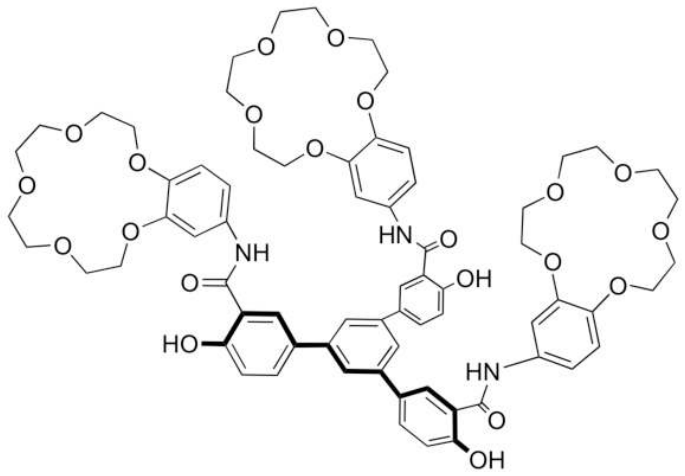

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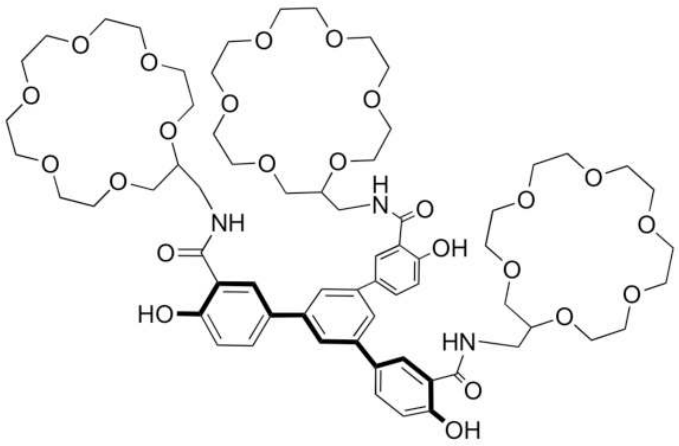

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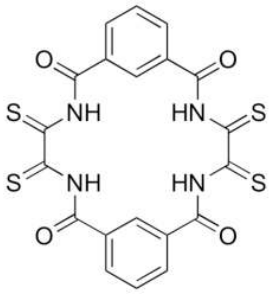

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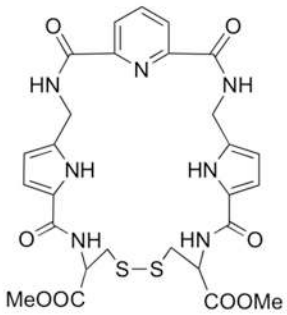

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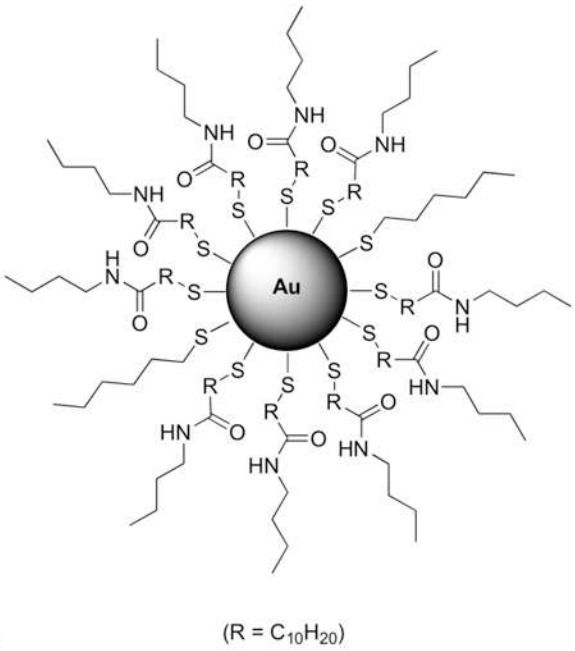
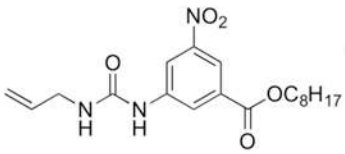
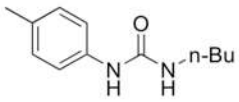


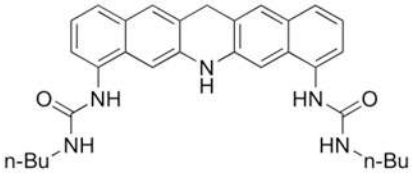
297



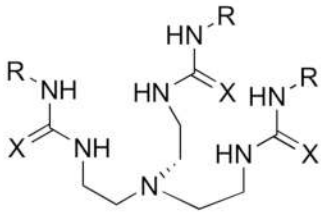
298



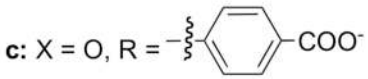
299

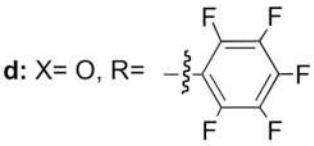


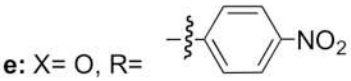
300



a: X = O, R =Ph
b: X = S, R = Ph







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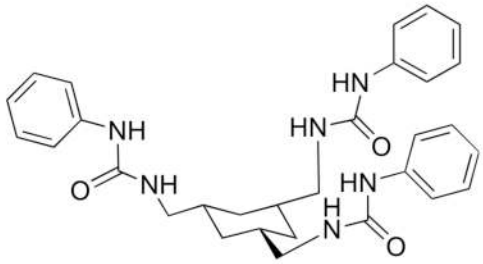
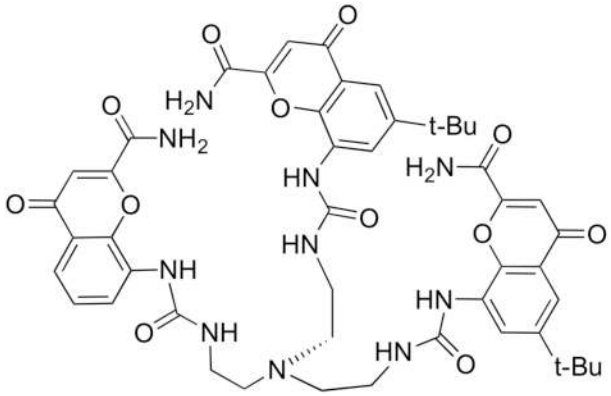
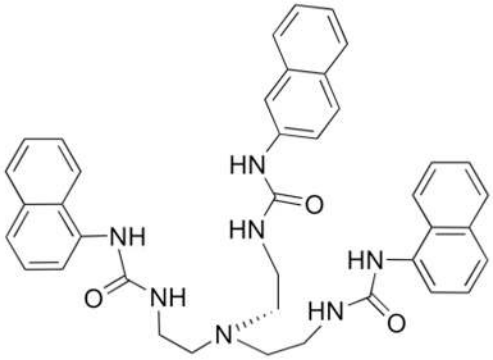


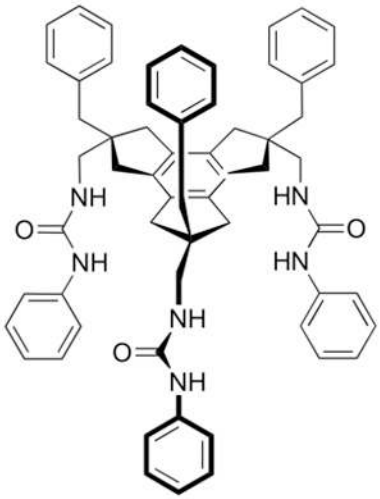
302



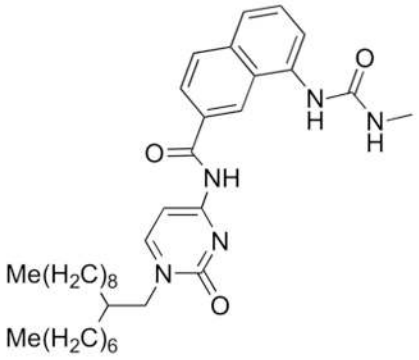
303



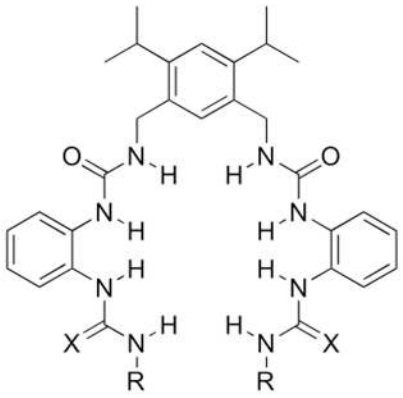
304



305

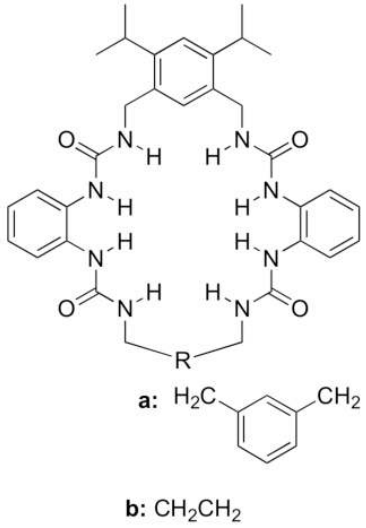


306

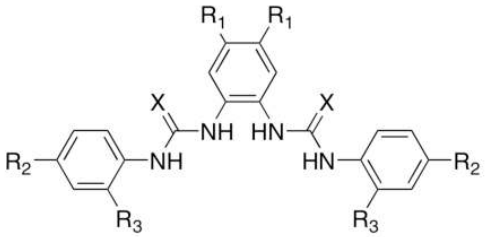


a: X = O, R = C_3H_7
b: X = O, R = Phenyl
c: X = O, R = SO_2Phenyl
d: X = S, R = Phenyl

307



308



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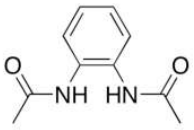

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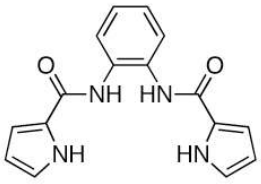
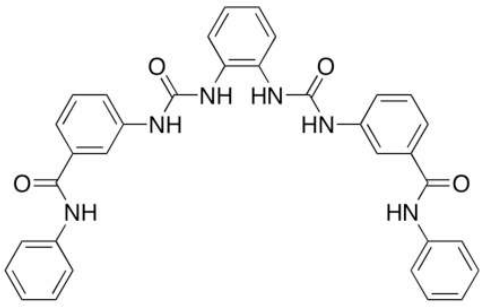
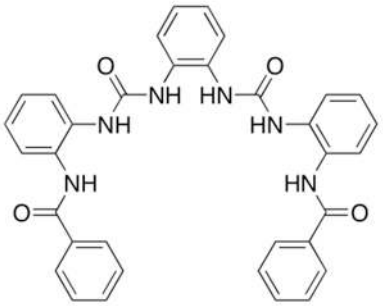


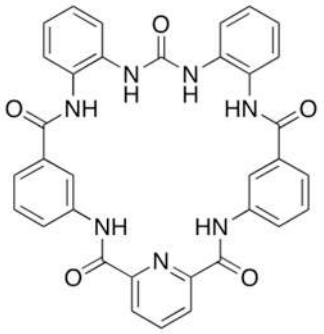
311



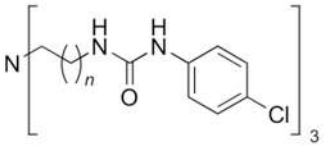
312



313

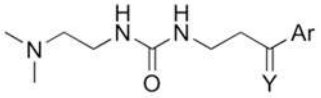


314



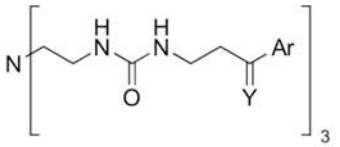
a: *n* = 1
b: *n* = 2

315



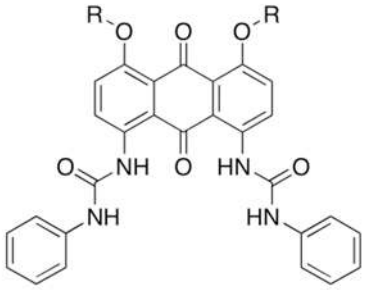
a: Ar = pyren-1-yl, Y = 2H
b: Ar = pyren-1-yl, Y = O
c: Ar = Ph, Y = 2H

316



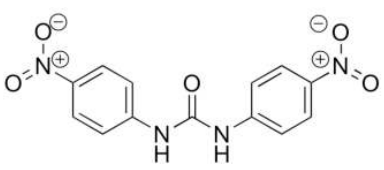
a: Ar = pyren-1-yl, Y = O
b: Ar = pyren-1-yl, Y = 2H
c: Ar = Ph, Y = 2H

317

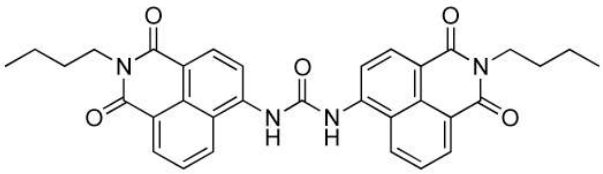


a: R = H
b: R = Me
c: R = Bu

318



319



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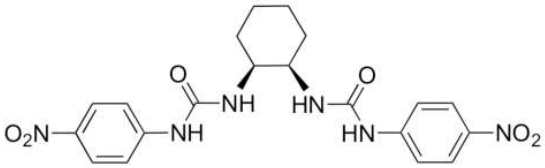

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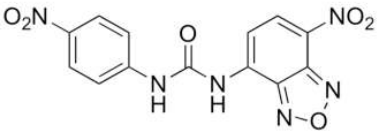
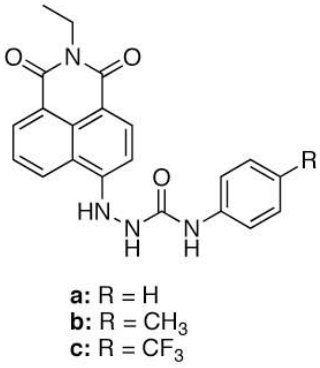
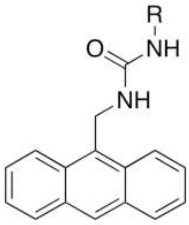


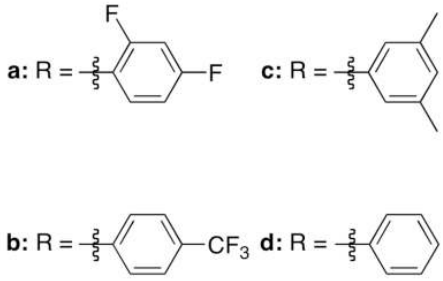
322



a: R = H
b: R = CH_3
c: R = CF_3

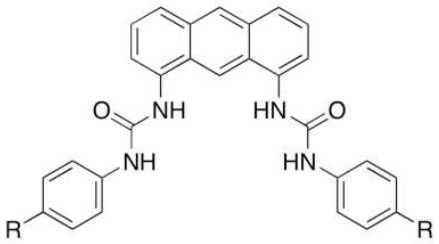


323



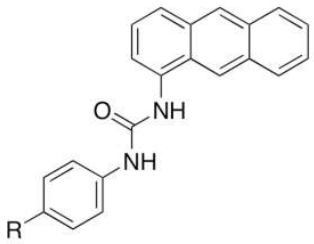
a: R = **b:** R = **c:** R = **d:** R =

324



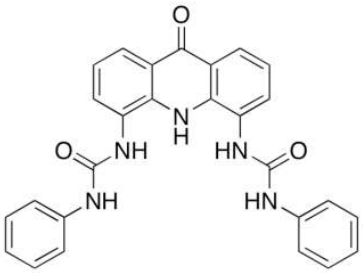
a: R = NO_2
b: R = H

325

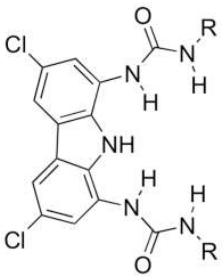


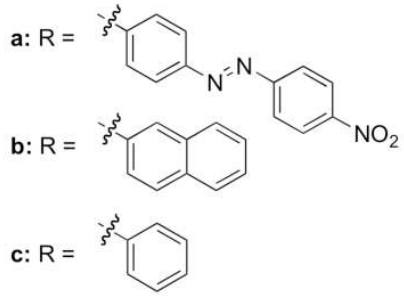
a: R = NO_2
b: R = H

326

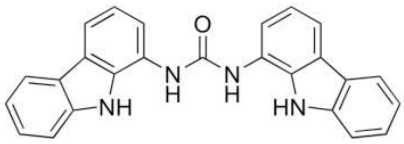


327

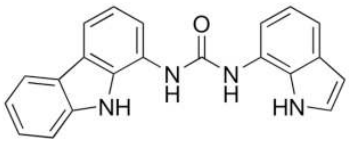




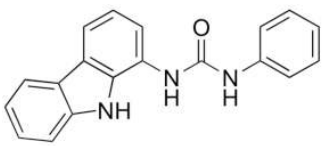
328



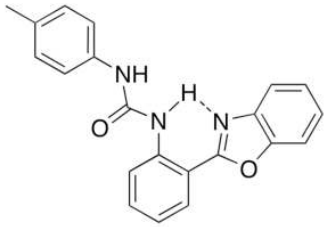
329



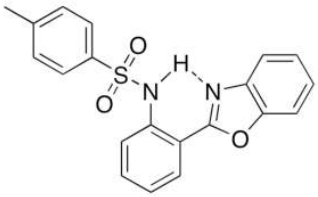
330



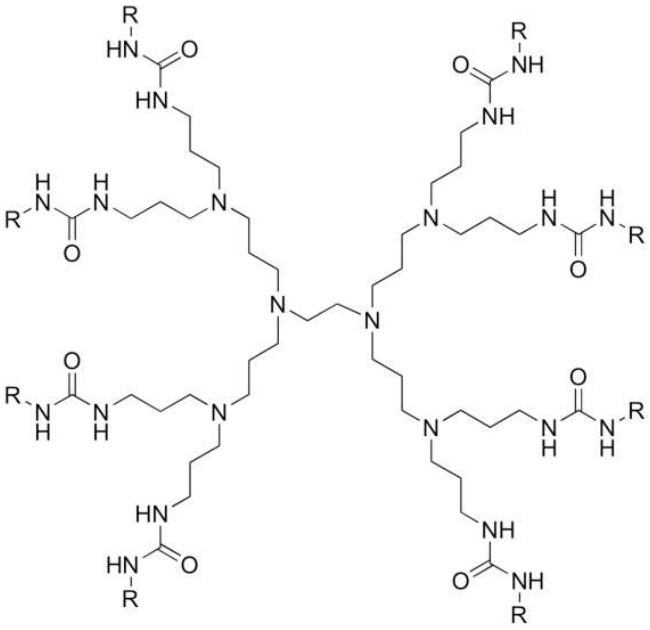
331



332

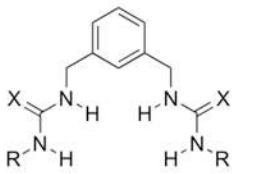


333



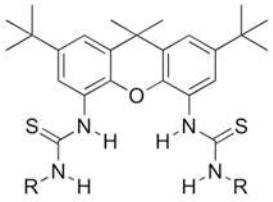
a: R = C_6H_{13}
b: R = C_8H_{17}
c: R = $C_{12}H_{25}$

334 (G2 shown)



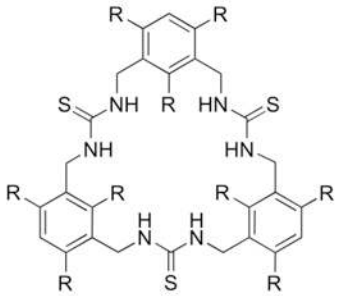
a: X = O, R = Bu
b: X = S, R = Bu
c: X = S, R = Ph
d: X = S, R = 1-naphthyl
e: X = S, R = *p*-nitrophenyl

335



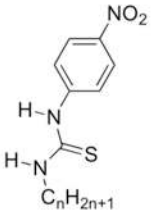
a: R = Bu
b: R = Ph

336



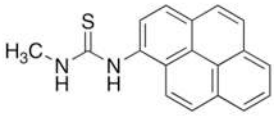
a: R = H
b: R = Et

337

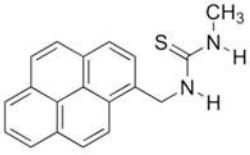


n = 1-8,12

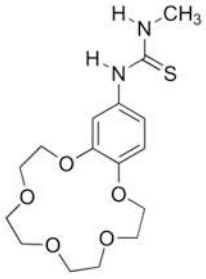
338



339



340



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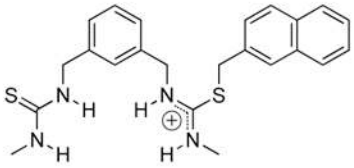

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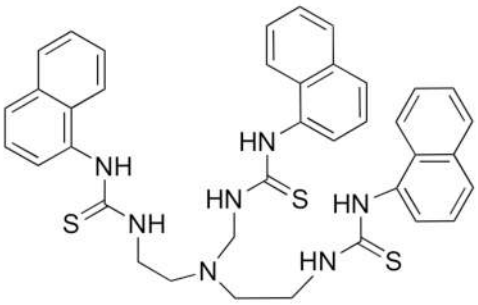

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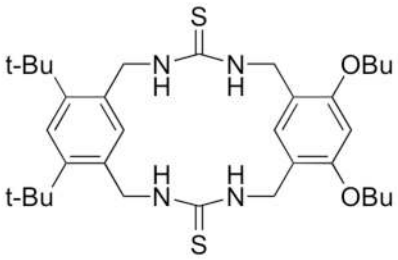

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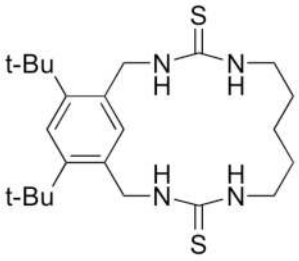

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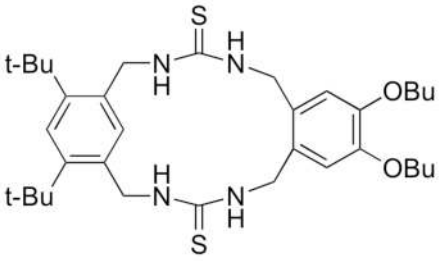

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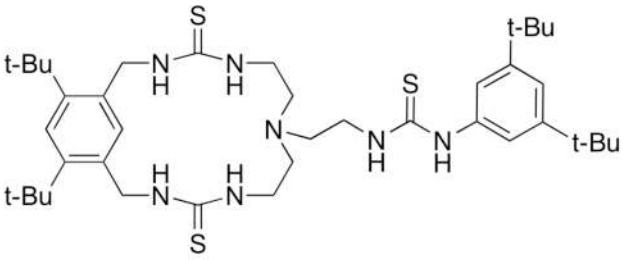

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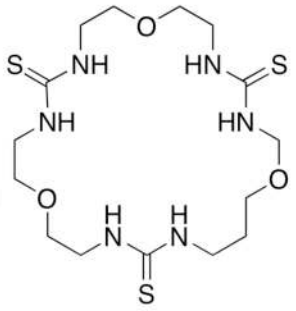

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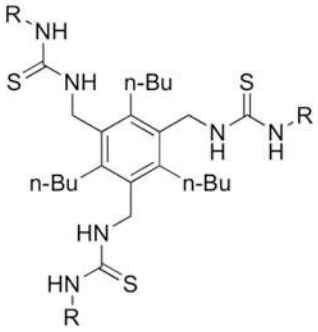
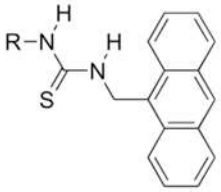


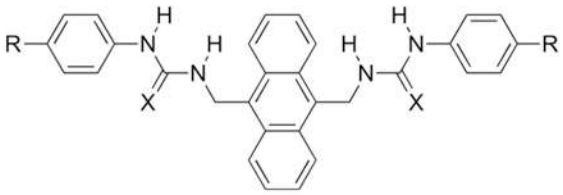
a: R = pyren-1-yl
b: R = 9-anthrylmethyl

349



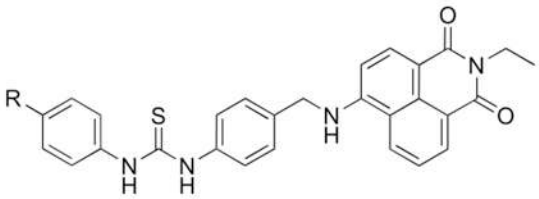
a: R = *p*-CF_3phenyl
b: R = phenyl
c: R = CH_3

350



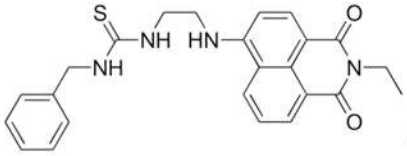
a: R = CF_3, X = S
b: R = H, X = S
c: R = CF_3, X = O

351



a: R = H
b: R = CF_3
c: R = CH_3

352



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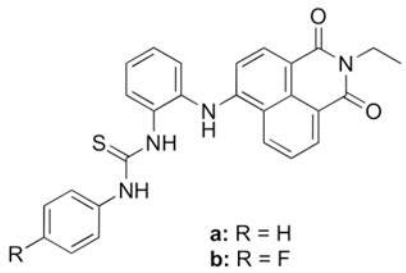

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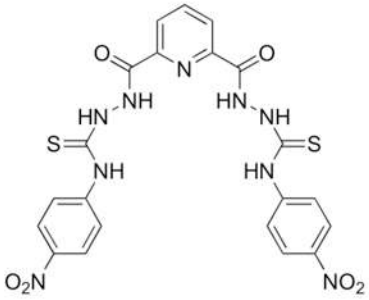

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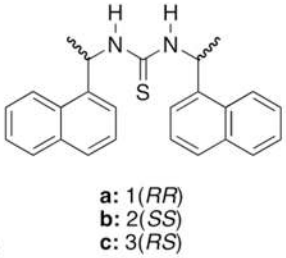

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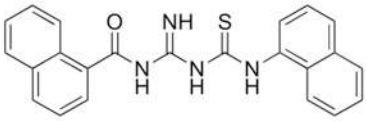

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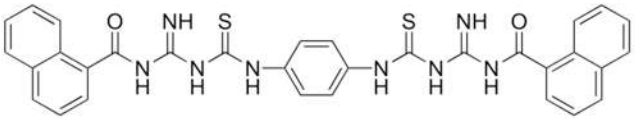
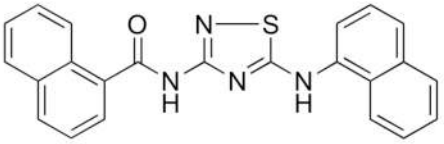


358



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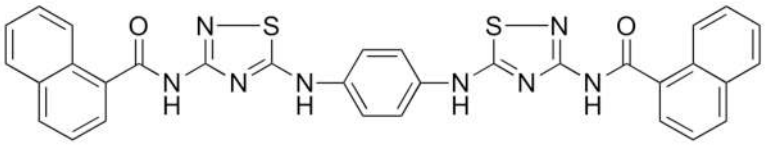

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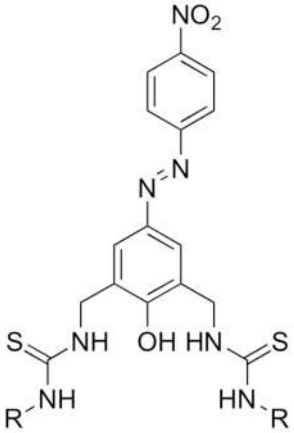
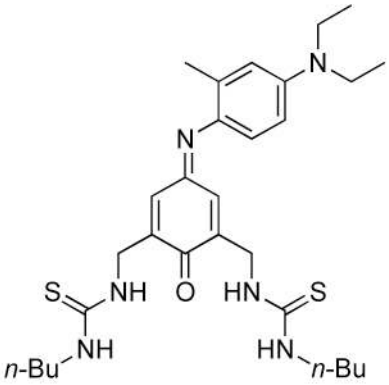


a: R = *n*-Bu
b: R = Ph
c: R = *p*-NO_2Ph

361



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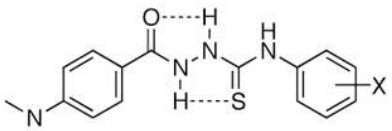
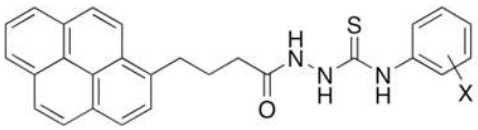


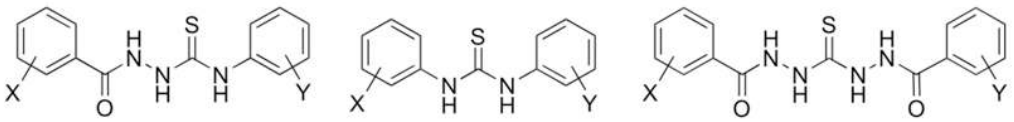
a: X = H
b: X = *p* - OC_2H_5
c: X = *p* - CH_3
d: X = *m* - CH_3
e: X = *p* - Cl
f: X = *p* - Br
g: X = *m* - Cl
h: X = *p* - NO_2

363



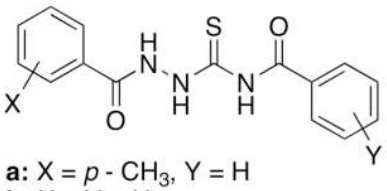
a: X = *p* - CH_3
b: X = H
c: X = *m* - CF_3

364



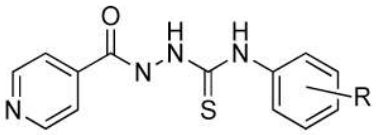
a: X = Y = H
b: X = *p* - OC_2H_5, Y = H
c: X = *p* - CH_3, Y = H
d: X = *m* - CH_3, Y = H
e: X = *p* - Cl, Y = H
f: X = *p* - Br, Y = H
g: X = *m* - Cl, Y = H
h: X = *p* - NO_2, Y = H
i: X = Y = *p* - OEt
j: X = Y = *p* - CH_3
k: X = Y = *p* - Cl
l: X = Y = *m* - Cl

365 **366** **367**



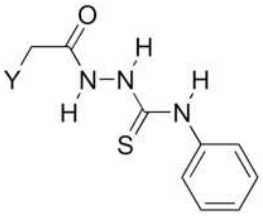
a: X = *p* - CH_3, Y = H
b: X = Y = H
c: X = *m* - Cl, Y = H
d: X = *p* - $N(CH_3)_2$, Y = H
e: X = *p* - $N(CH_3)_2$, Y = *p* - $N(CH_3)_2$
f: X = *p* - $N(CH_3)_2$, Y = *p* - OCH_2CH_3
g: X = *p* - $N(CH_3)_2$, Y = *p* - CH_3
h: X = *p* - $N(CH_3)_2$, Y = *p* - Cl

368



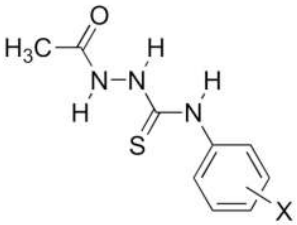
a: R = *p* - OCH_3
b: R = *p* - CH_3
c: R = H
d: R = *m* - Br
e: R = *m* - CF_3

369



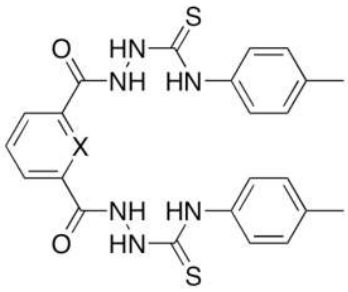
a: Y = H
b: Y = $C_{10}H_{21}$
c: Y = $N(CH_3)_2$
d: Y = OCH_3

370



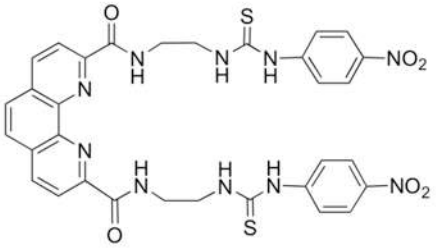
a: X = *p* - OCH_3
b: X = *p* - OCH_3
c: X = H
d: X = *m* - Br
e: X = *m* - CF_3
f: X = *m* - NO_2

371



a: X = CH
b: X = N

372



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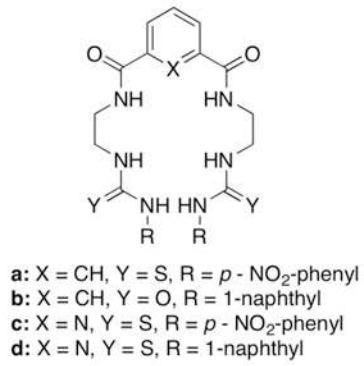

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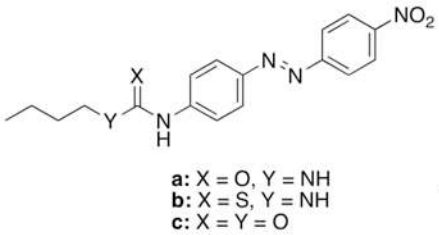

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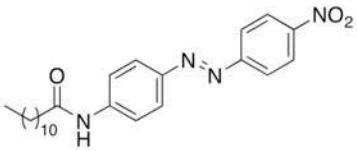

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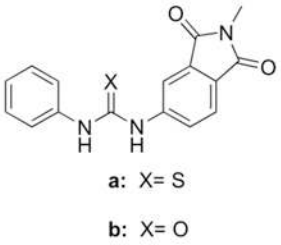

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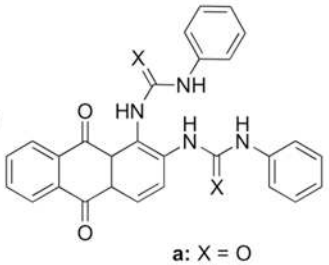

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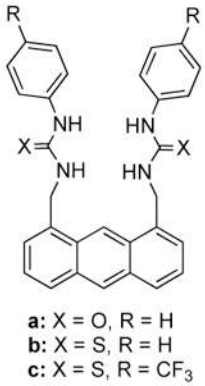

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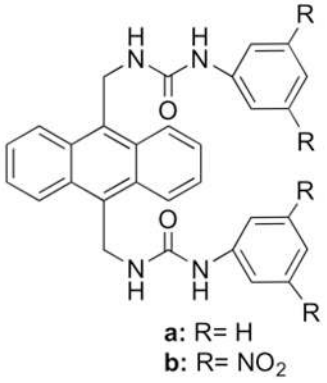

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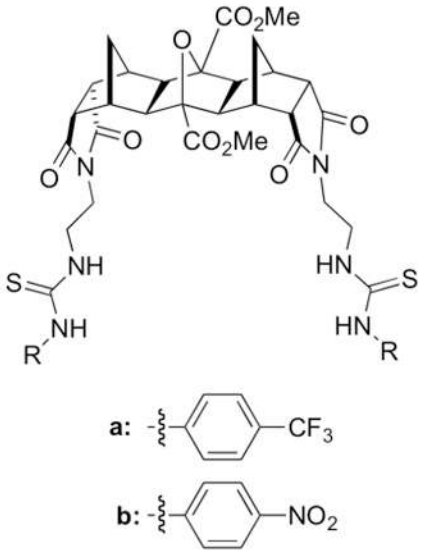

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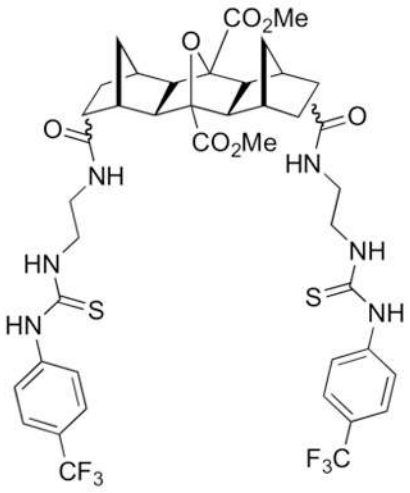

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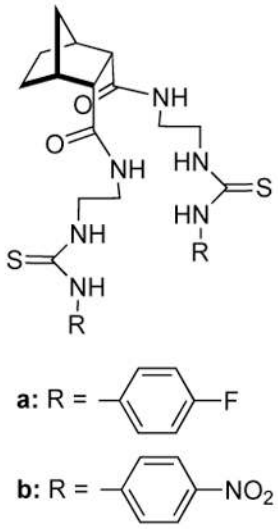

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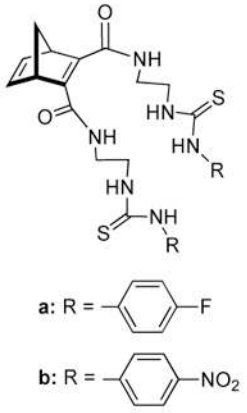

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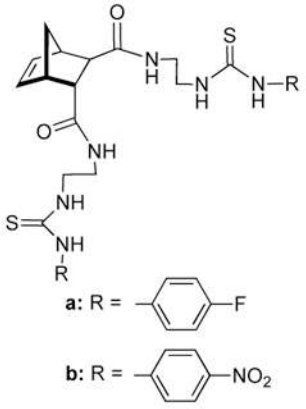

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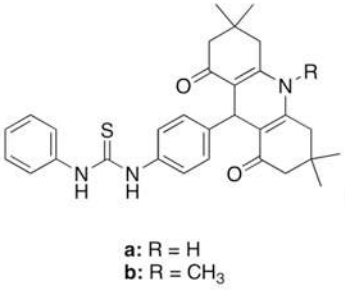

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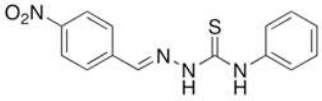
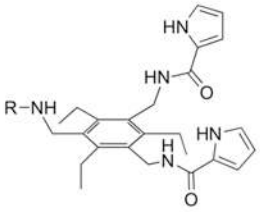
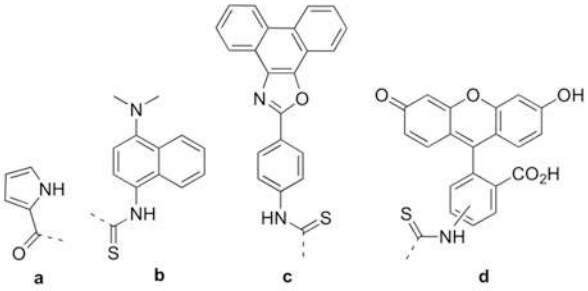


387





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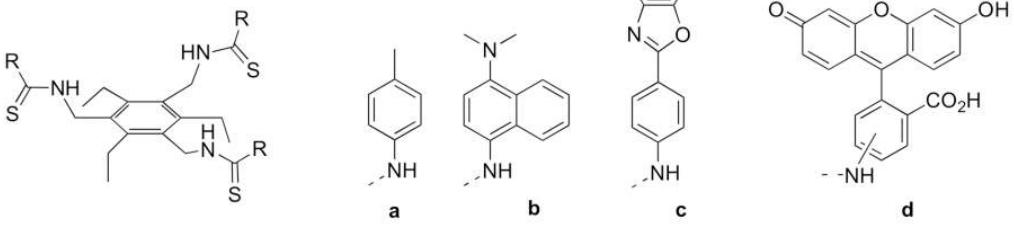

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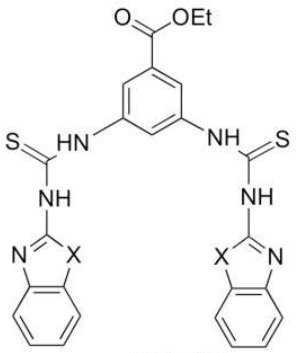

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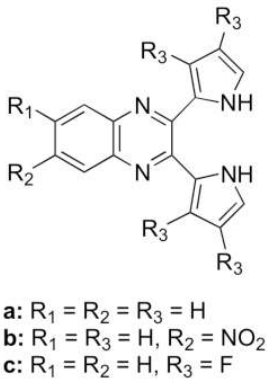

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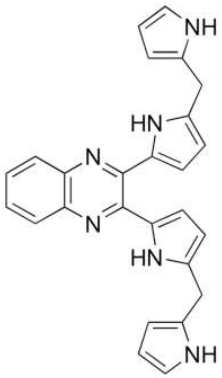

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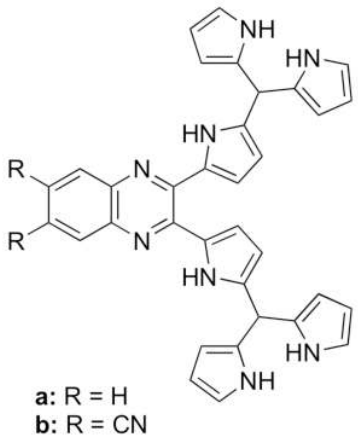

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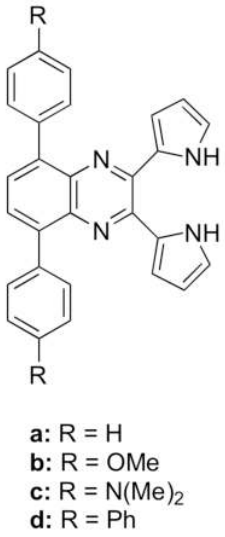

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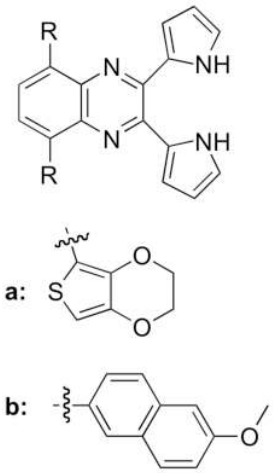

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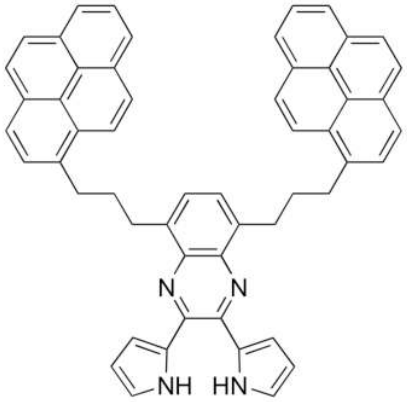

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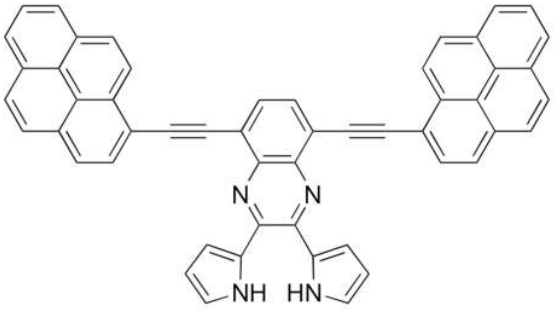

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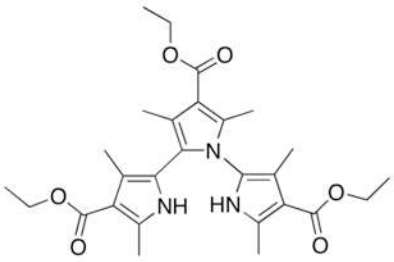

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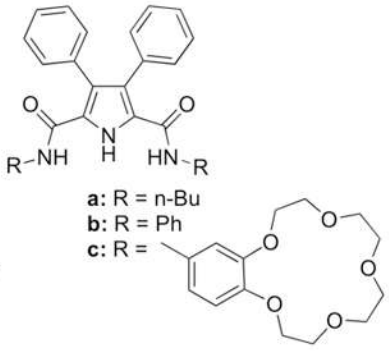

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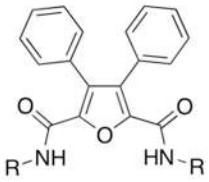

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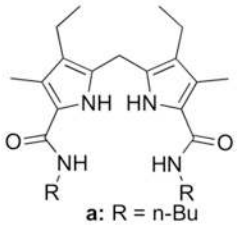

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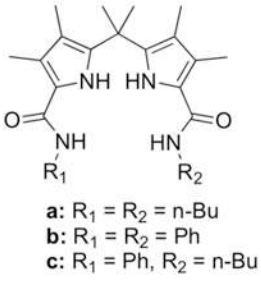

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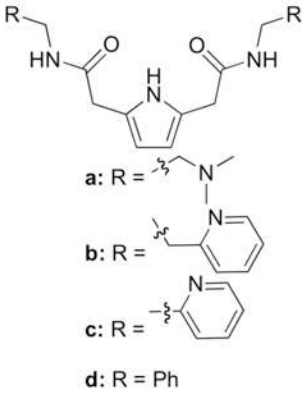
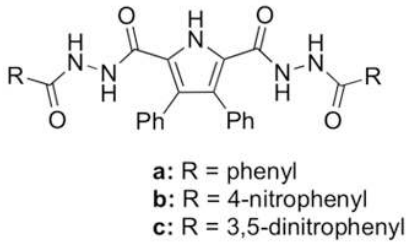


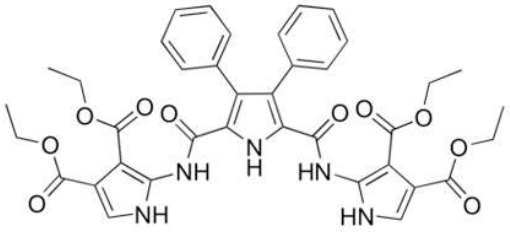
403

404

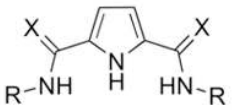


a: R = phenyl
b: R = 4-nitrophenyl
c: R = 3,5-dinitrophenyl

405

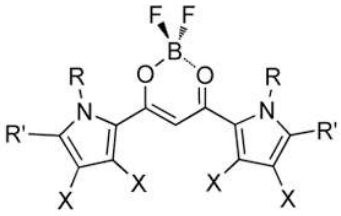


406



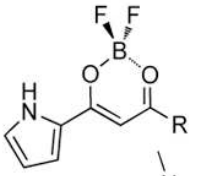
a: X = O, R = n-Bu
b: X = O, R = Ph
c: X = S, R = n-Bu
d: X = S, R = Ph

407



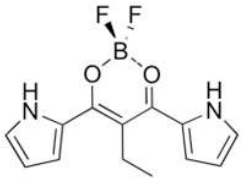
a: R = H, R' = neopentyl, X = H
b: R = R' = H, X = F
c: R = R' = H, X = ethyl
d: R = H, R' = CO_2Et, X = ethyl
e: R = H, R' = Ph, X = ethyl

408

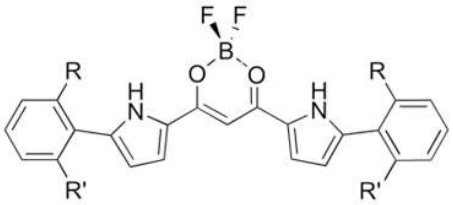


a: R =
b: R = CH_3

409

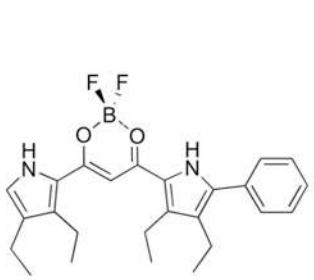


410



a: R = R' = H
b: R = H, R' = Me
c: R = R' = Me

411



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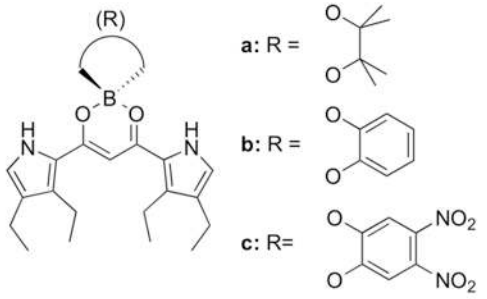

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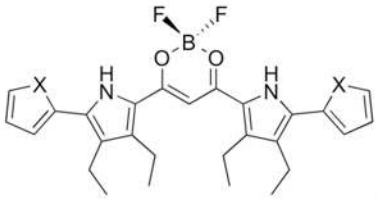

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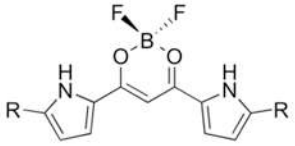

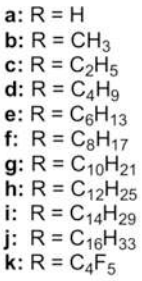

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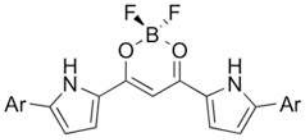

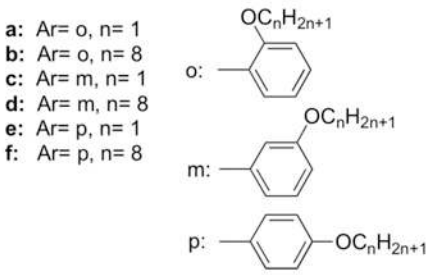

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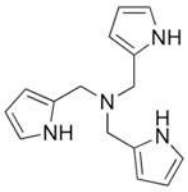

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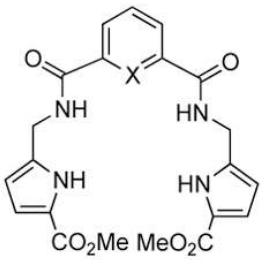
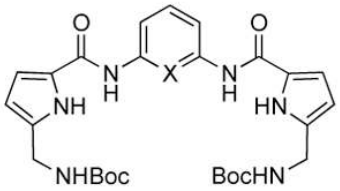


a: X = N

b: X = CH

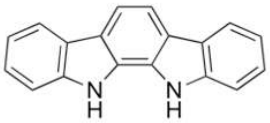
418



a: X = N

b: X = CH

419



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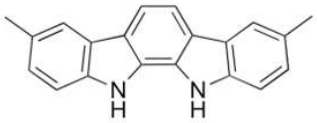

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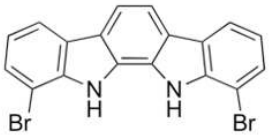

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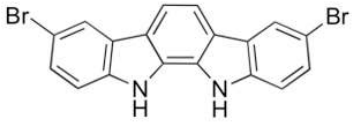

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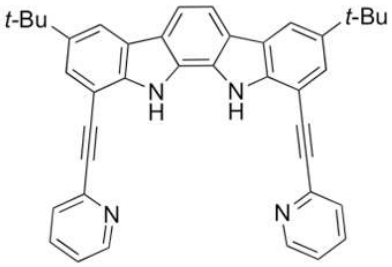

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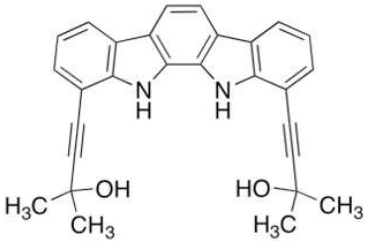

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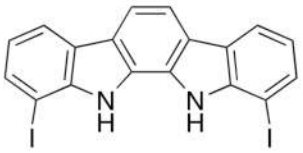

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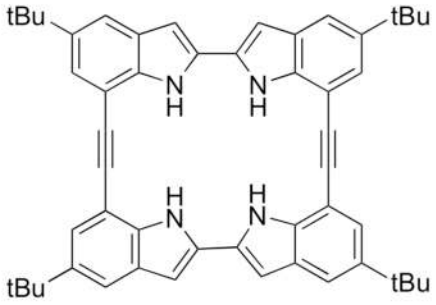

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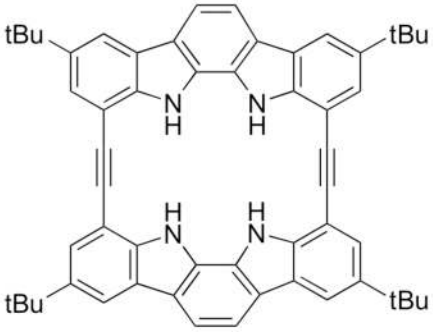

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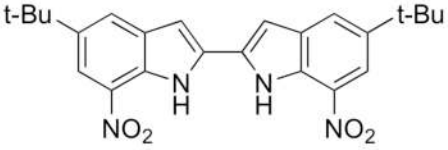

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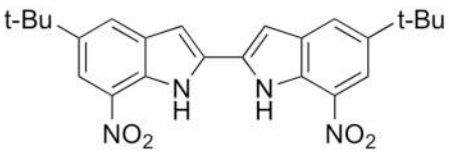

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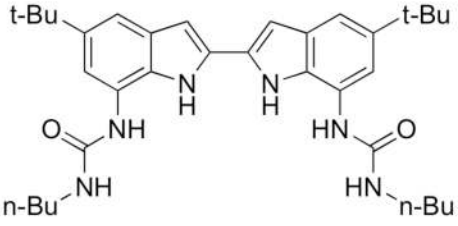

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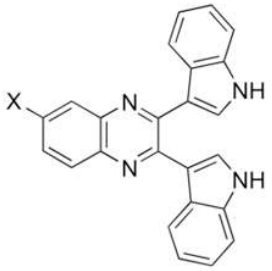
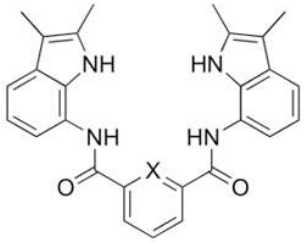


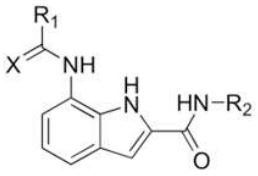
a: X = H
b: X = NO_2

432



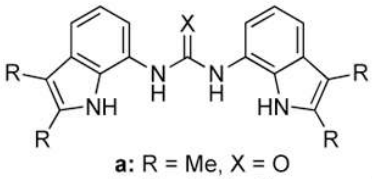
a: X = N
b: X = CH

433



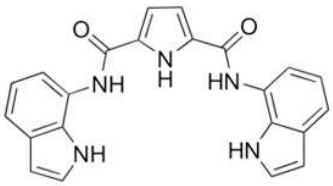
a: $R_1 = R_2$ = Bu, X = O
b: $R_1 = R_2$ = Ph, X = O
c: $R_1 = CH_2Ph$, R_2 = Ph, X = O
d: R_1 = NHBu, R_2 = Bu, X = O
e: R_1 = NHPh, R_2 = Ph, X = O
f: R_1 = NHPh, R_2 = Ph, X = S

434



a: R = Me, X = O
b: R = H, X = O
c: R = Me, X = S
d: R = H, X = S

435



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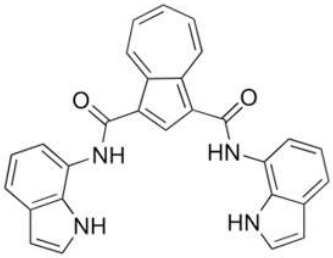
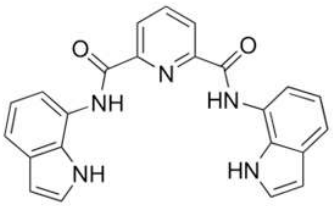


437



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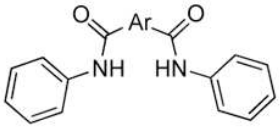
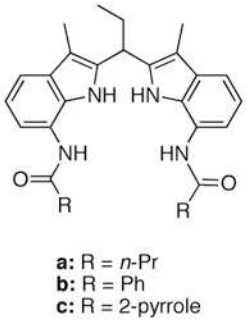


a: Ar = 2,5-pyrrole
b: Ar = 1,3-azulene
c: Ar = 2,6-pyridine

439



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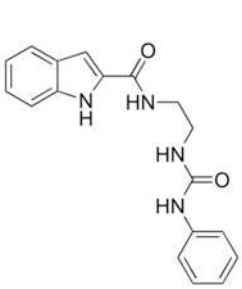

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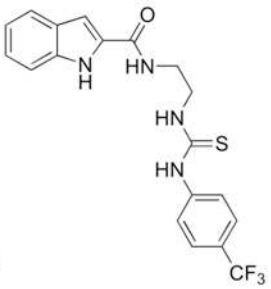

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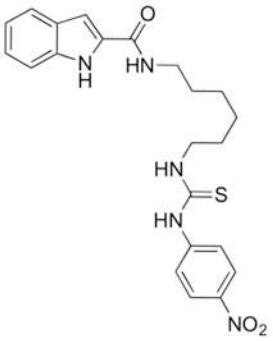

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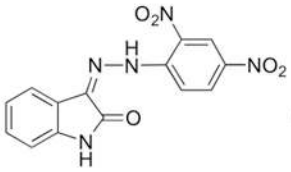

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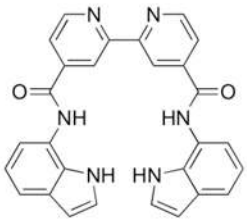

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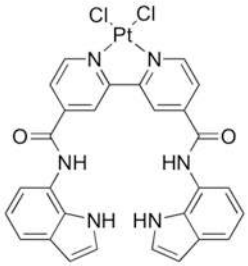

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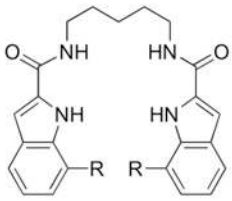

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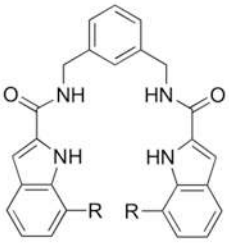

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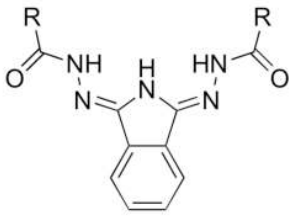
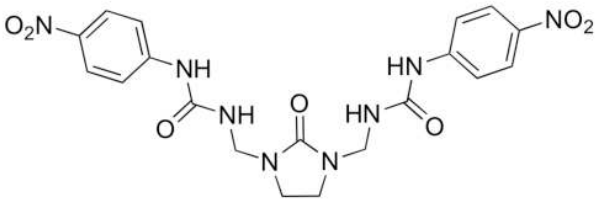


a: R = n-C_5H_{11}
b: R = Ph
c: R = 2-pyridine
d: R = 2-pyrrole

449



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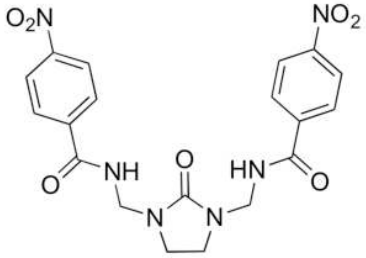

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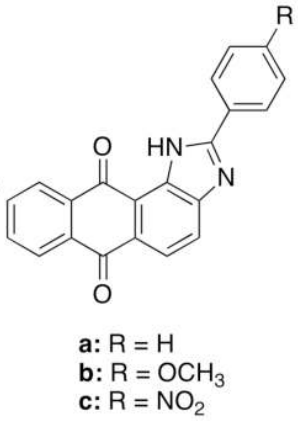
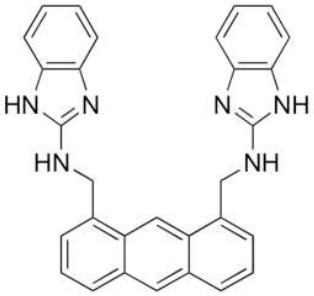


a: R = H
b: R = OCH_3
c: R = NO_2

452



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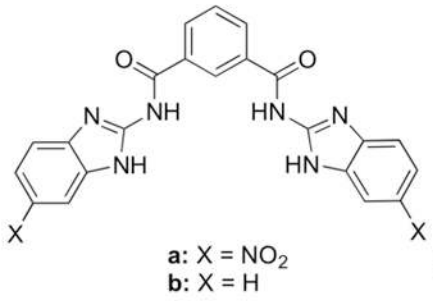

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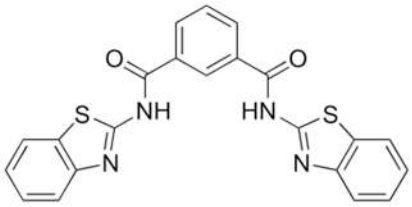

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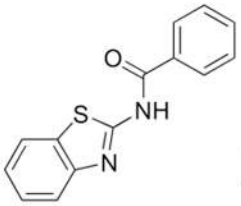

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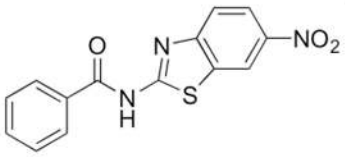

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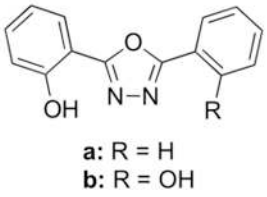

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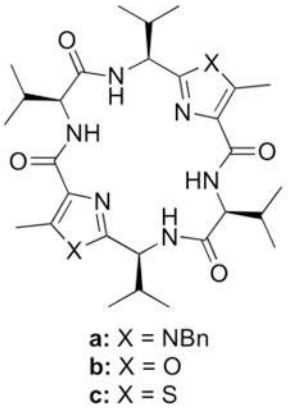

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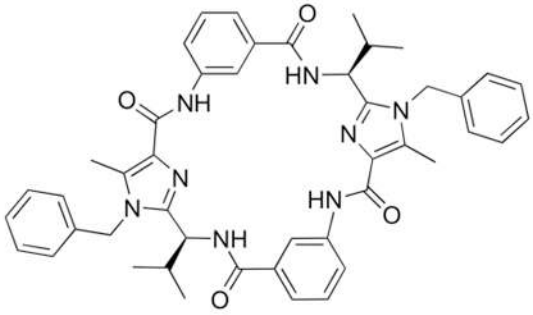

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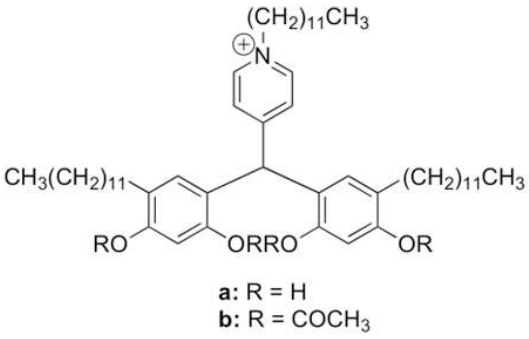

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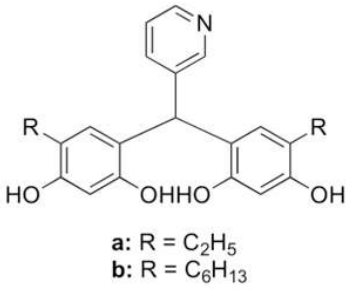

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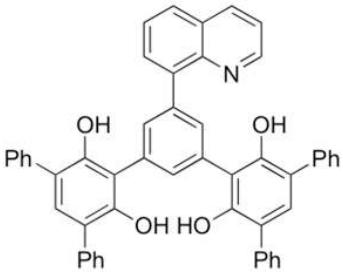

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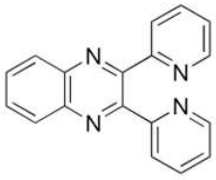

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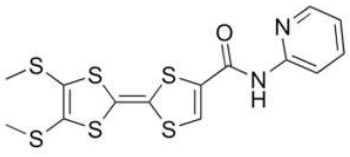

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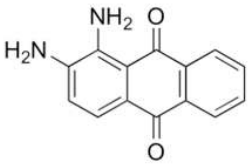

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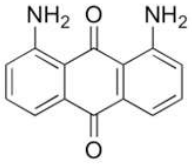

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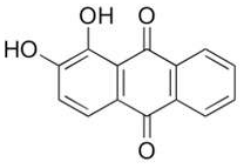

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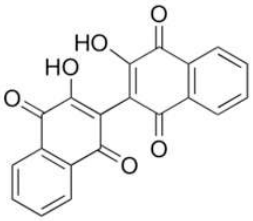

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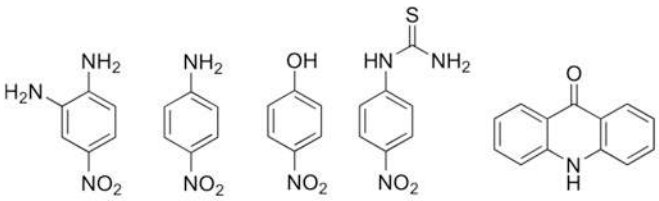

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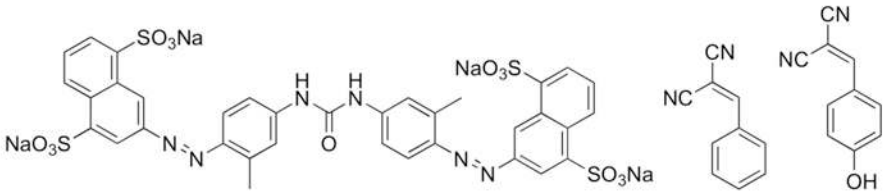

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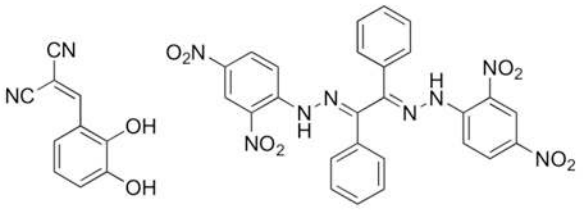

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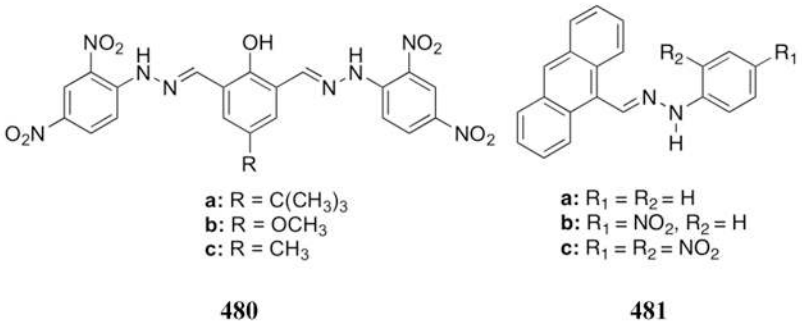
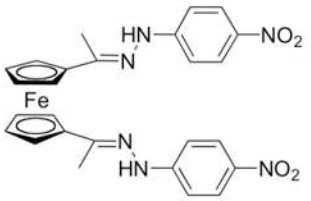


a: R = $C(CH_3)_3$
b: R = OCH_3
c: R = CH_3

a: $R_1 = R_2 = H$
b: $R_1 = NO_2$, $R_2 = H$
c: $R_1 = R_2 = NO_2$

480 481



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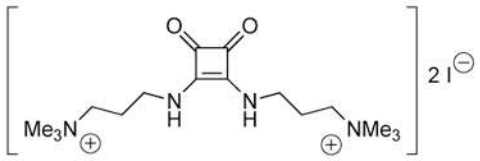

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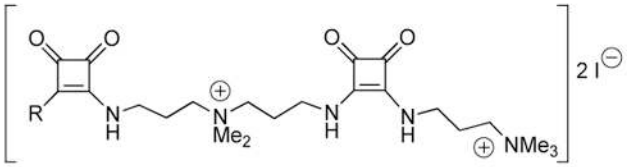
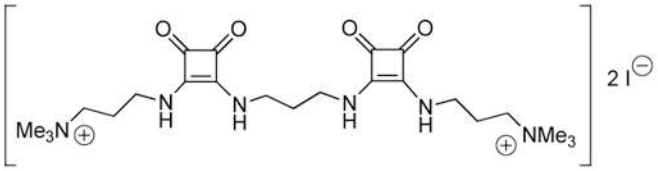


a: R = OEt
b: R = $NH(CH_2)_3NMe_2$

484



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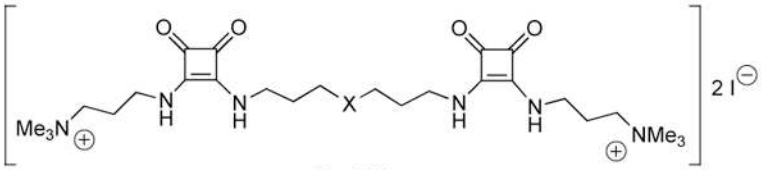
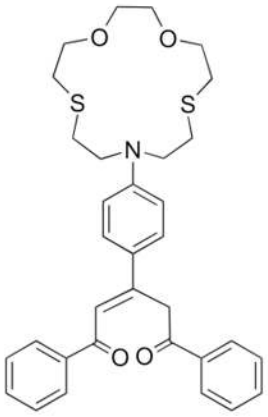


a: R = NH
b: R = CH_2

486



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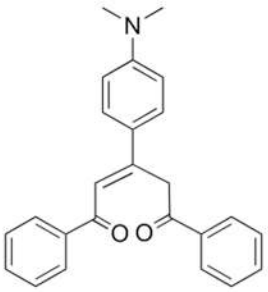

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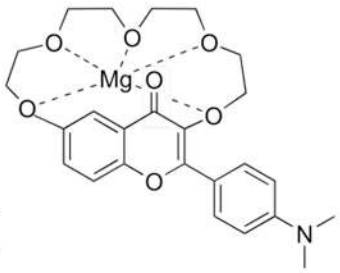

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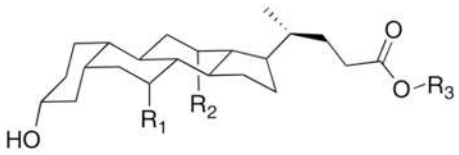
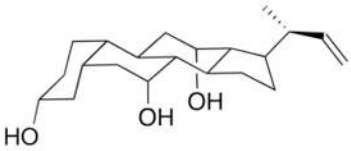


a: R_1 = OH, R_2 = OH, R_3 = CH_3
b: R_1 = OH, R_2 = OH, R_3 = n-C_8H_{17}
c: R_1 = H, R_2 = OH, R_3 = CH_3
d: R_1 = OH, R_2 = H, R_3 = CH_3
e: R_1 = R_2 = H, R_3 = CH_3

490



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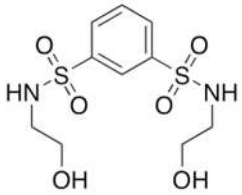

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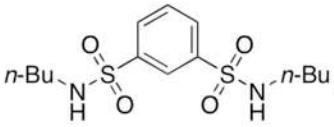

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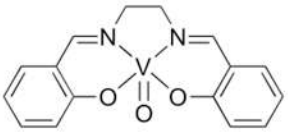

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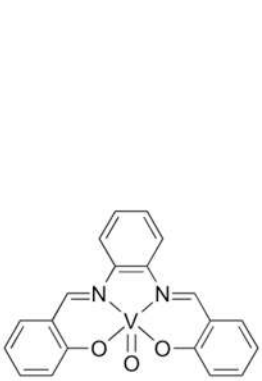

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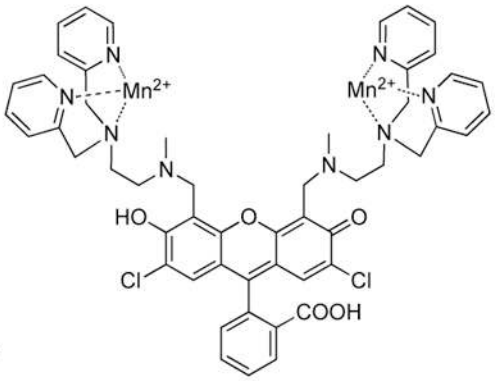

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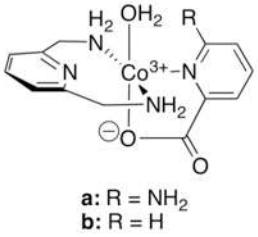
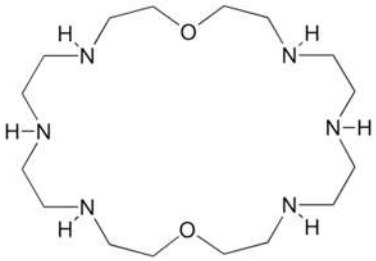


a: R = NH_2
b: R = H

497



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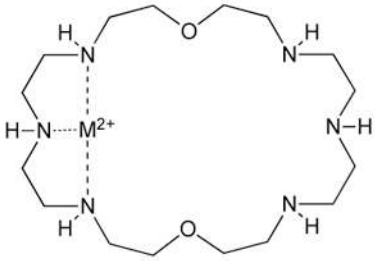

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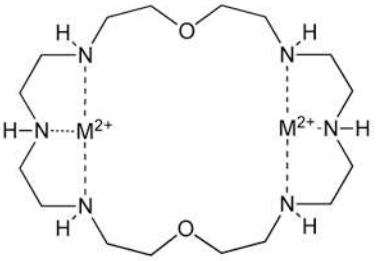

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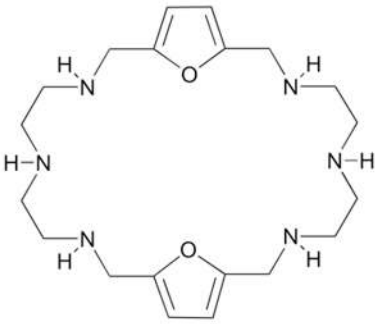

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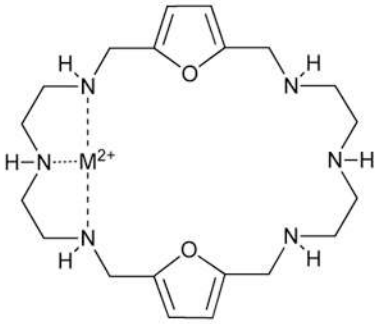

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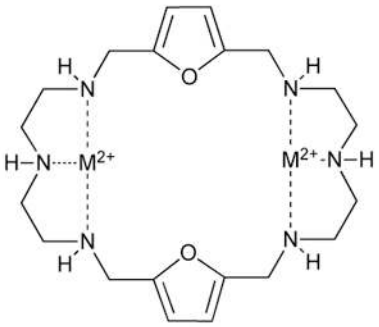

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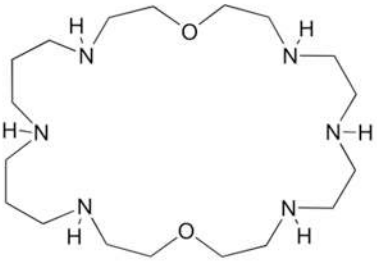

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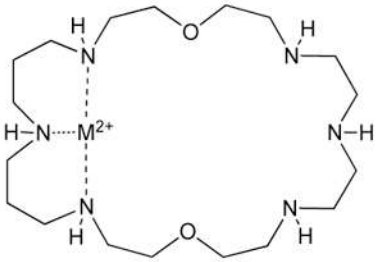

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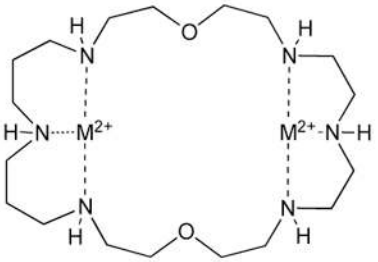

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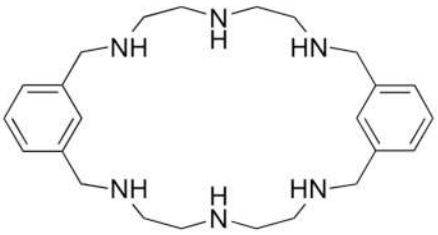

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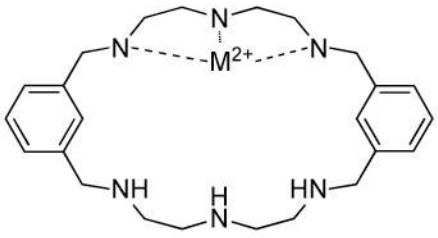

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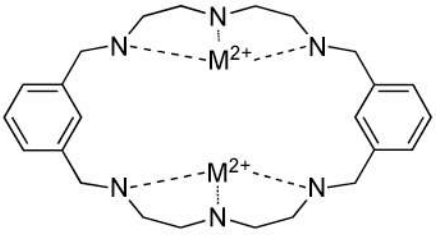

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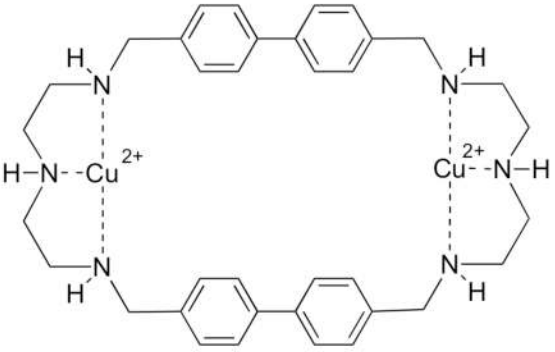

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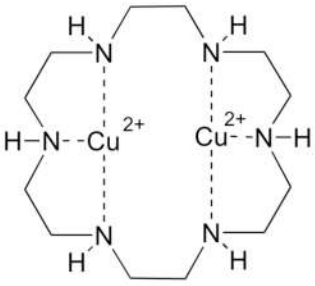

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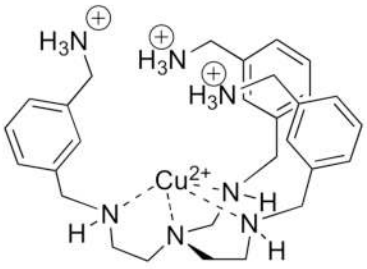

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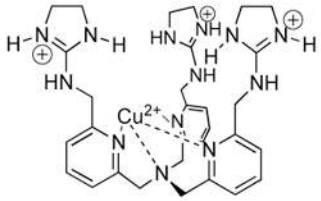

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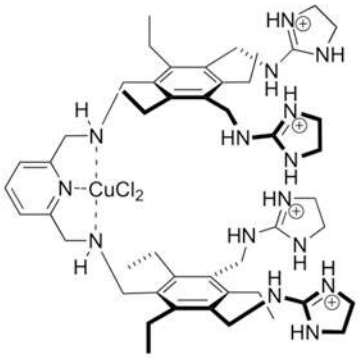

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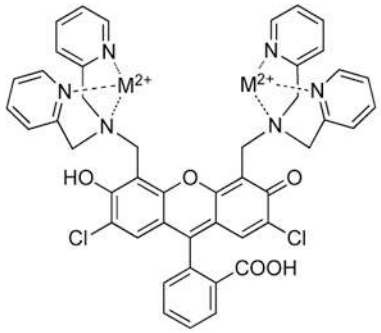
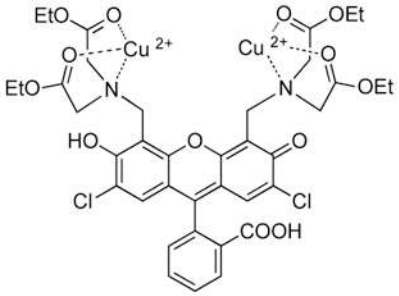


a: M = Zn
b: M = Cu

515



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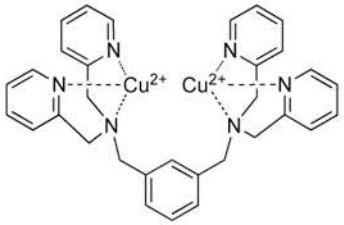

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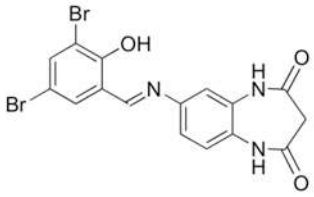

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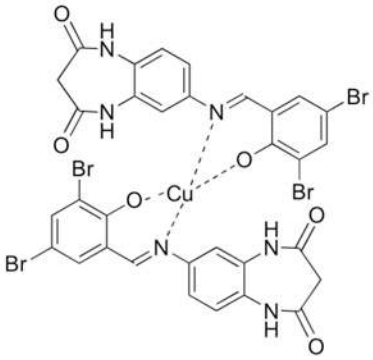

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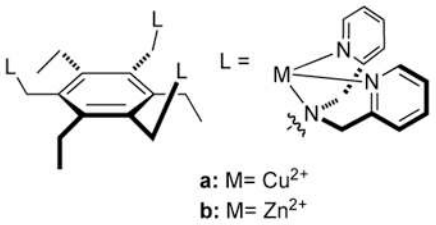

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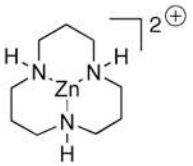

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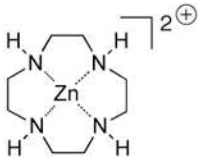

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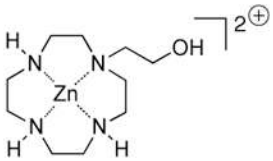

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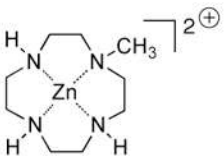

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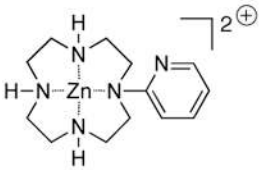

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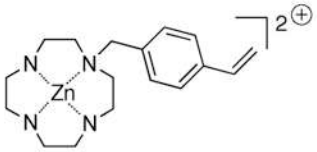

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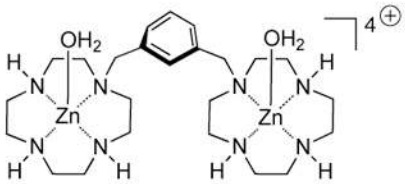

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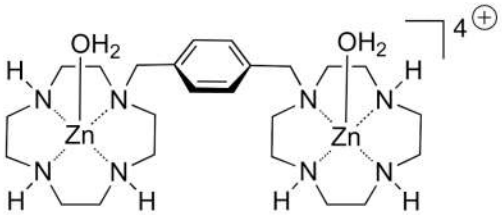

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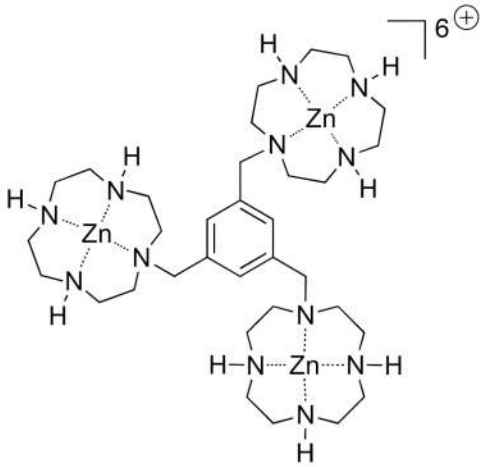

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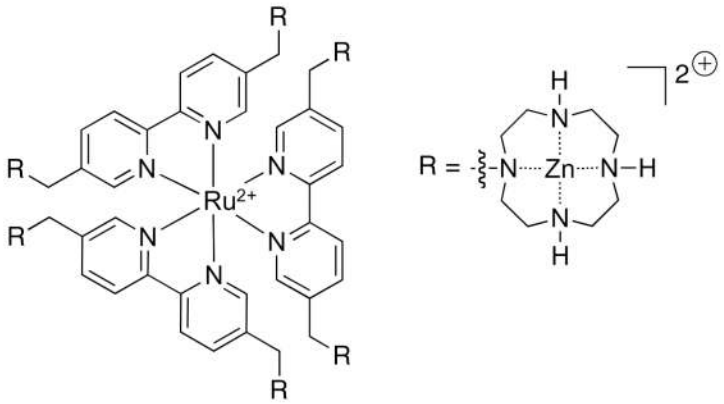

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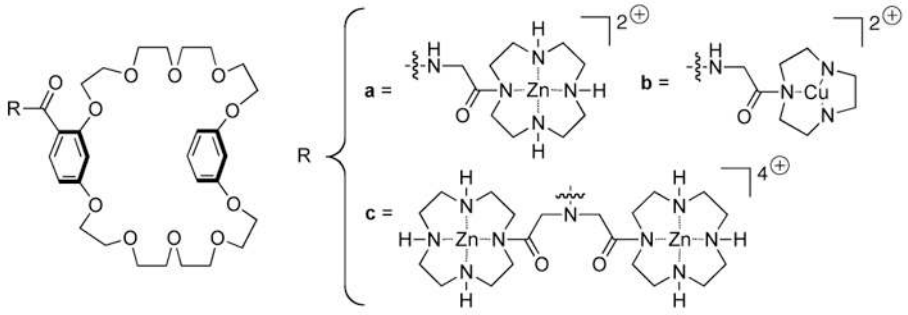

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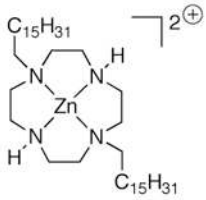

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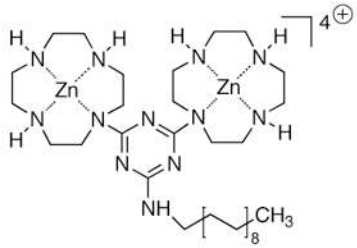

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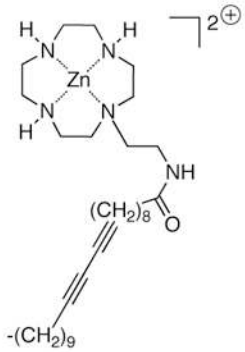

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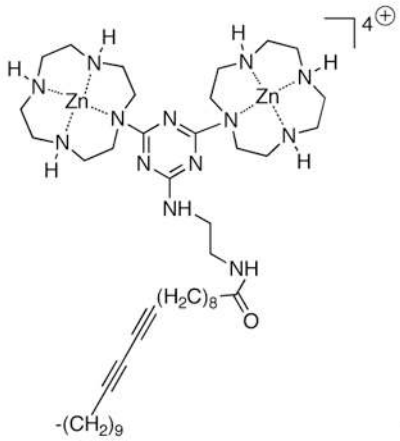

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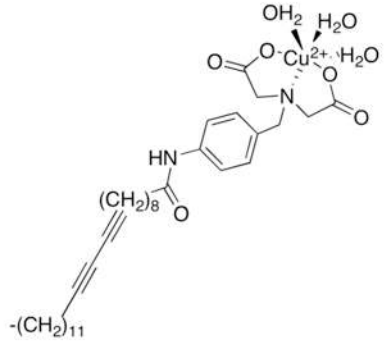

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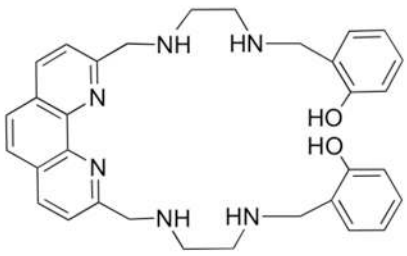

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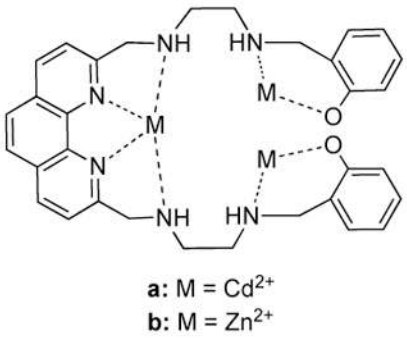

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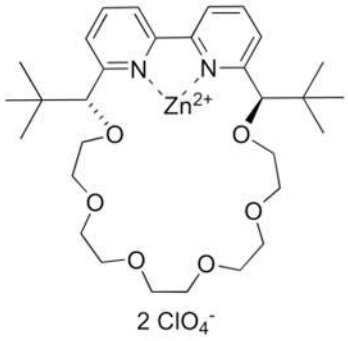

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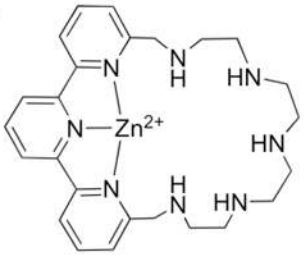

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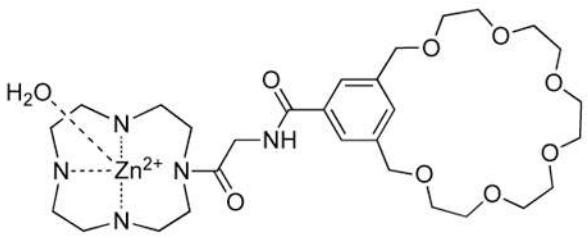

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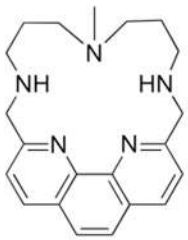

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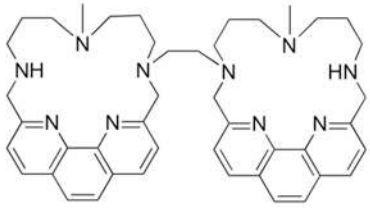

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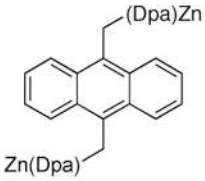

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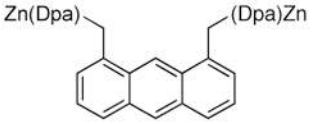

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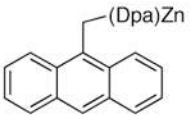

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a: Glu-Glu-Glu-Ile-**p**Tyr-Glu-Glu-Phe-Asp
b: Asp-Glu-Glu-Ile-**p**Tyr-Gly-Glu-Phe-Phe
c: Arg-Arg-Phe-Gly-**p**Ser-Ile-Arg-Arg-Phe
d: Lys-Ser-Gly-**p**Tyr-Leu-Ser-Ser-Glu
e: Ala-Glu-Glu-Ile-**p**Tyr-Gly-Val-Leu-Phe
f: Ala-Arg-Arg-Gly-**p**Ser-Ile-Ala-Ala-Phe
g: Glu-Glu-Glu-Ile-Tyr-Glu-Glu-Phe-Asp

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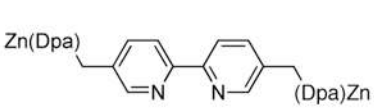

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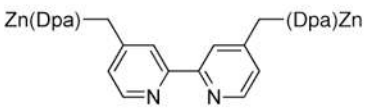

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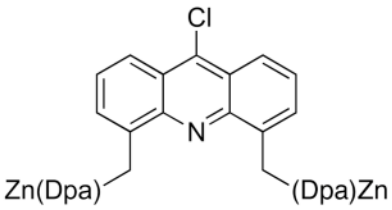

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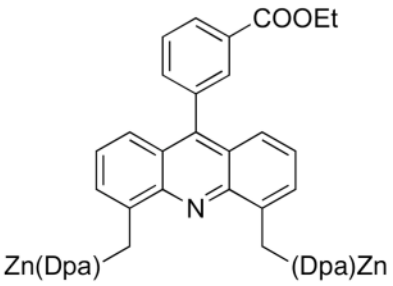

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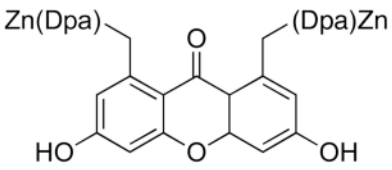

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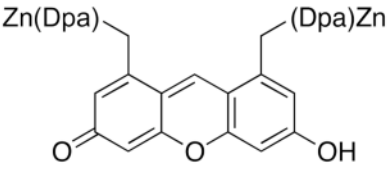

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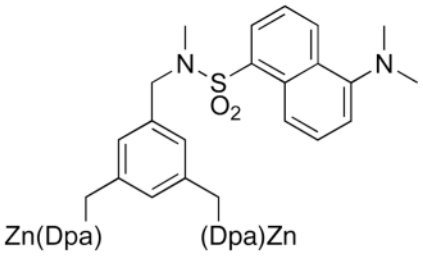

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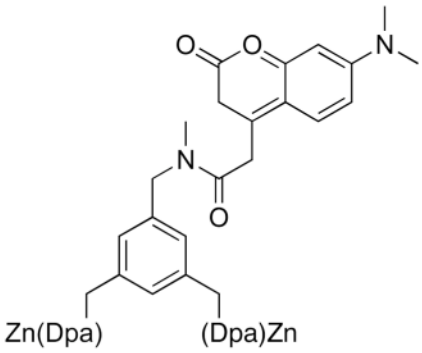

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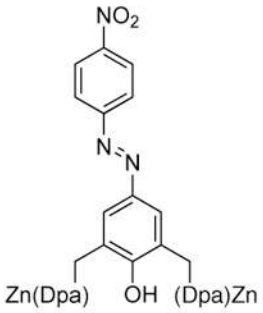

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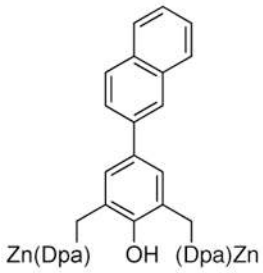

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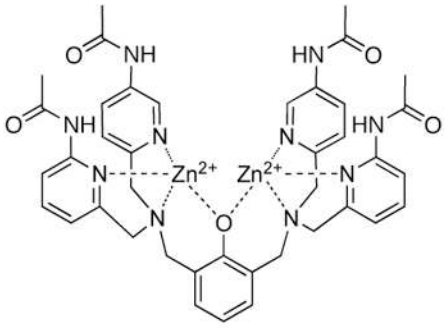

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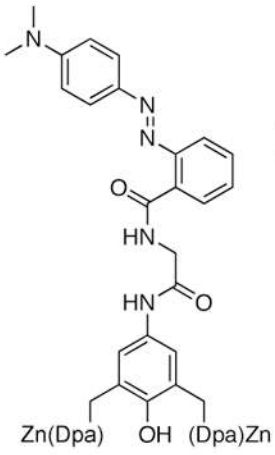

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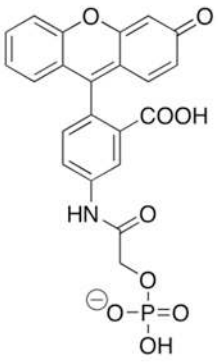

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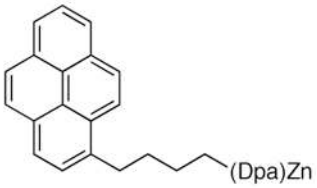

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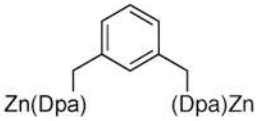

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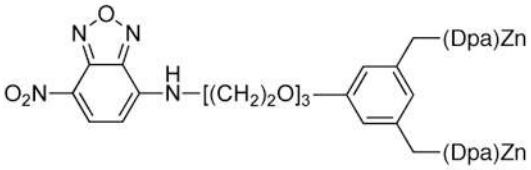

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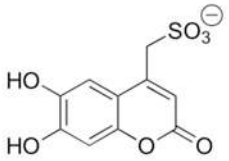

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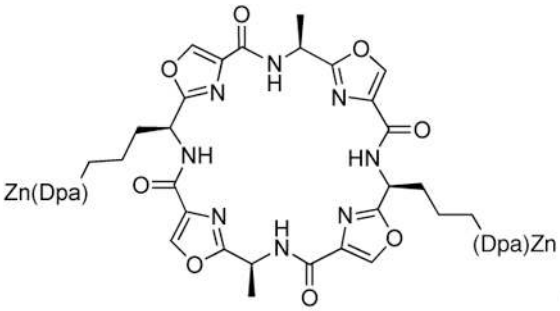

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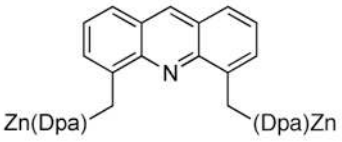

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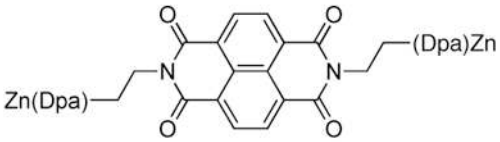

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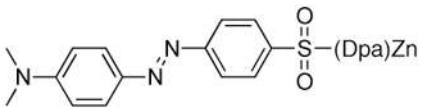

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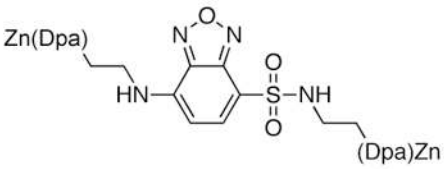

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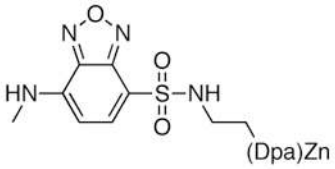

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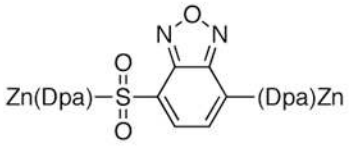

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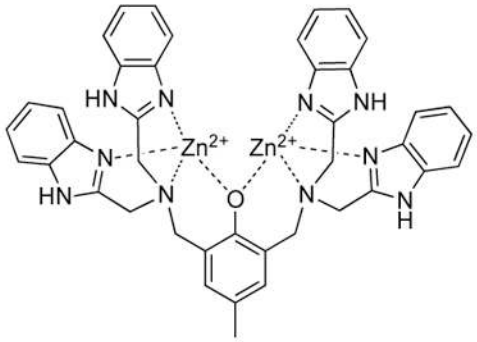

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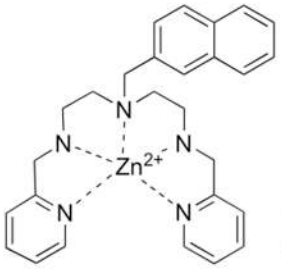

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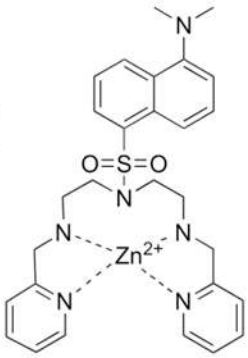

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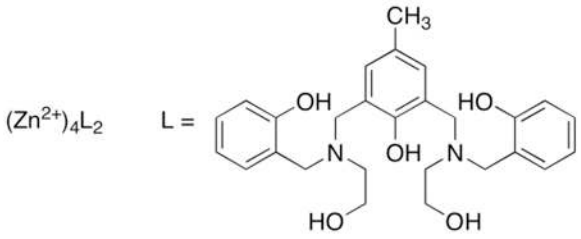

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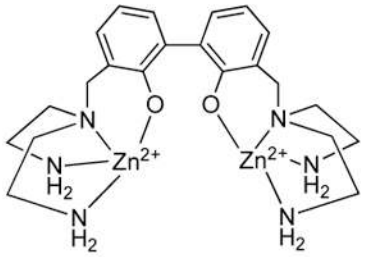

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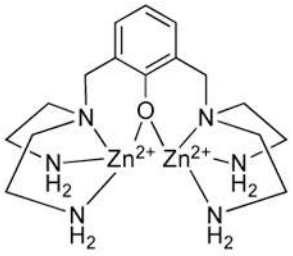

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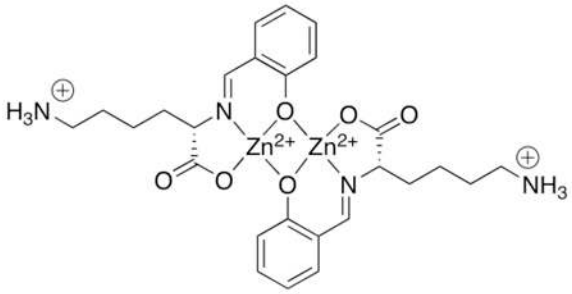

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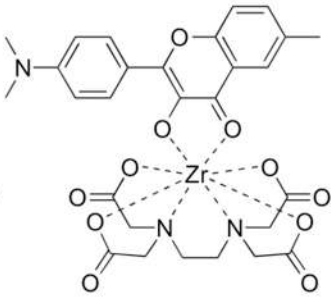

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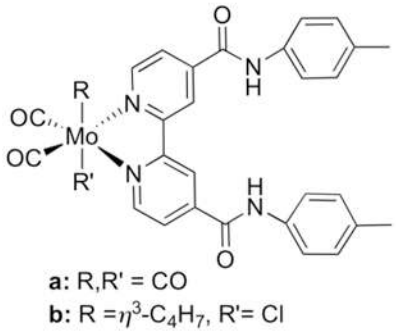

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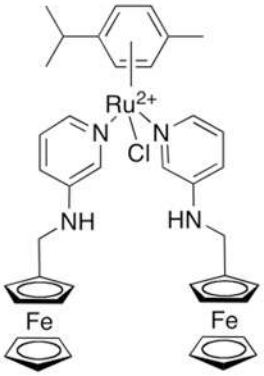

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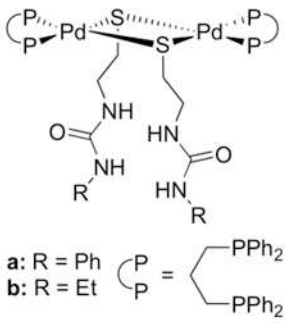

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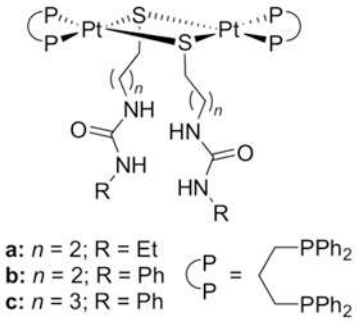

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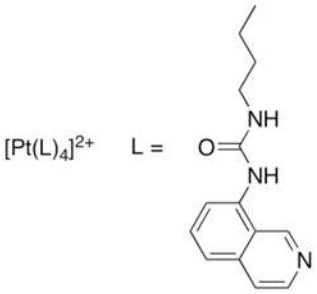

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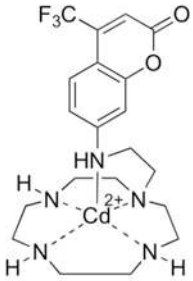

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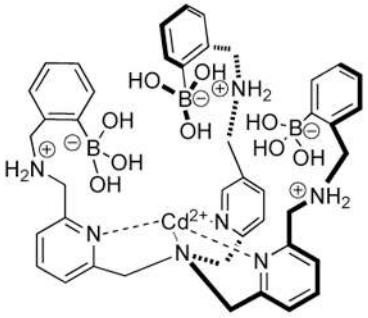

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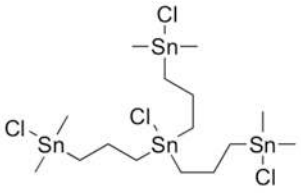

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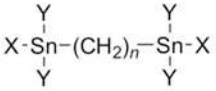
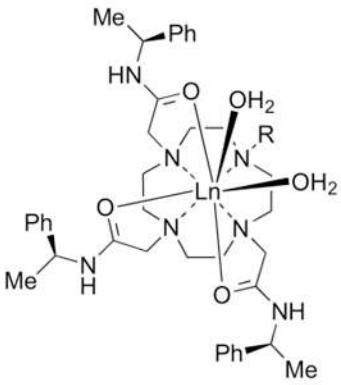


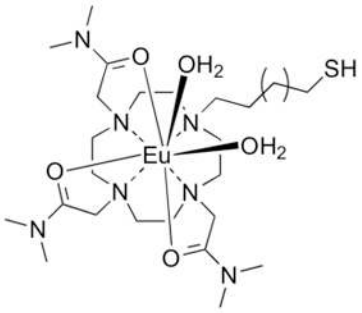
a: X = Ph, Y = Br, *n* = 1
b: X = Ph, Y = Br, *n* = 2
c: X = Me_3SiCH_2, Y = Cl, *n* = 3
d: X = Me_3SiCH_2, Y = Cl, *n* = 4
e: X = Cl, Y = Me, *n* = 1
f: X = Cl, Y = Me, *n* = 3
g: X = Bu, Y = Cl, *n* = 1
h: X = Cl, Y = Ph, *n* = 1
i: X = Cl, Y = Ph, *n* = 2
j: X = Octyl, Y = Cl, *n* = 1
k: X = Ph, Y = Cl, *n* = 2
l: X = Ph, Y = Cl, *n* = 3

589

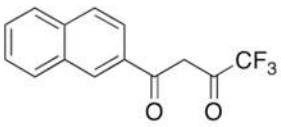


a: R = H
b: R = Me

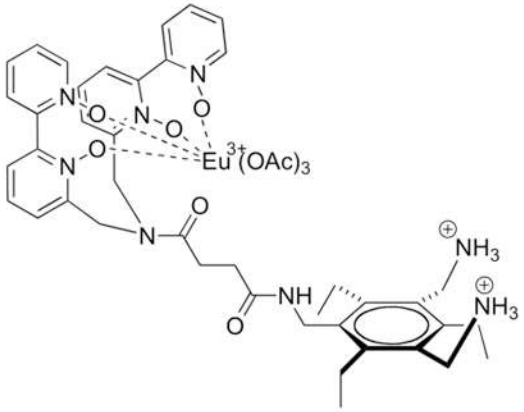
590



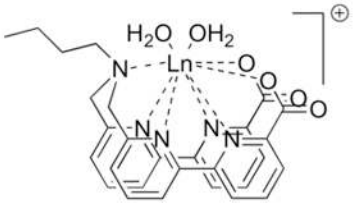
591



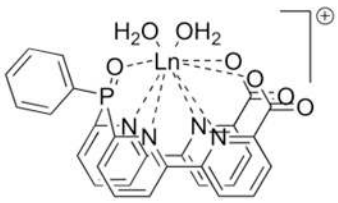
592



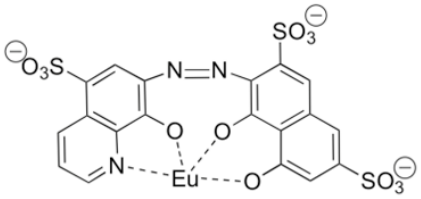
593



594



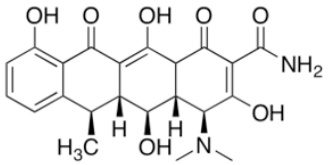
595



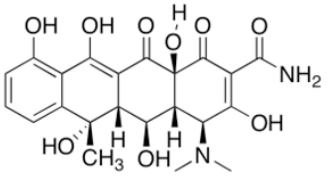
596

$Eu^{3+}(H_2O)_nL$

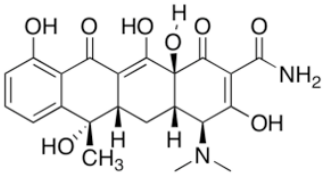
a: L =



b: L =

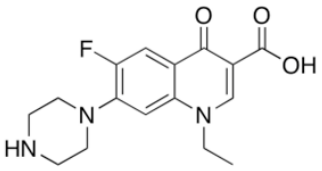


c: L =

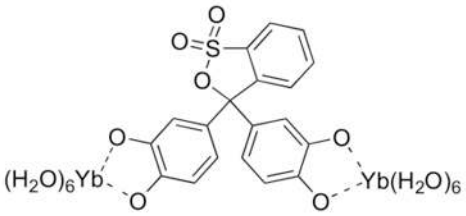


597

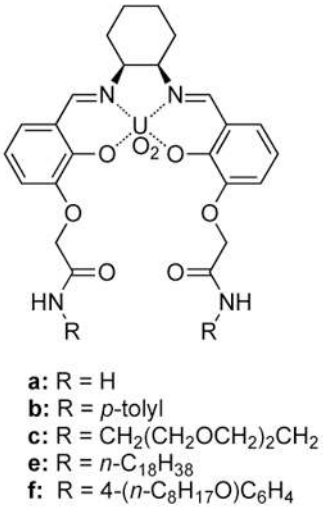
$Tb^{3+}(H_2O)_nL$ L =



598

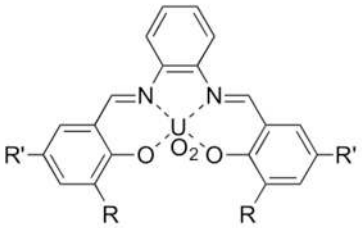


599



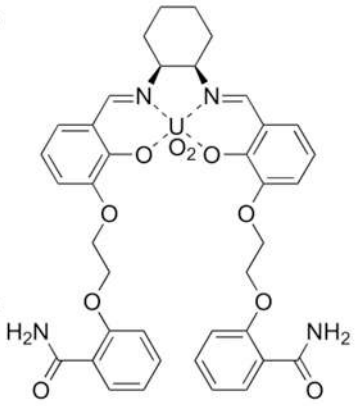
a: R = H
b: R = *p*-tolyl
c: R = $CH_2(CH_2OCH_2)_2CH_2$
e: R = *n*-$C_{18}H_{38}$
f: R = 4-(*n*-$C_8H_{17}O$)C_6H_4

600

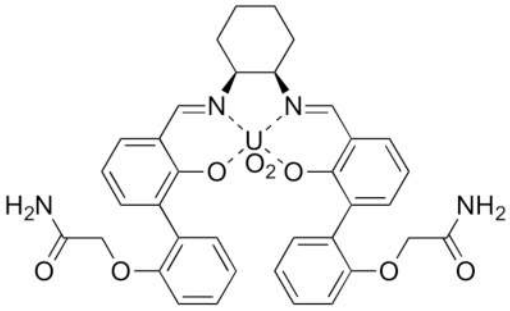


a: R = H, R' = H
b: R = OMe, R' = H
c: R = H, R' = NO_2
d:
e: R = H, R' = *t*-butyl
f: R = R' = *t*-butyl

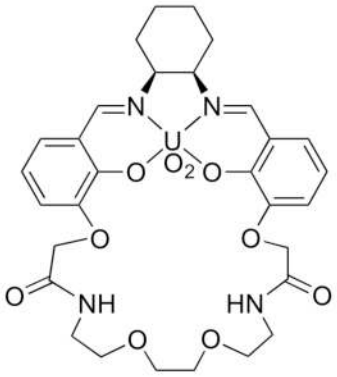
601



602



603



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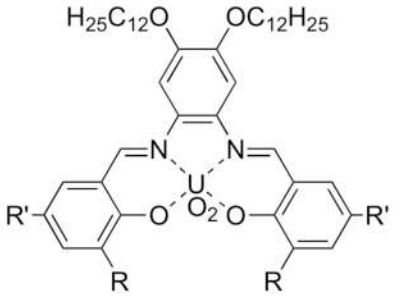
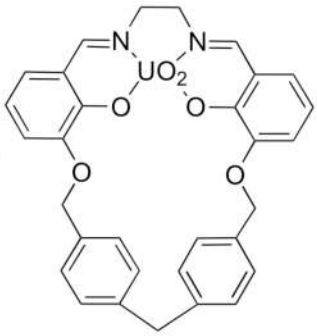
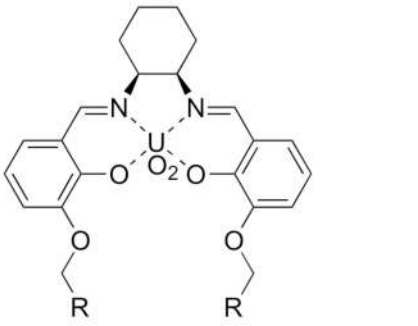


a: R, R' = H
b: R = OMe, R' = H
c: R = H, R' = OMe

605



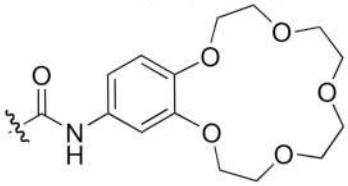
606



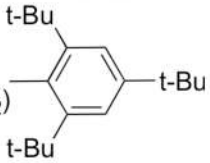
a: (CO)NH-*n*-(C_8H_{16})—O-(O_2N)

b: $CH_2CH_2NH(SO_2)$-*p*-tolyl

c: (CO)NH-*p*-C_6H_4-O-*n*-C_8H_{17}



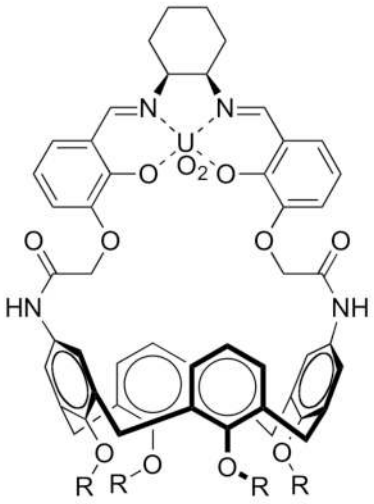
e: $CH_2CH_2NH(SO_2)$-*n*-$C_{16}H_{33}$



f: $CH_2CH_2NH(SO_2)$

g: (CO)NH-*m*-C_6H_4-O-C_8H_{17}

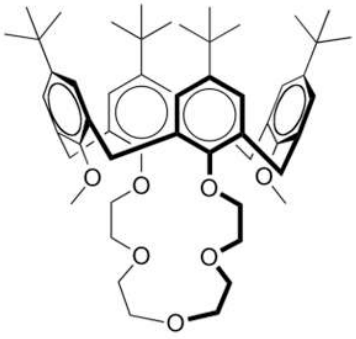
607



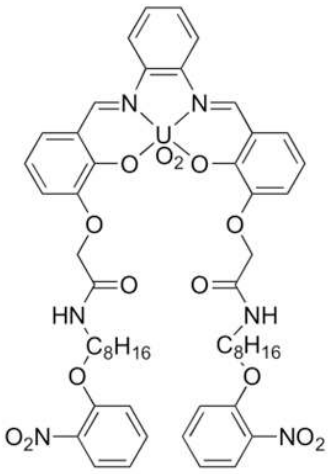
a: R = n-Pr

b: R = $CH_2C(O)Et$

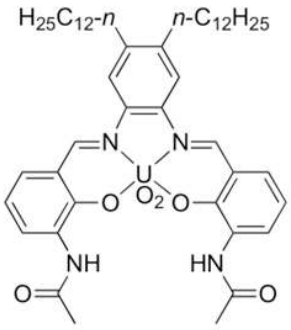
608



609



610



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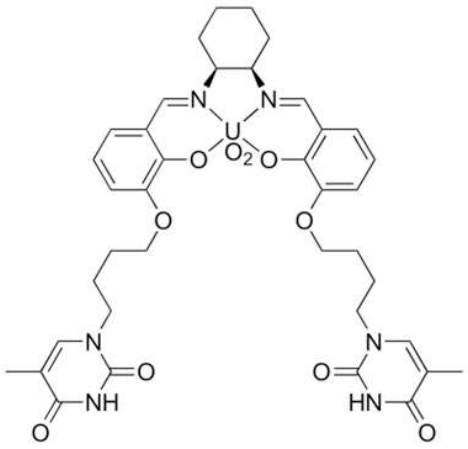

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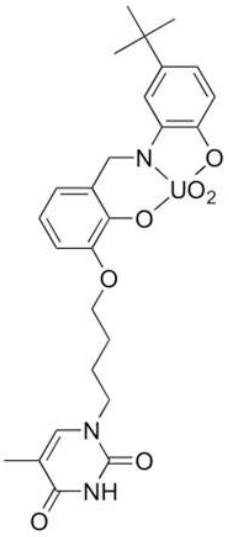

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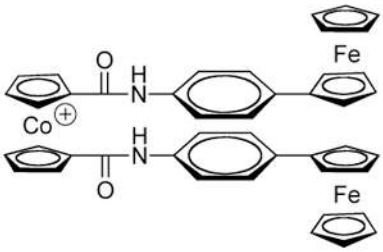

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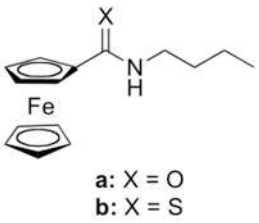

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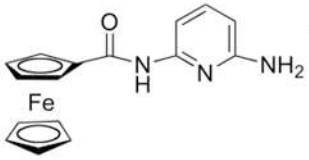

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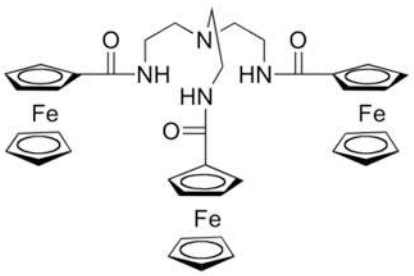

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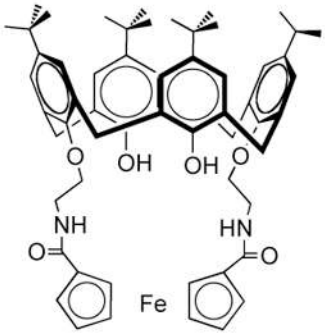

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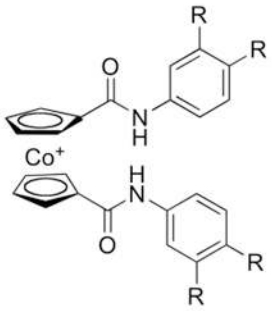

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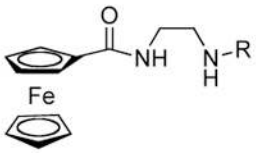

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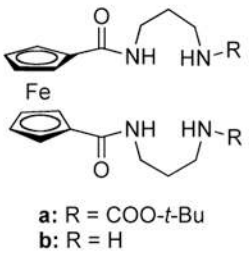

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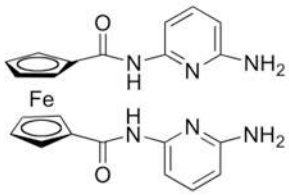

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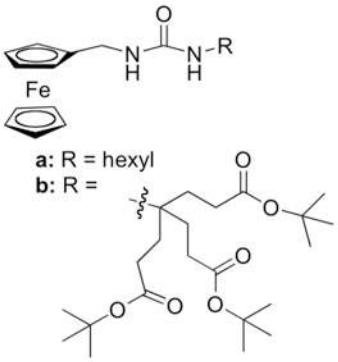

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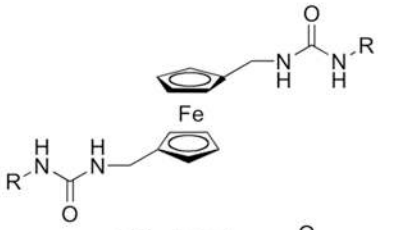

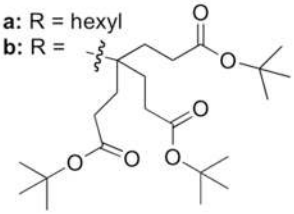

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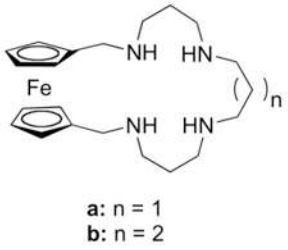

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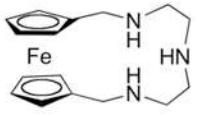

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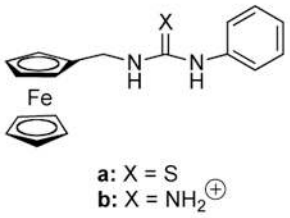

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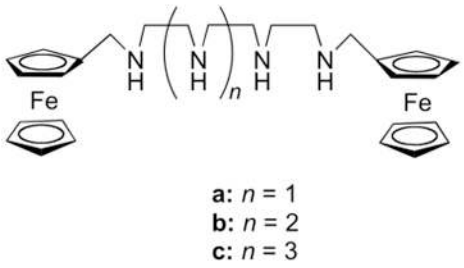
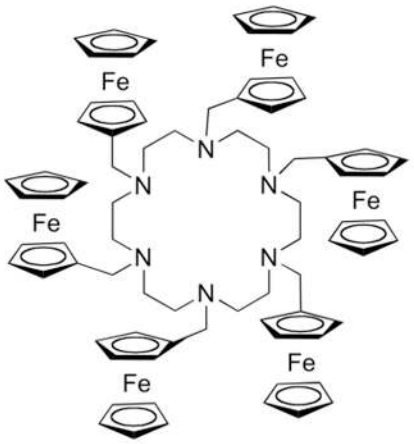


a: *n* = 1
b: *n* = 2
c: *n* = 3

628



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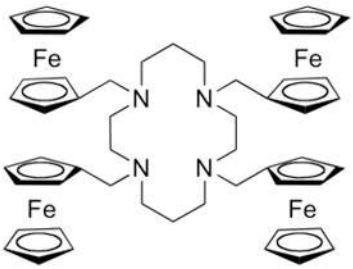

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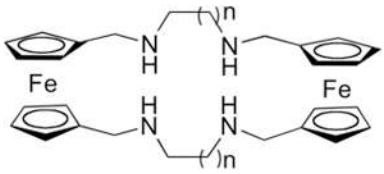
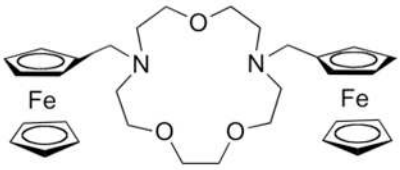


a: n = 1
b: n = 3

631



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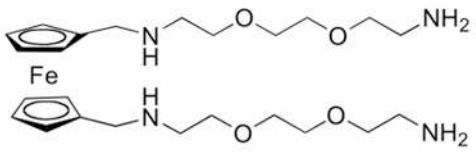

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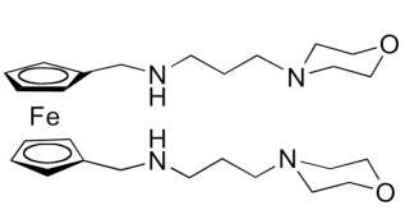

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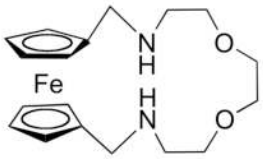

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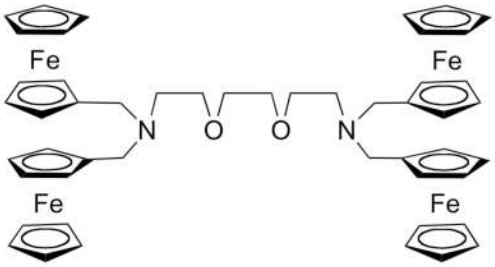

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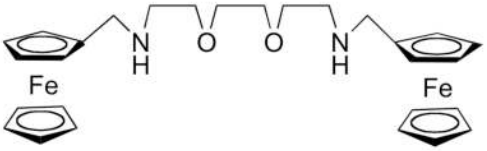

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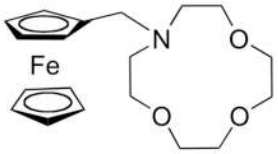

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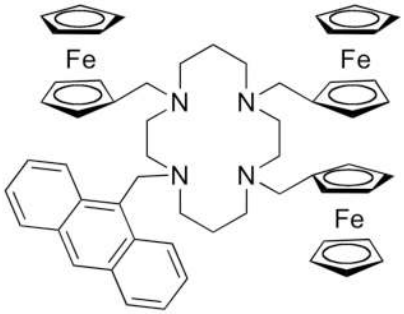

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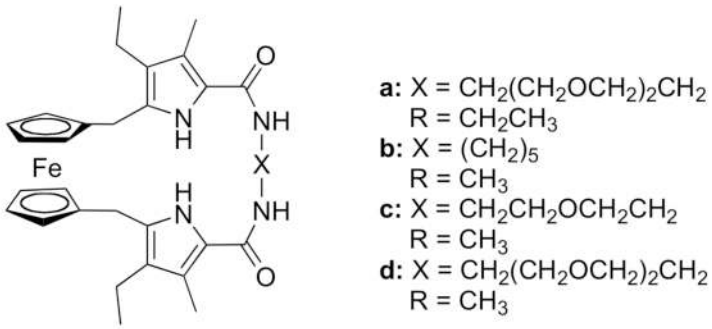

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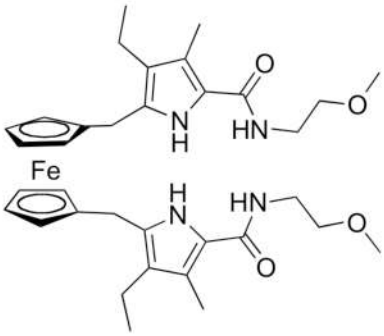

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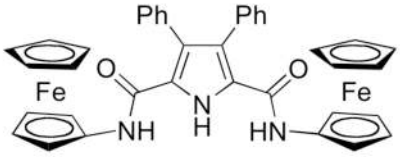

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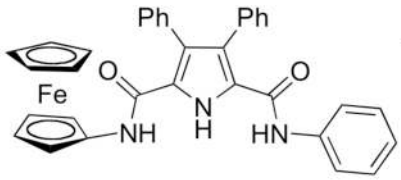

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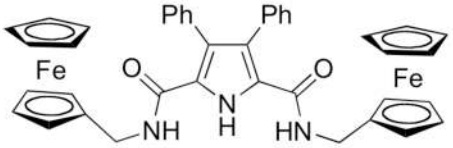

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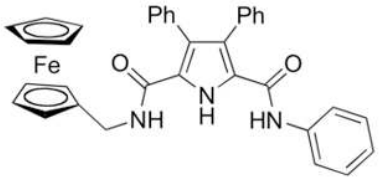

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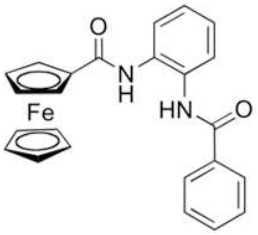

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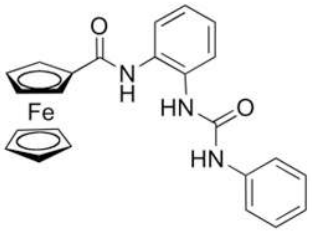

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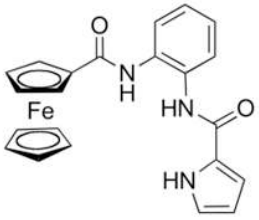

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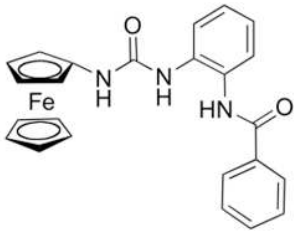

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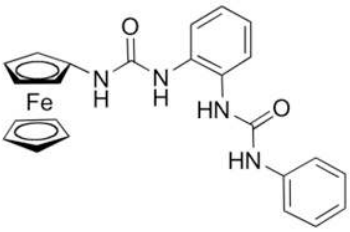

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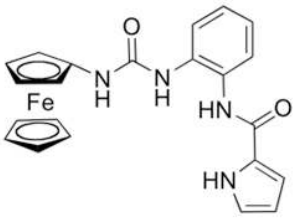

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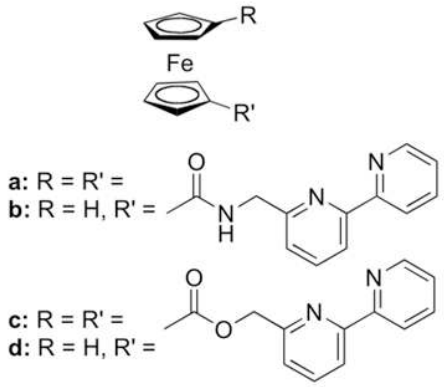

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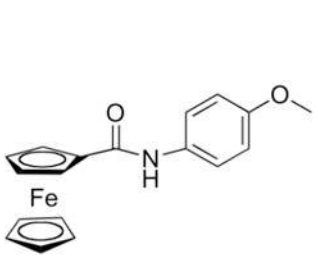

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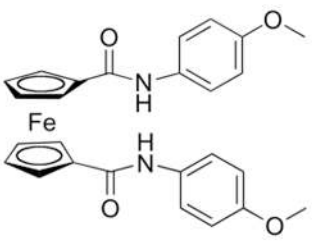

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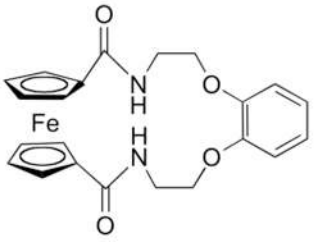

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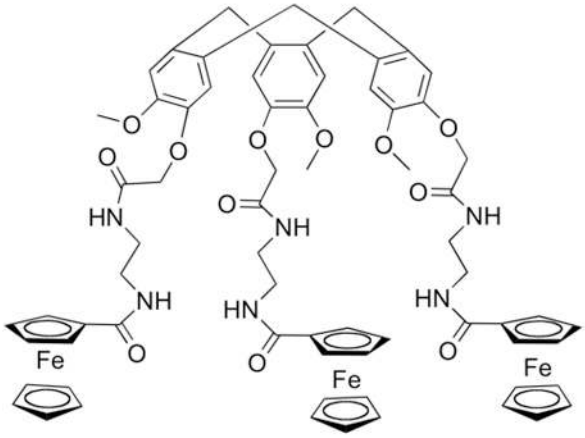

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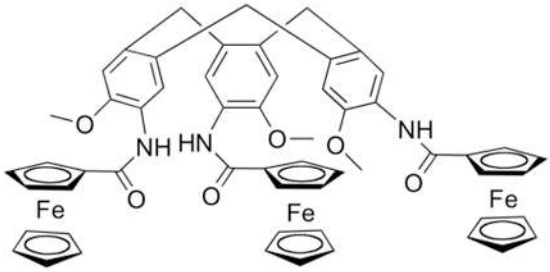

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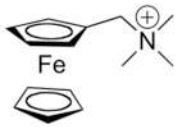

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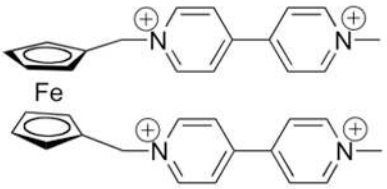

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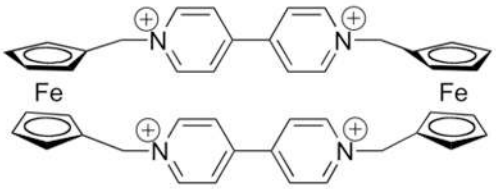

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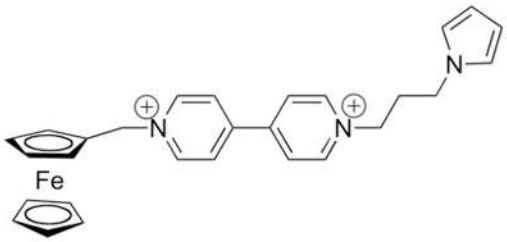

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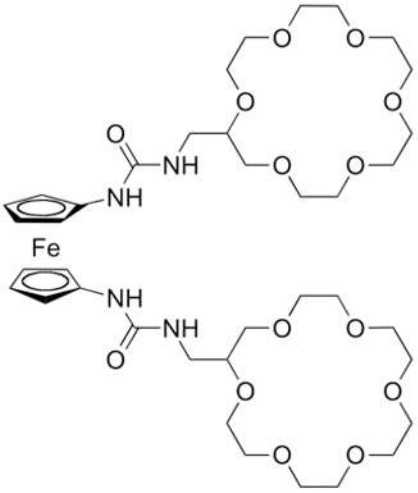

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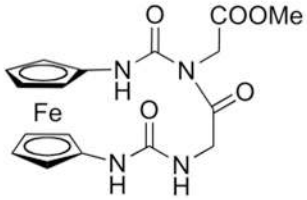

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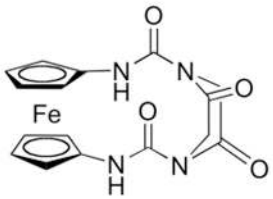

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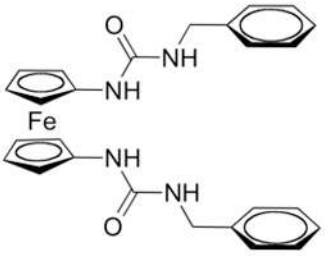

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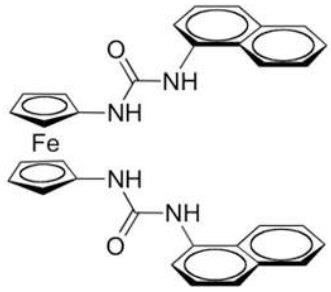

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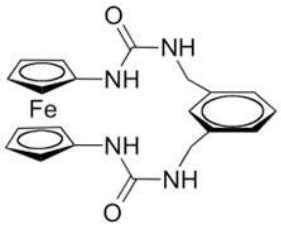

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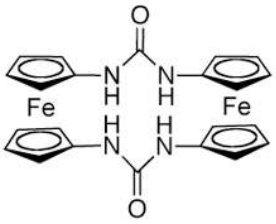

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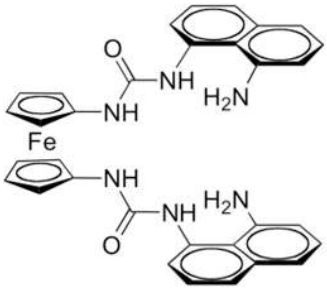

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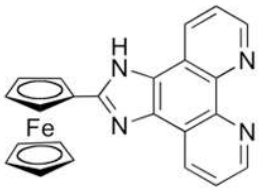

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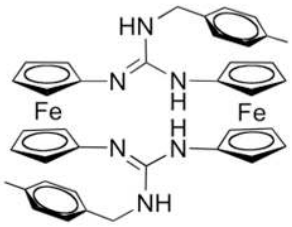

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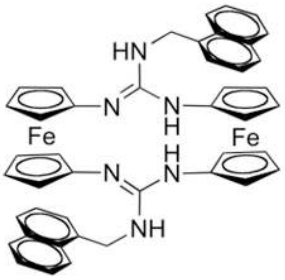

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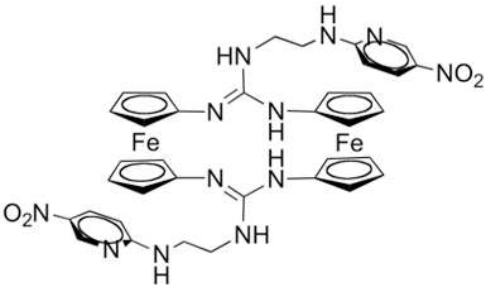

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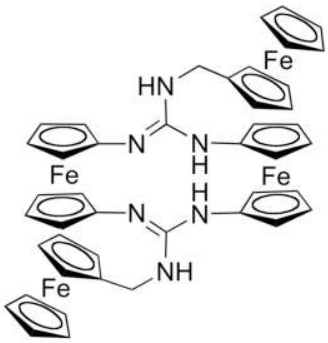

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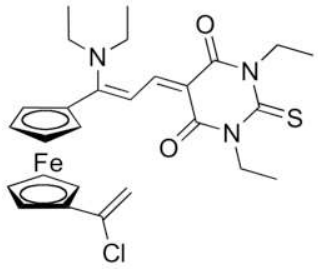

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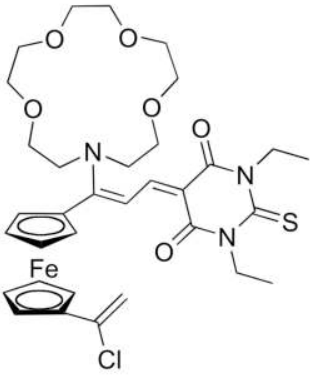

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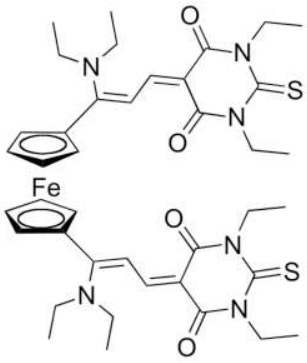

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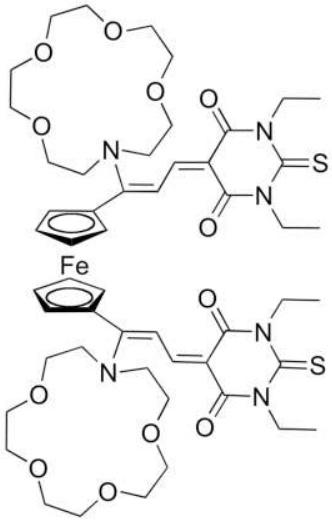

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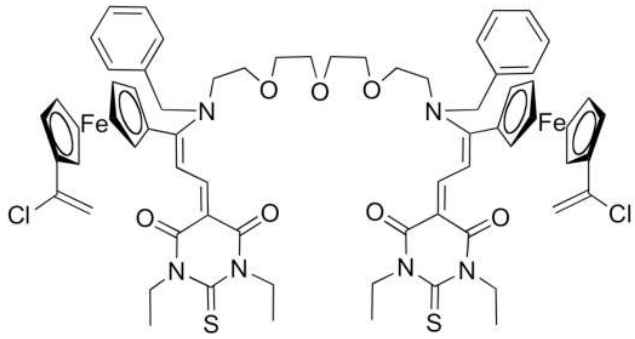

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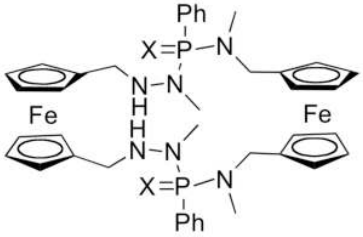

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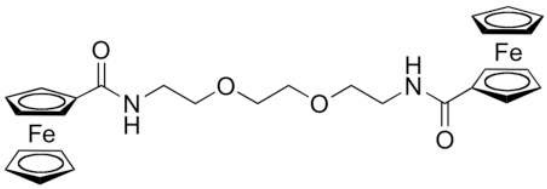

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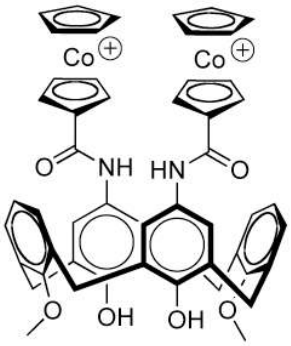

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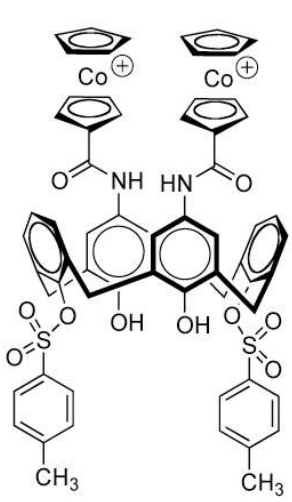

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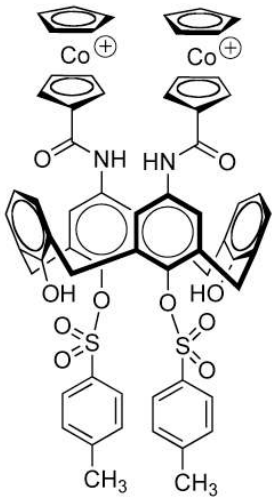

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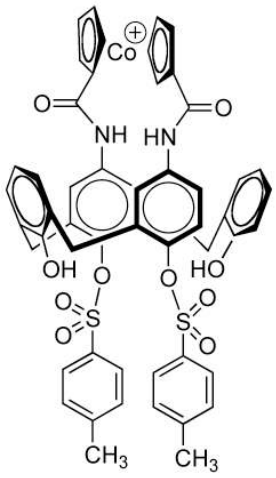

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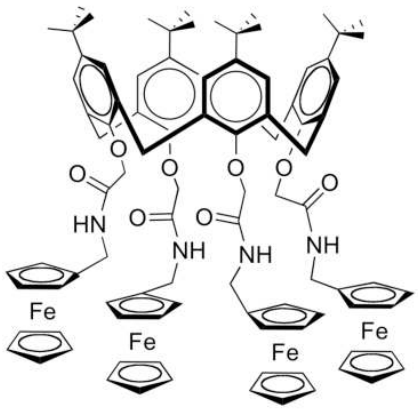

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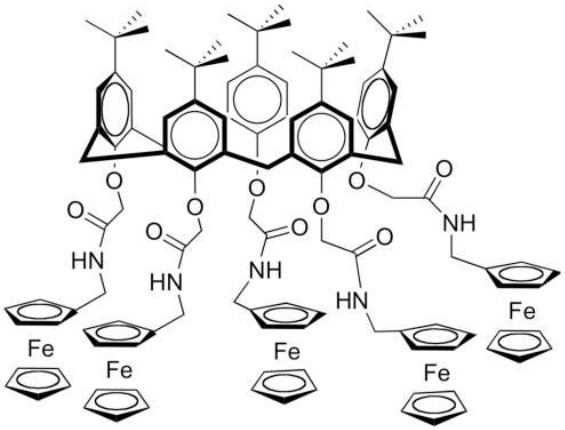

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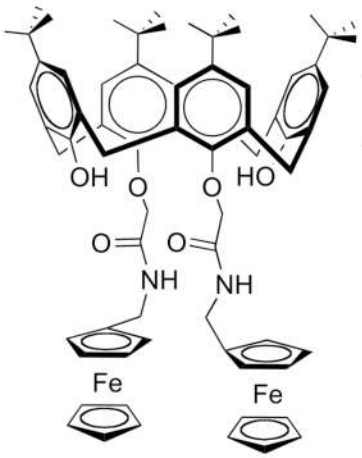

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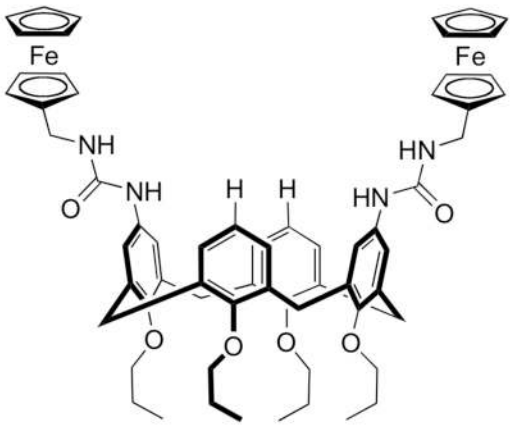

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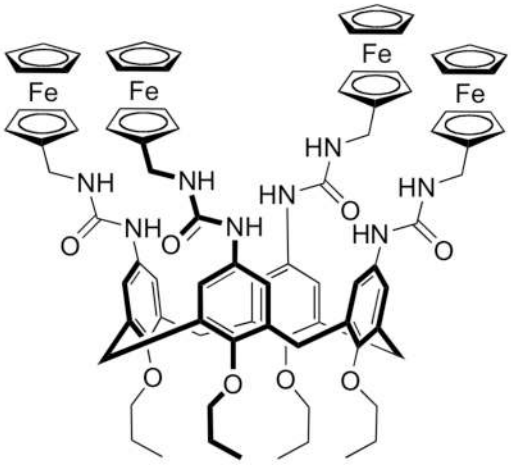

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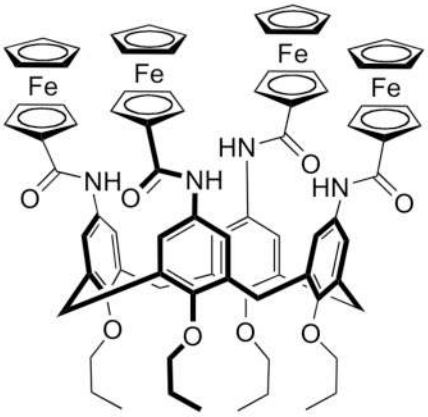

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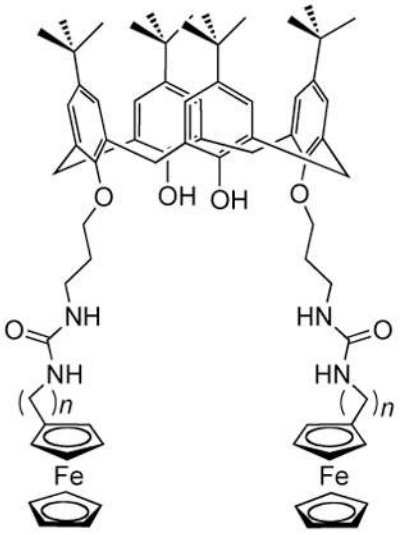

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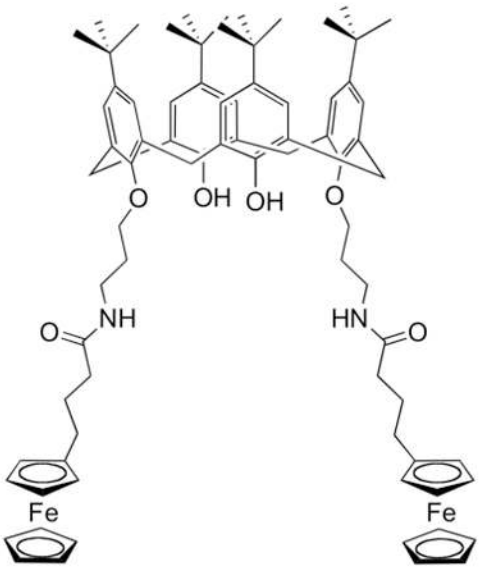

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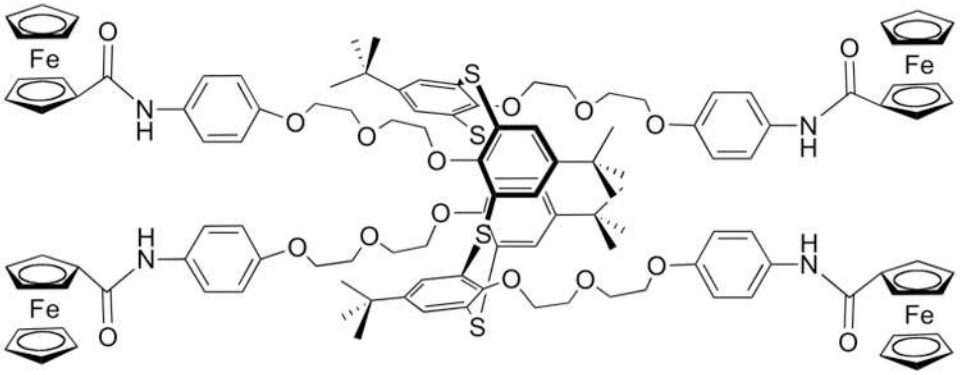

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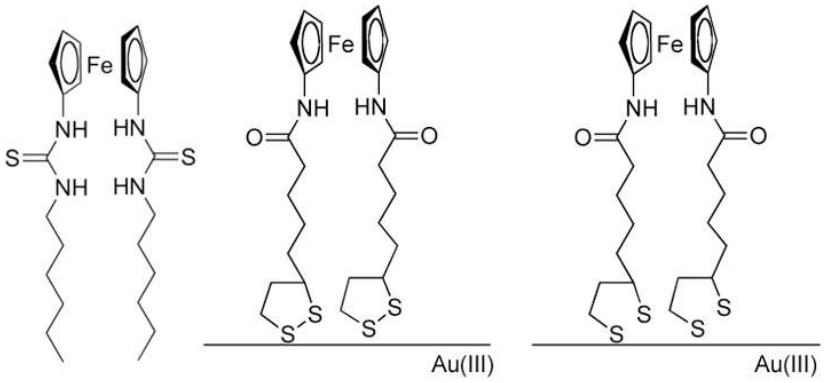

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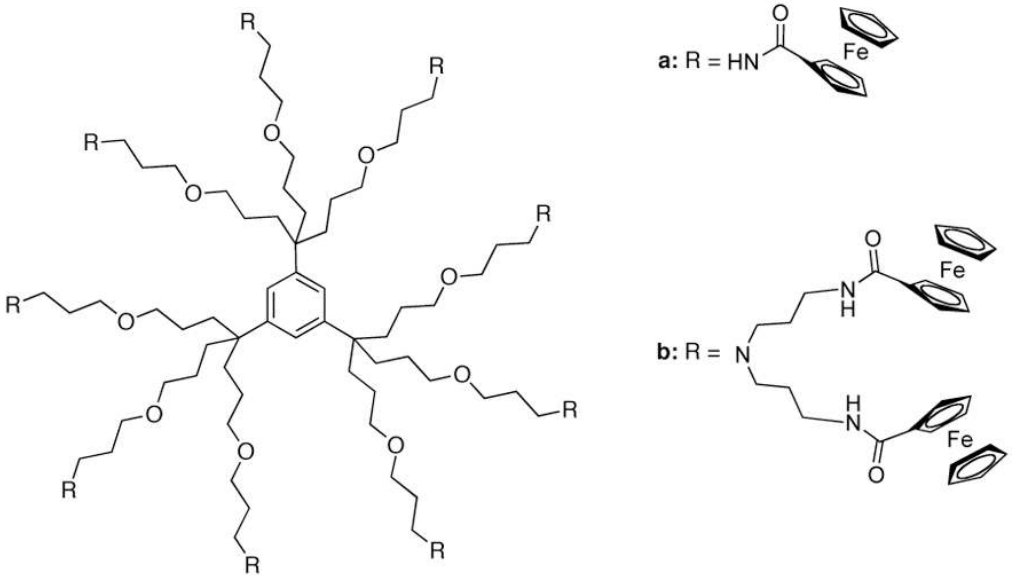

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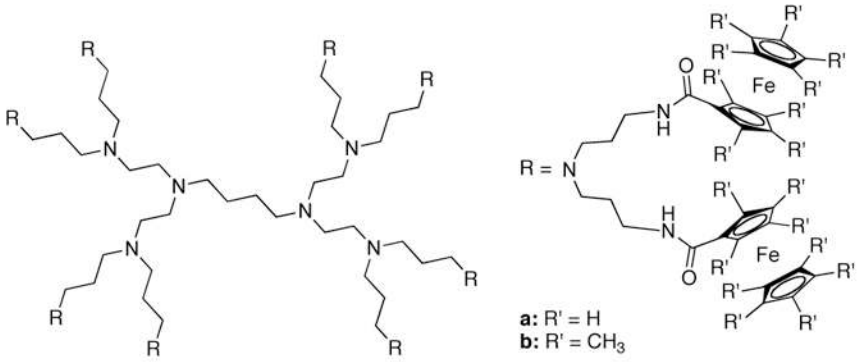

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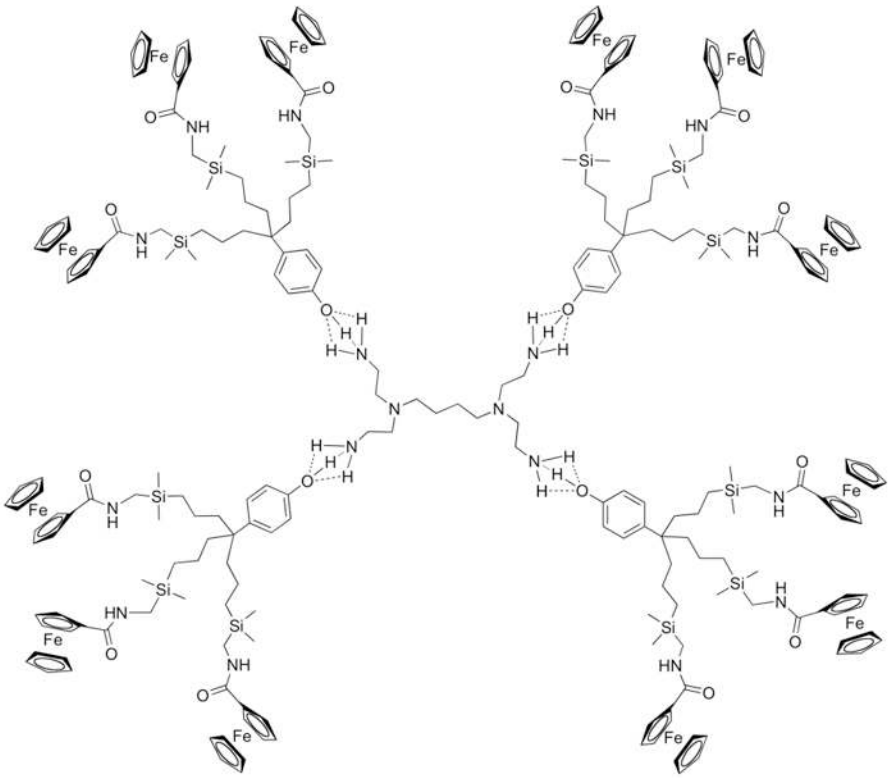

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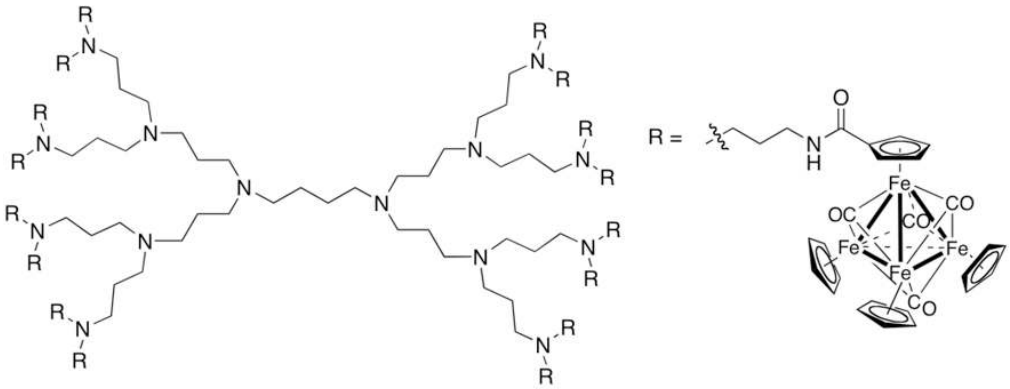

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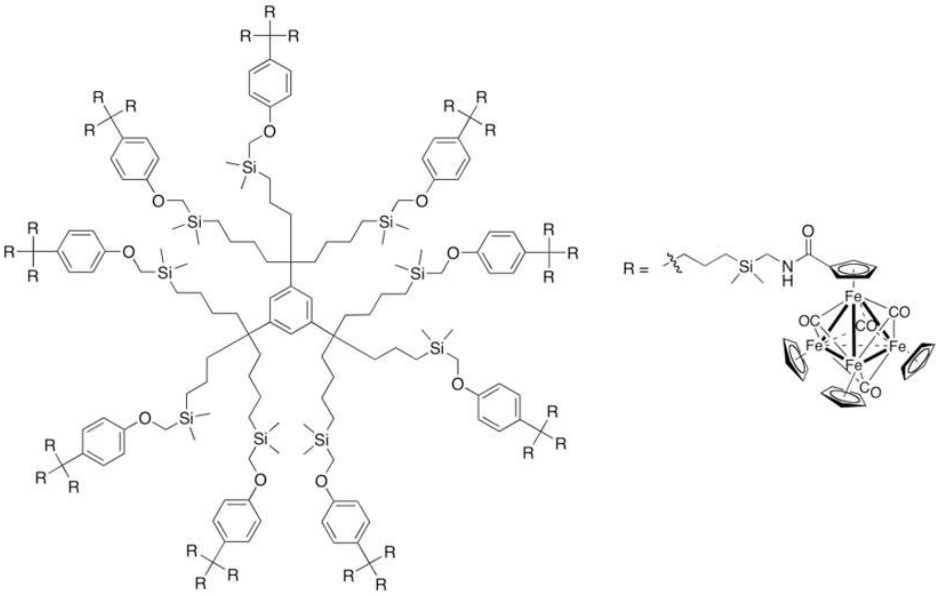

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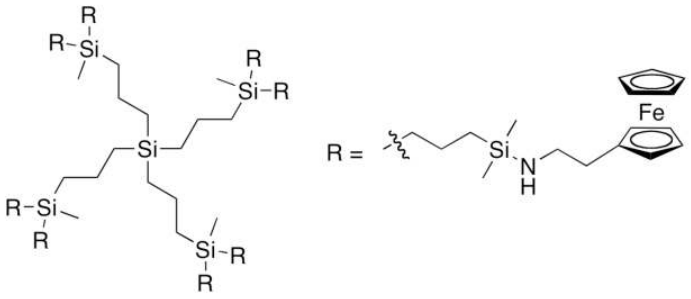

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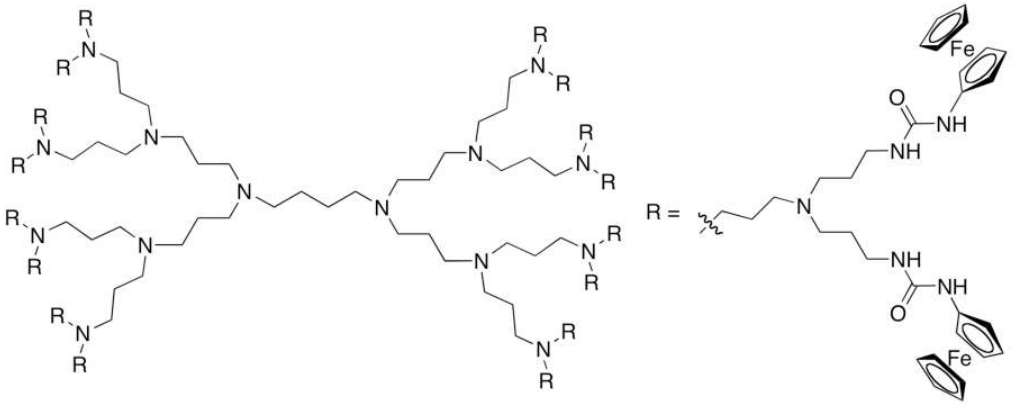

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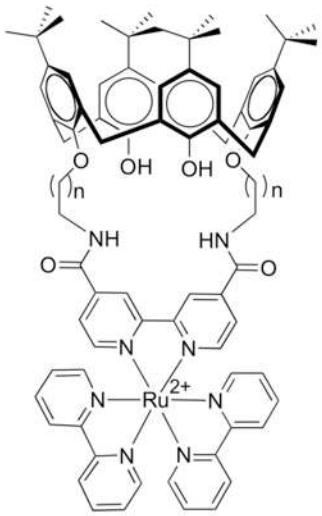

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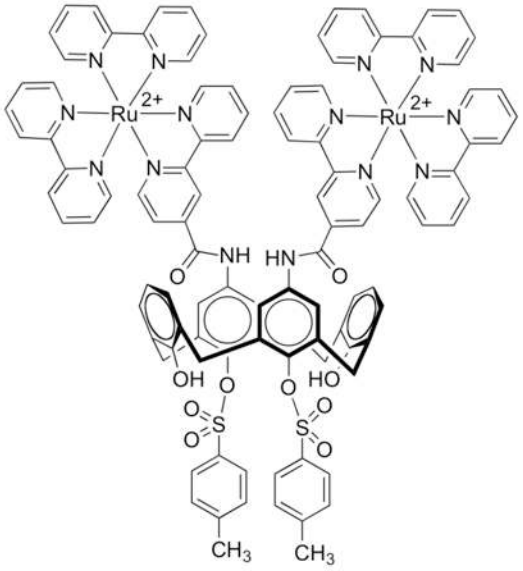

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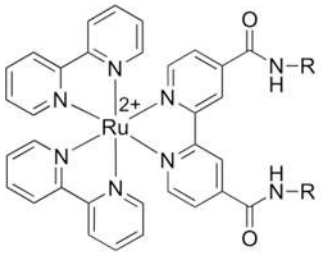

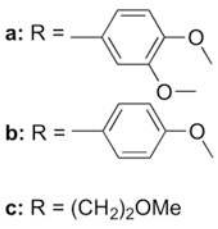

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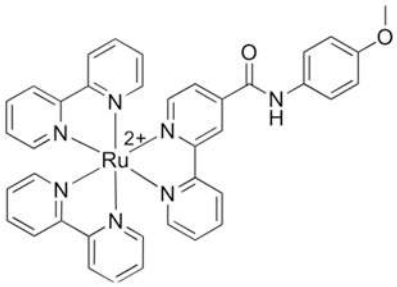

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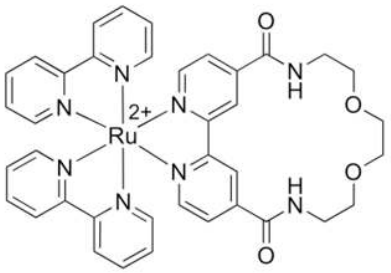

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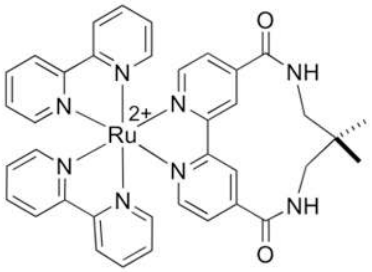

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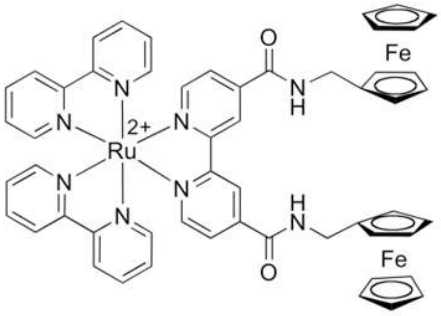

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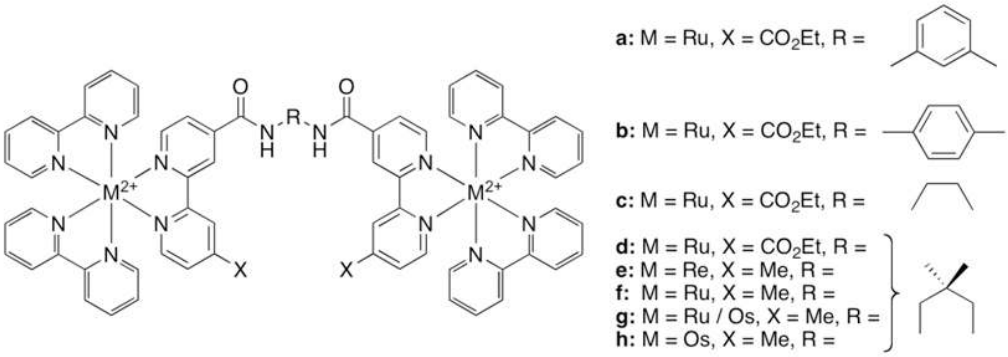

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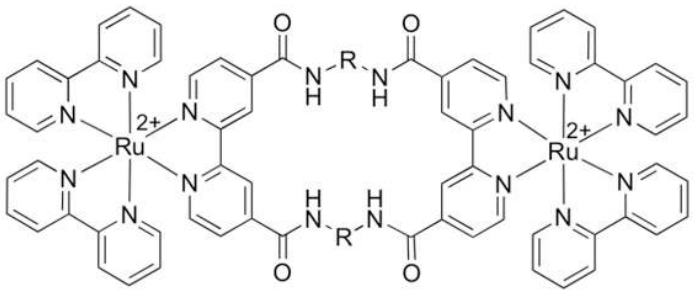
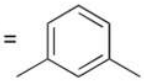
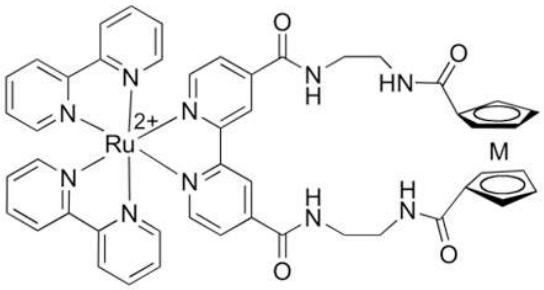


a: R = 

b: R = 

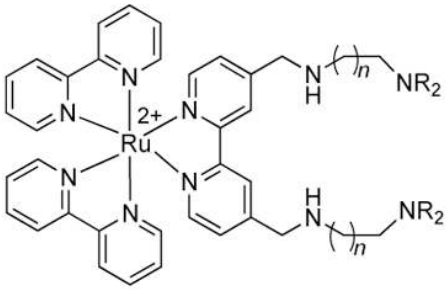
c: R = $(CH_2)_3$
d: R = $(CH_2)_4$

713



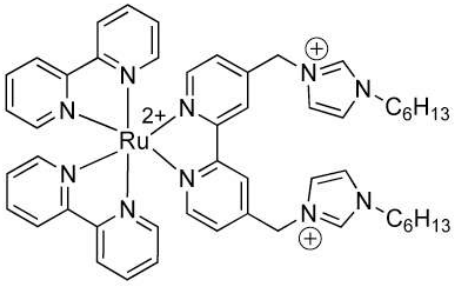
a: M = Fe
b: M = Co^+

714



a: n = 1, R = H
b: n = 2, R = H
c: n = 3, R = H
d: n = 1, R = Me

715



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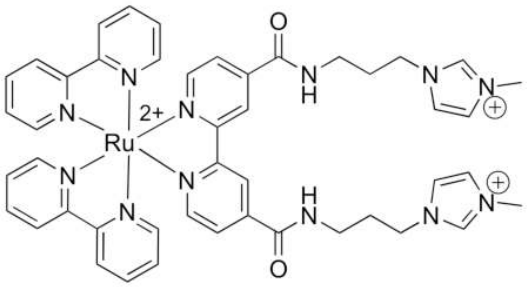

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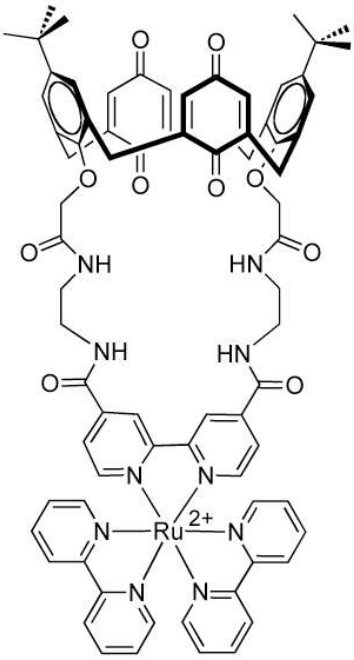

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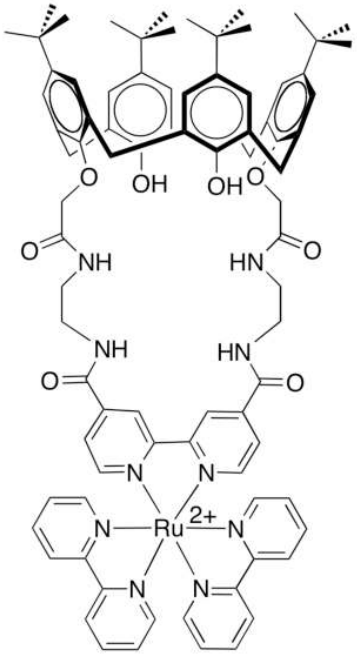

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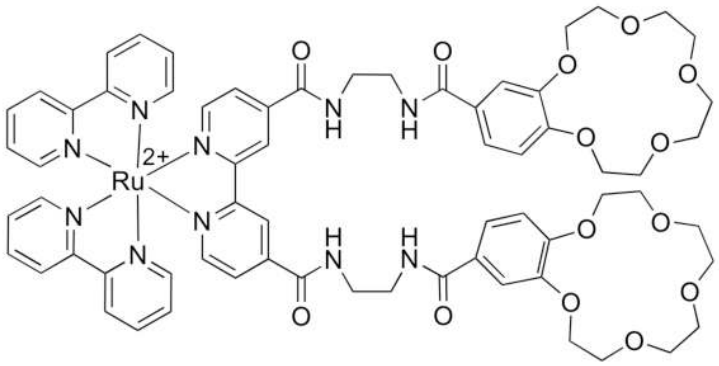

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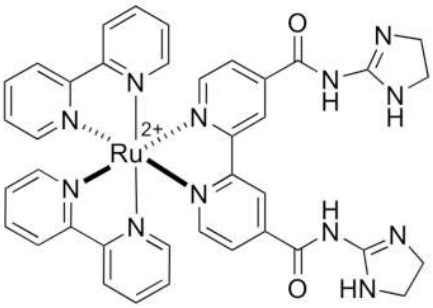

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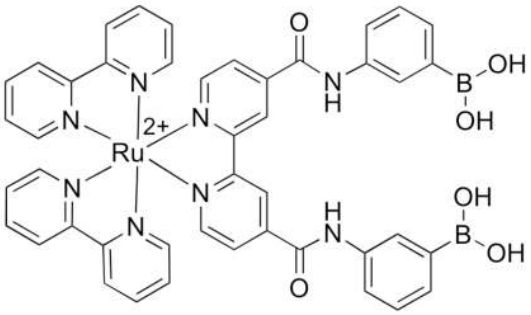

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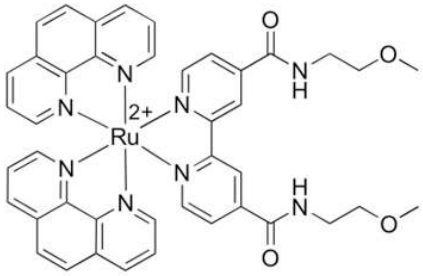

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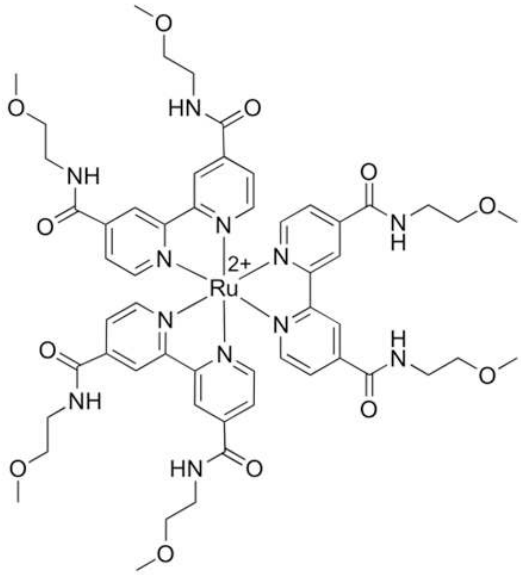

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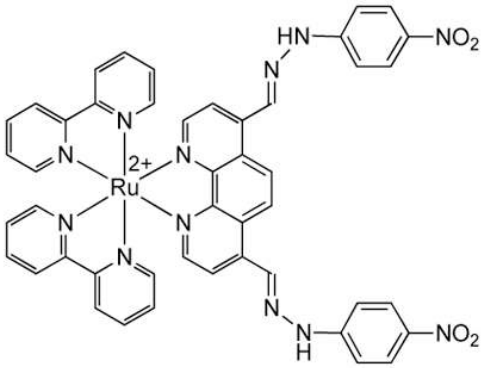

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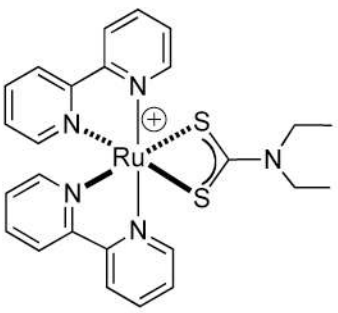

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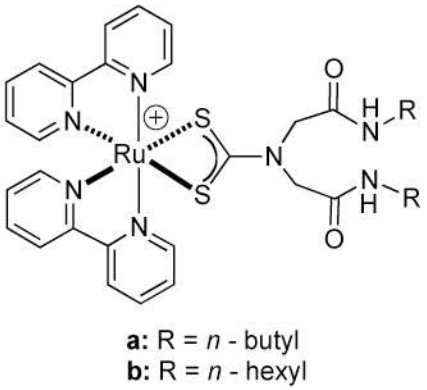

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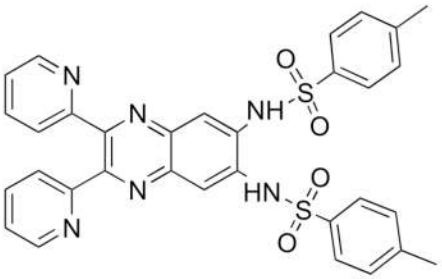

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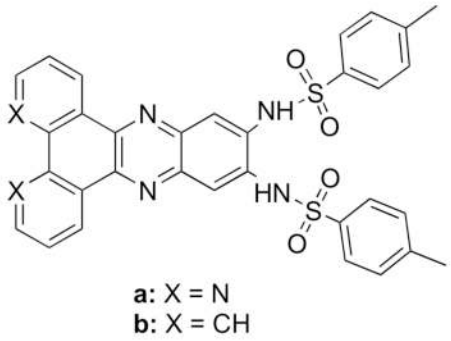

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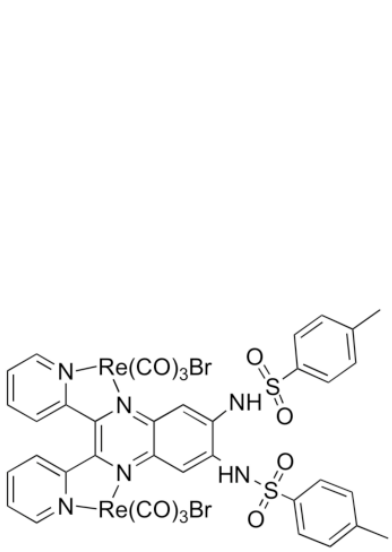

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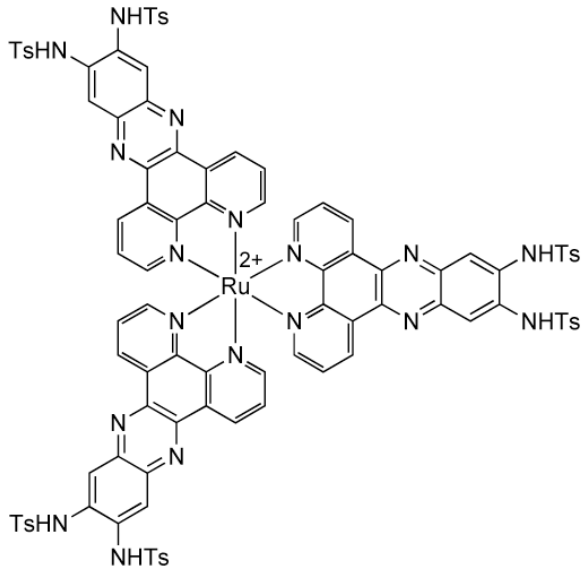
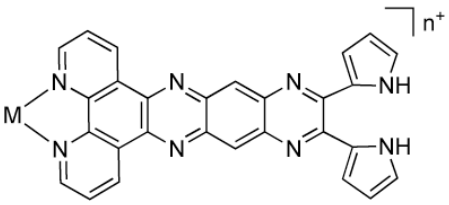
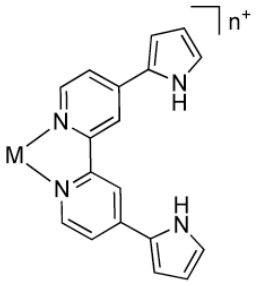


731



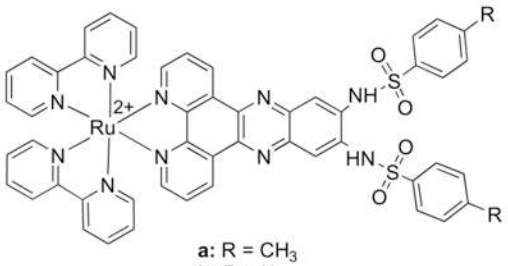
a: M = bpy_2Ru, n = 2
b: M = $phen_2Co$, n = 3

732



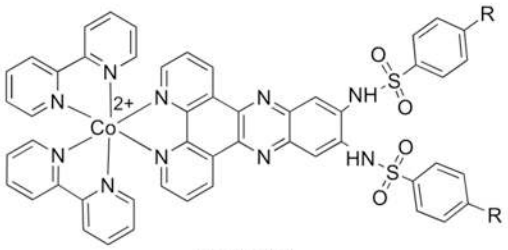
a: M = bpy_2Ru, n = 2
b: M = bpy_2Rh, n = 3

733



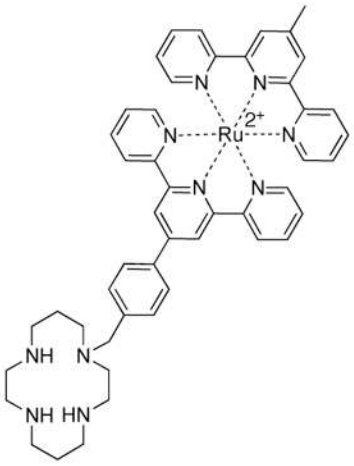
a: R = CH_3
b: R = H

734

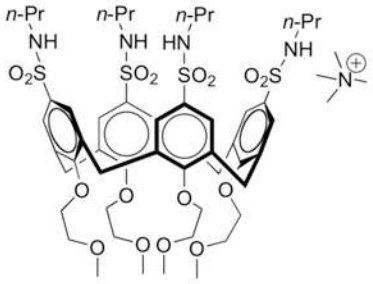


a: R = CH_3
b: R = H

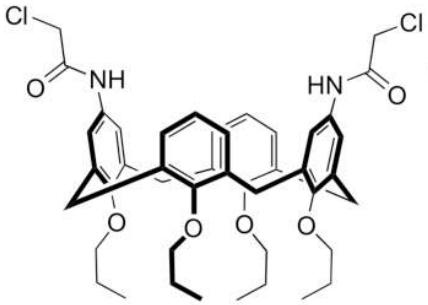
735



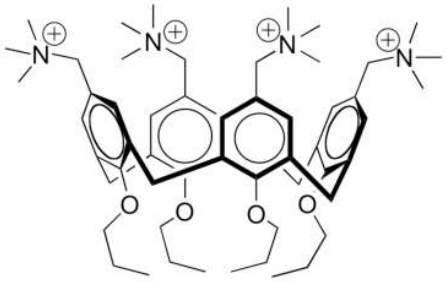
736



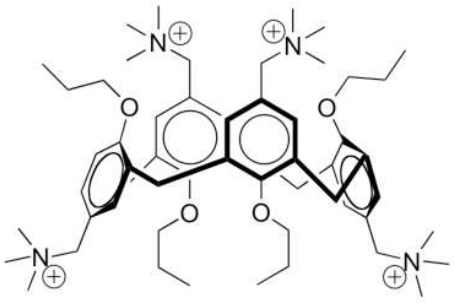
737



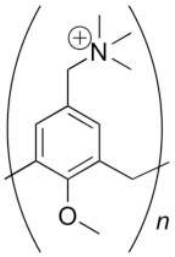
738



739



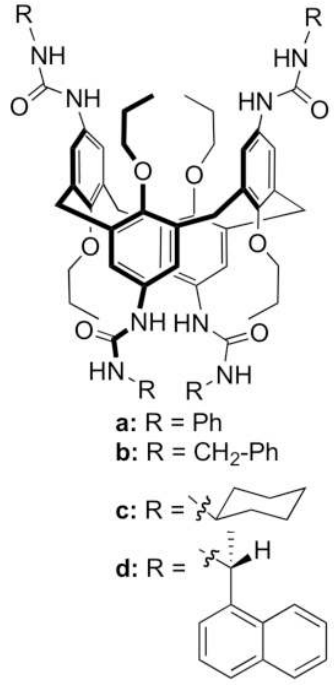
740



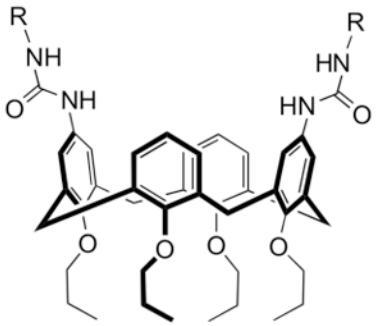
a: $n = 6$

b: $n = 8$

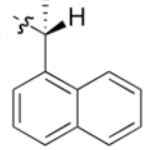
741



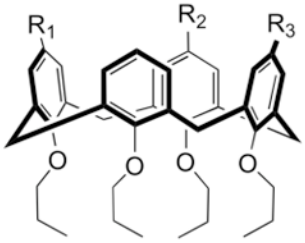
742



a: R = Ph
b: R = CH_2-Ph
c: R =
d: R =

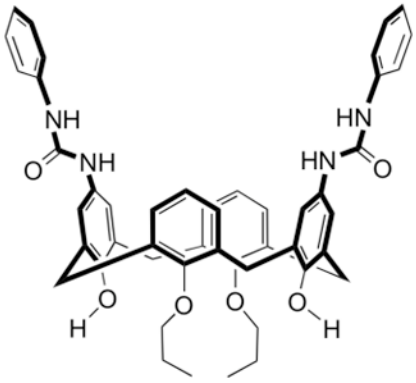


743

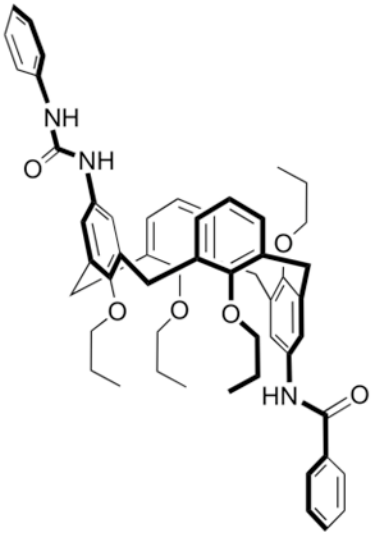


a: R_1 = NH-CO-NH-Ph
R_2 = R_3 = H
b: R_1 = R_2 = NH-CO-NH-Ph
R_3 = H
c: R_1 = R_2 = R_3 = NH-CO-NH-Ph

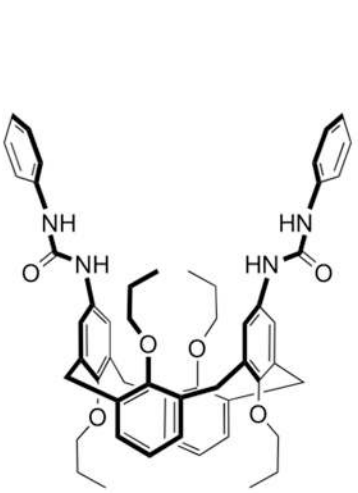
744



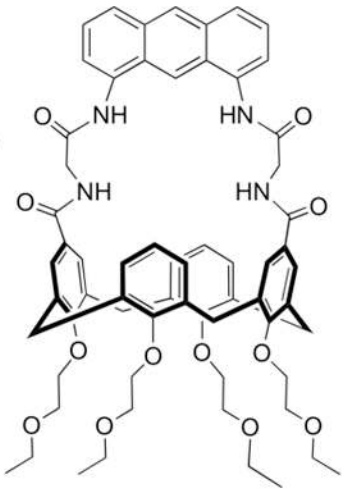
745



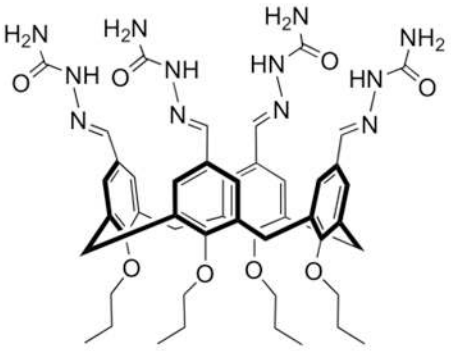
746



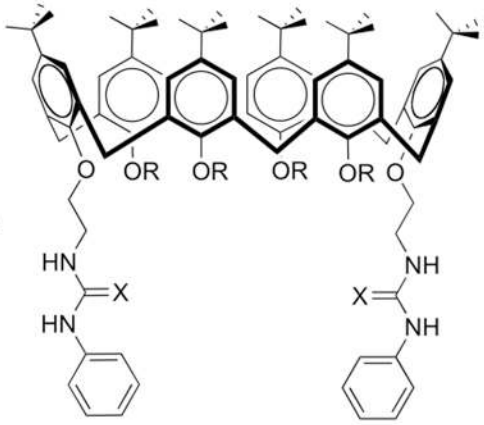
747



748

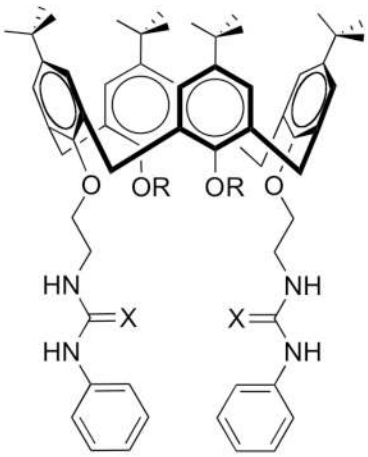


749

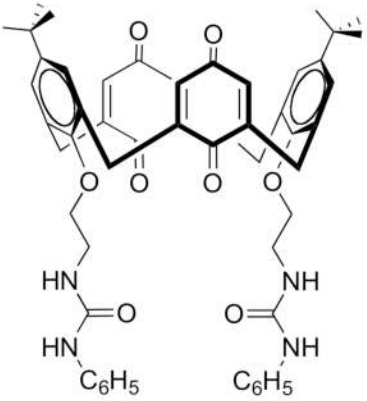


a: R = H, X = O
b: R = CH_3, X = O
c: R = H, X = S

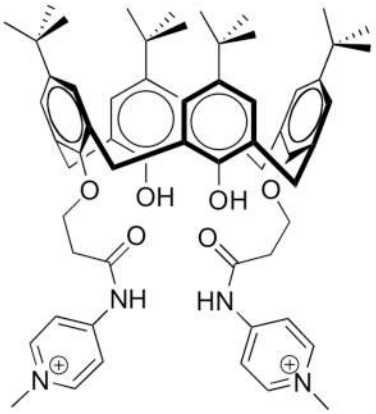
750



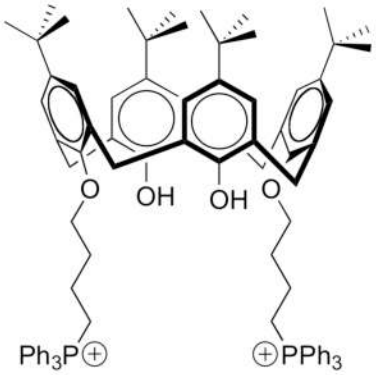
751



752



753



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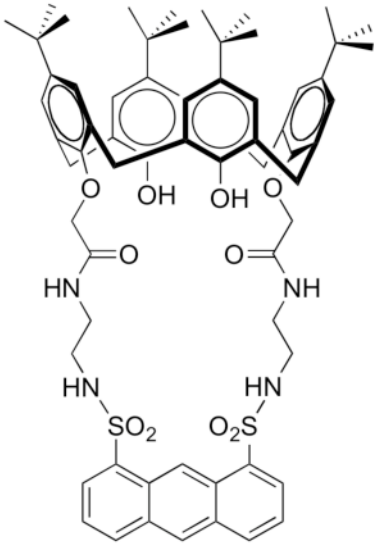

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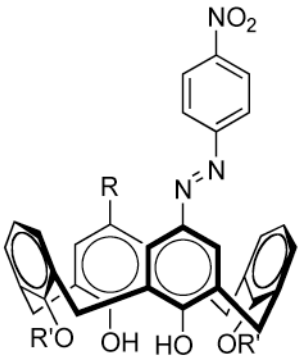


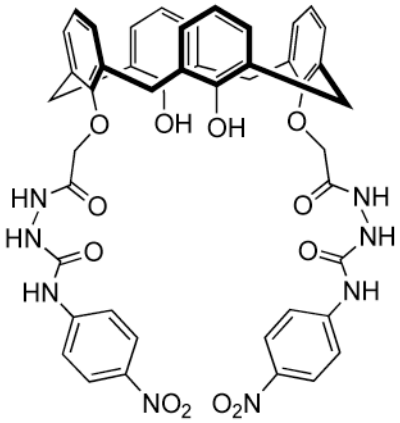
a: R = —N=N— NO_2

R' = $CH_2CO_2C_2H_5$

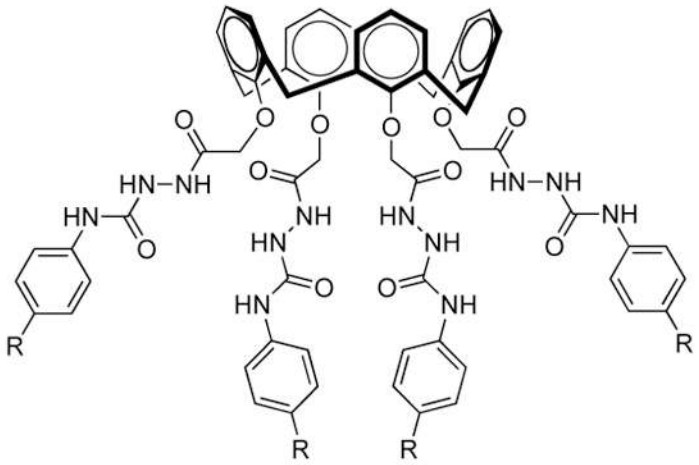
b: R = H, R' = $CH_2CO_2CH_2CH_2CO_2CCH_2$

c: R = H, R' = $CH_2CONHCH_3$

756

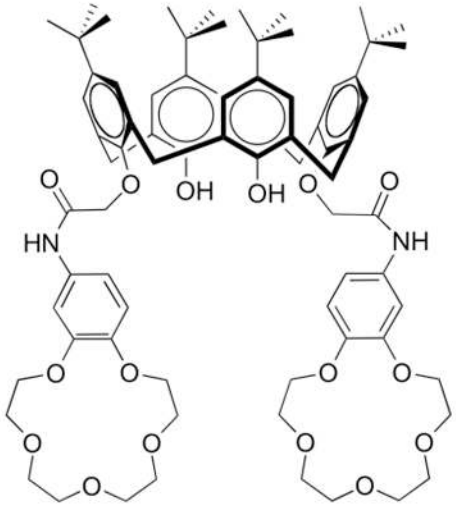


757

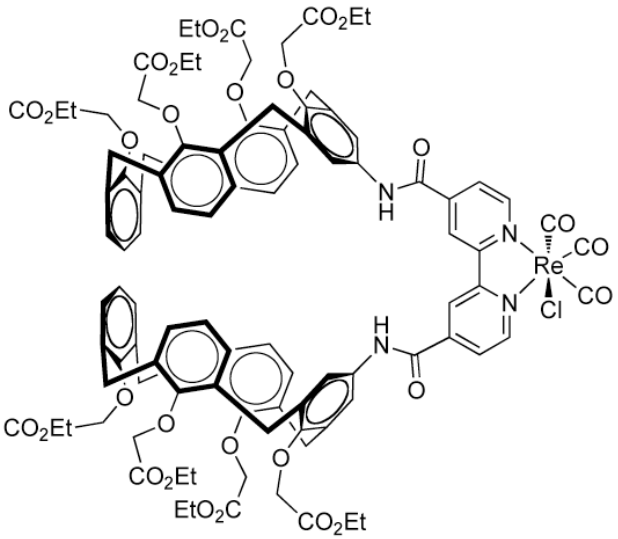


a: R = NO_2
b: R = H

758



759



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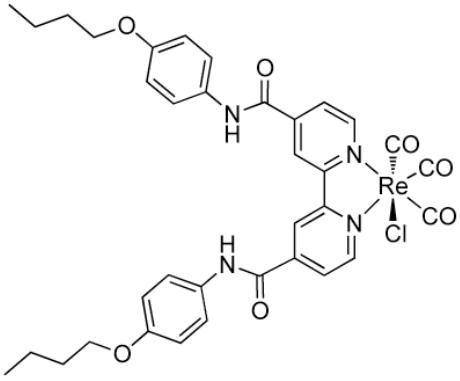
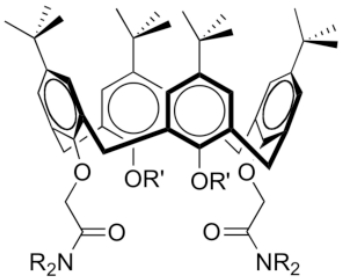
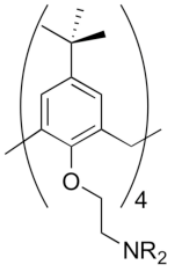


761



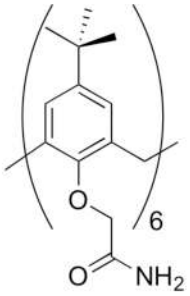
a: R = Et, R' = Me
b: R = Et, R' = H

762

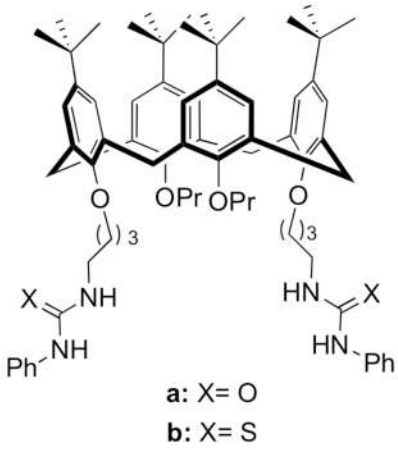


a: R = H
b: R = Et

763



764



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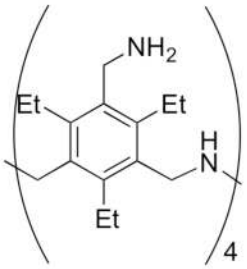

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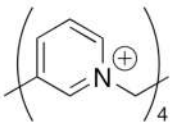

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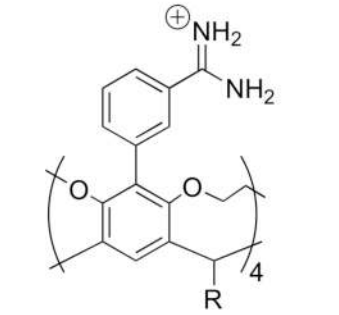
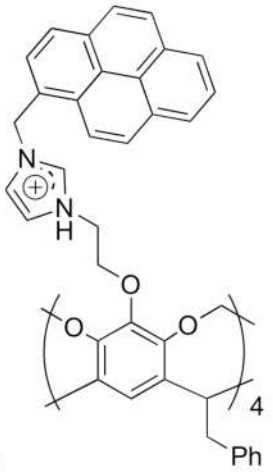


768



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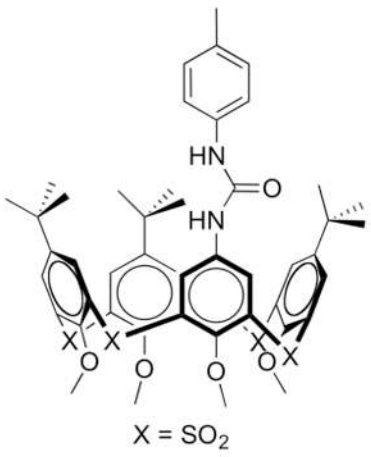
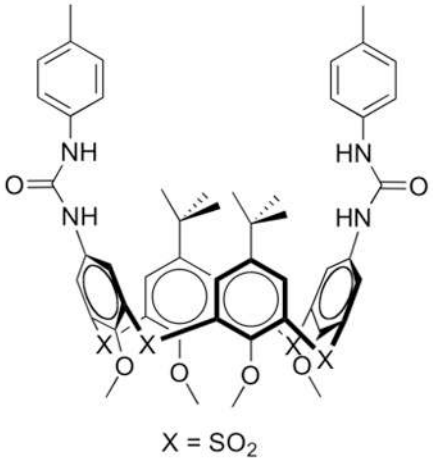
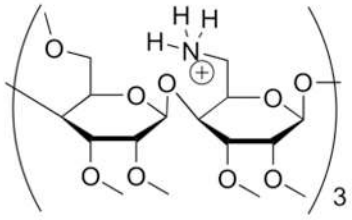


770



771



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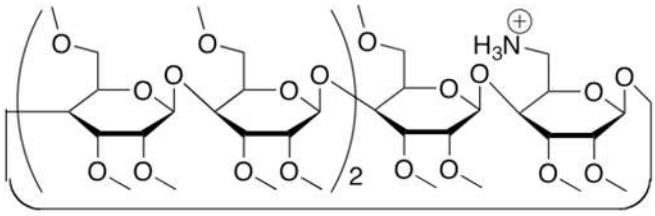

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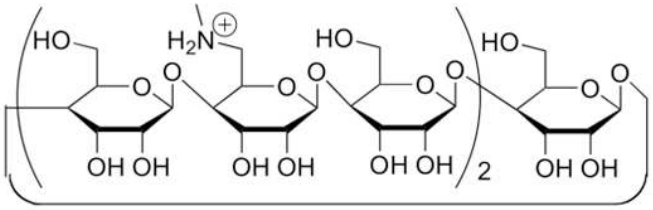

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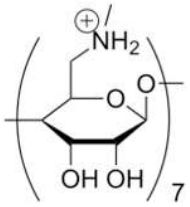

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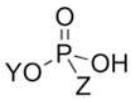
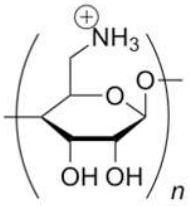


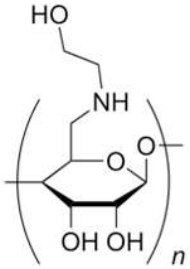
a: Y = 4-iPrPh, Z = -1-*myo*-inositol
b: Y = 4-iPrPh, Z = -OCH_3
c: Y = 4-iPrPh, Z = -O-
d: Y = -CH_3, Z = -O-
e: Y = 4-NO_2Ph, Z = -O-

776



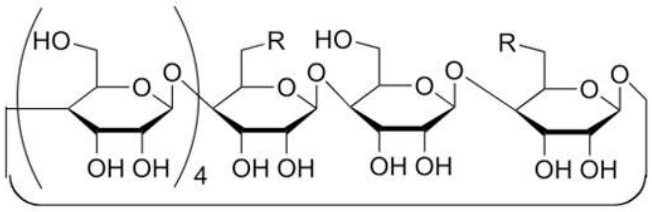
a: *n* = 7
b: *n* = 6

777



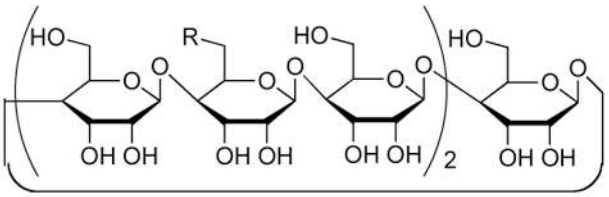
a: *n* = 7
b: *n* = 6

778



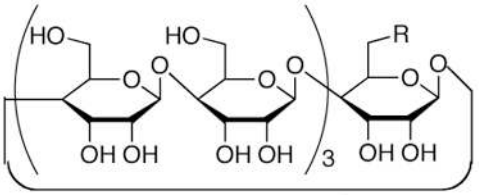
a: R = NH_3^+
b: R = $NHC(NH_2)_2^+$

779



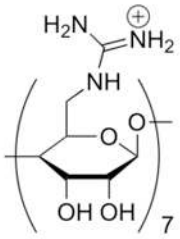
a: R = $NH_3^{\oplus}$
b: R = $NHC(NH_2)_2^{\oplus}$

780



R = $NHC(NH_2)_2^{\oplus}$

781



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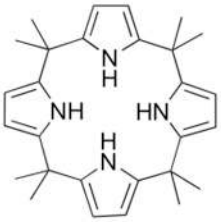

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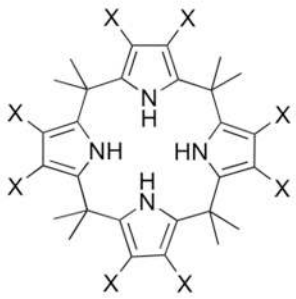
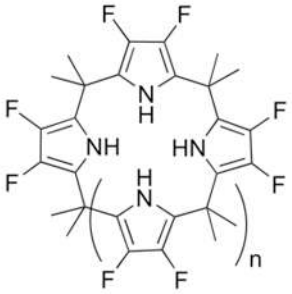


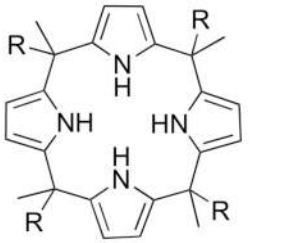
a: X = Br
b: X = F

784



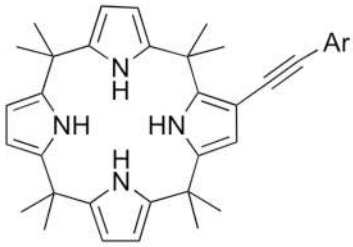
a: n = 2
b: n = 3

785

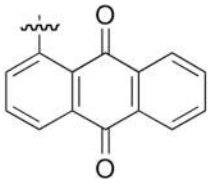


a: R = 4-hydroxyphenyl
b: R = 4-methoxyphenyl
c: R = 4-bromophenyl

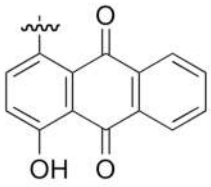
786



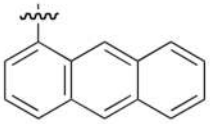
a: Ar =



b: Ar =



c: Ar =



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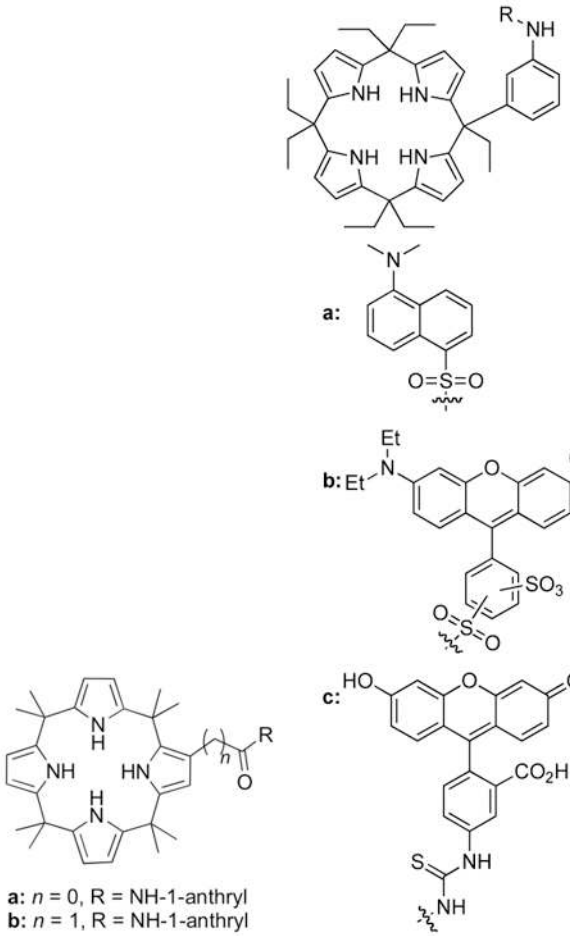
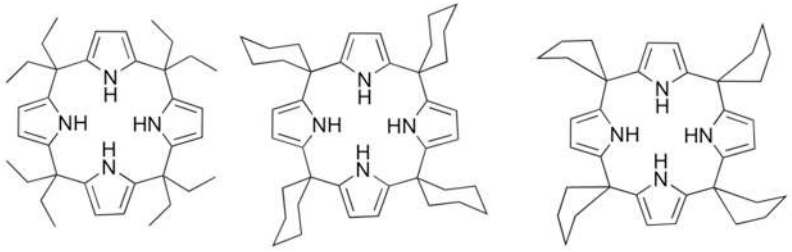
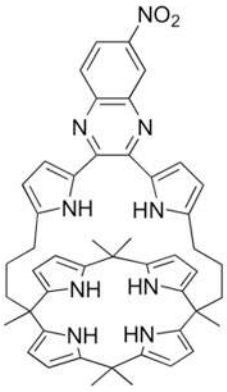


788 789



790 791 792



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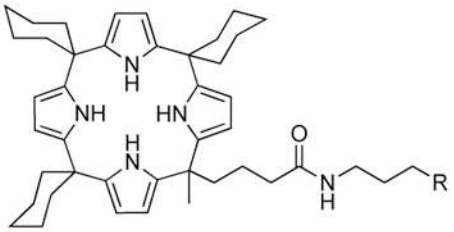
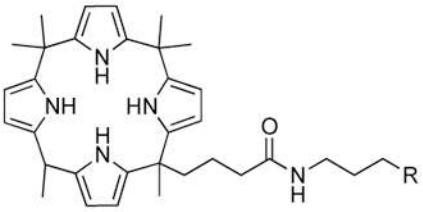


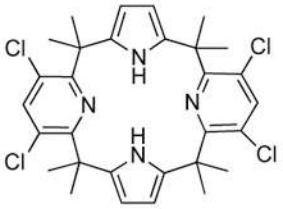
a: R = CH_3
b: R = silica gel

794



a: R = CH_3
b: R = silica gel

795



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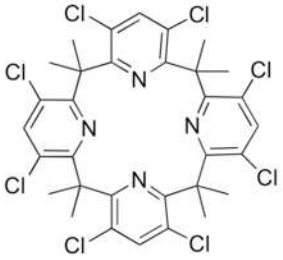

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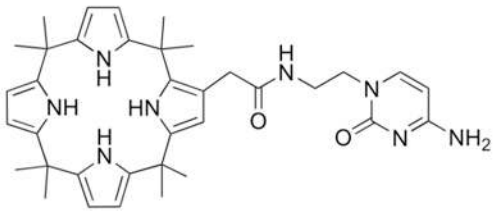

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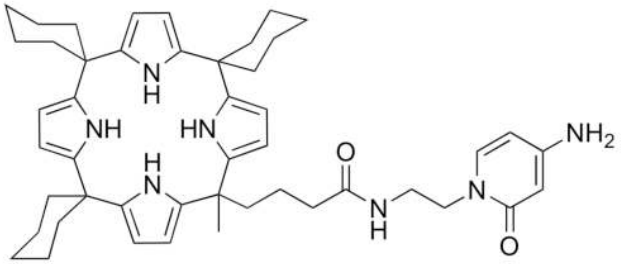

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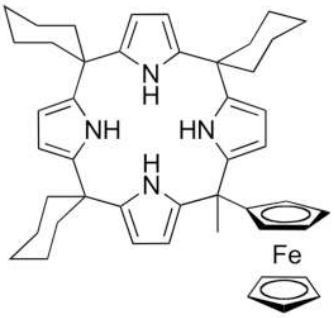

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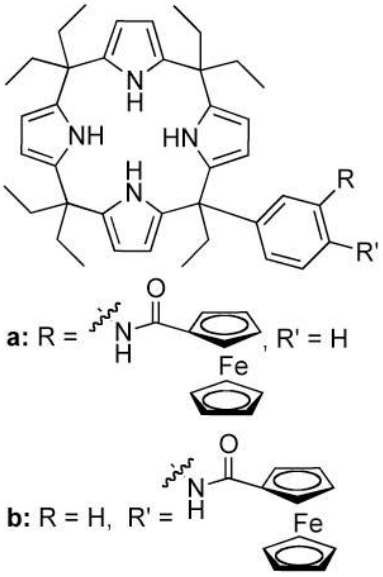

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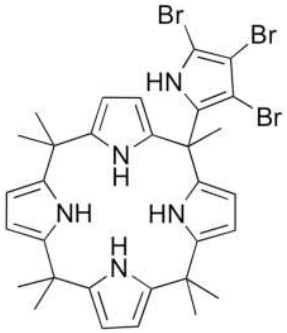

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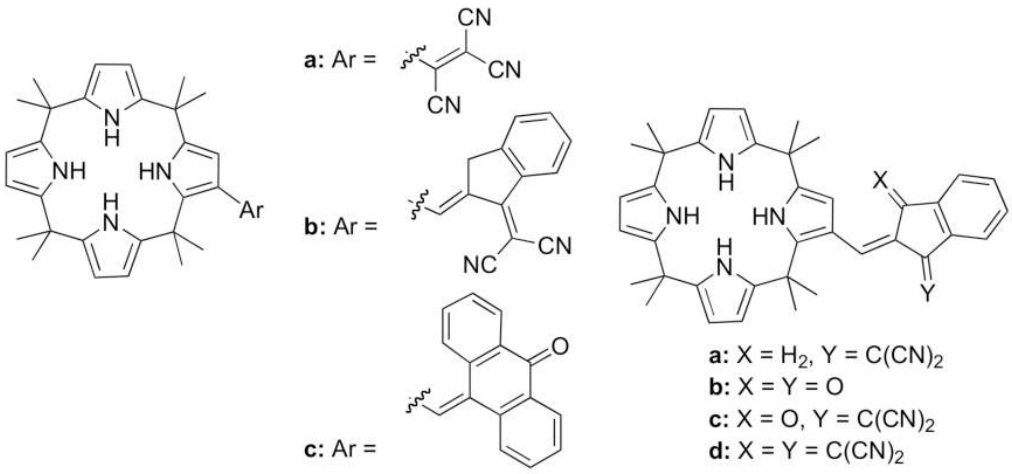
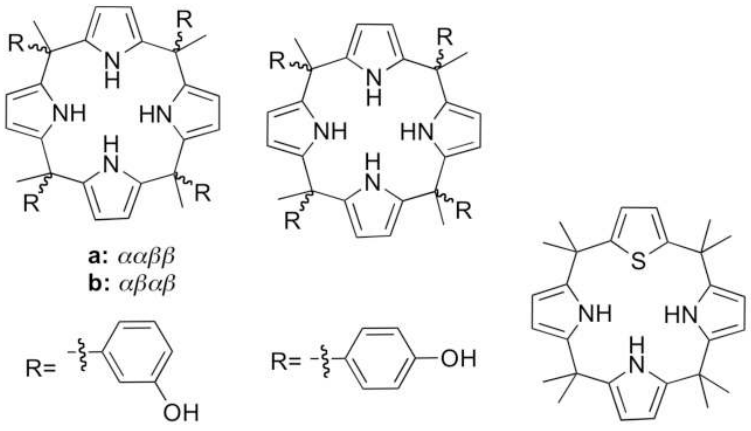
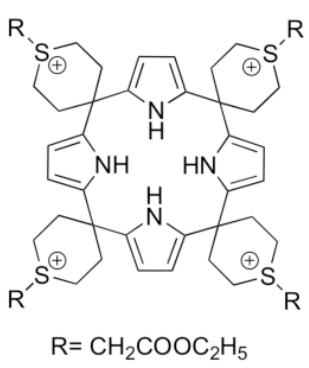


803 **804**



805 **806** **807**



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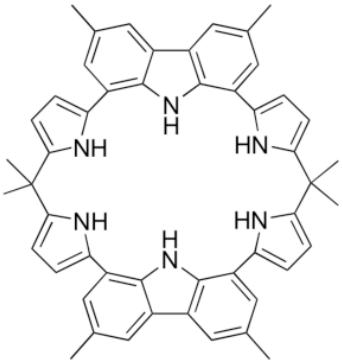

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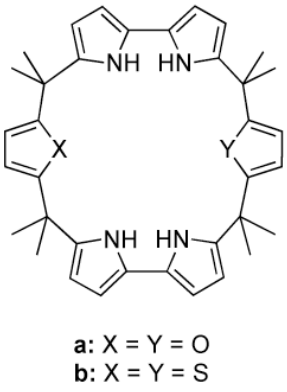

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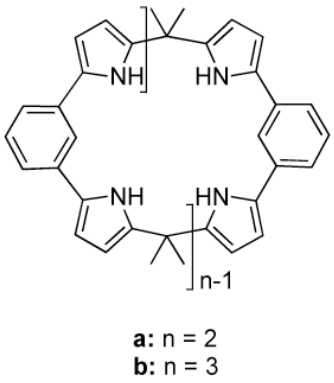

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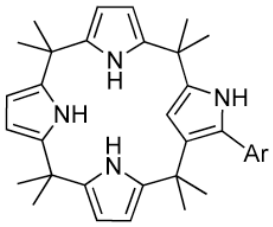

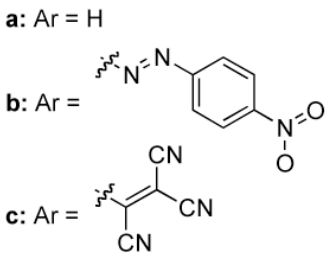

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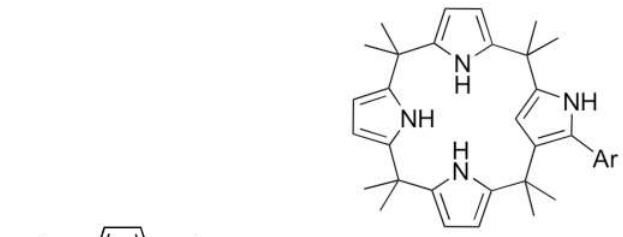
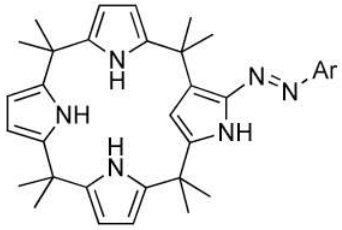
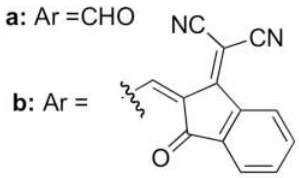
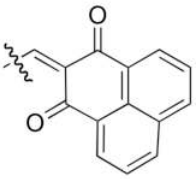


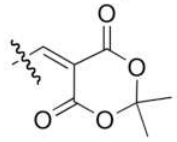


a: Ar = C_6H_5
b: Ar = *p*-C_6H_4-OCH_3
c: Ar = *p*-C_6H_4-Br
d: Ar = *p*-C_6H_4-NO_2
e: Ar = C_6F_5

813

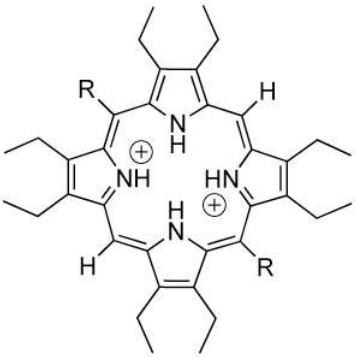


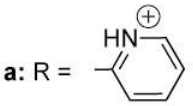




a: Ar =CHO
b: Ar =
c: Ar =
d: Ar =
e: Ar = H

814





a: R =
b: R =

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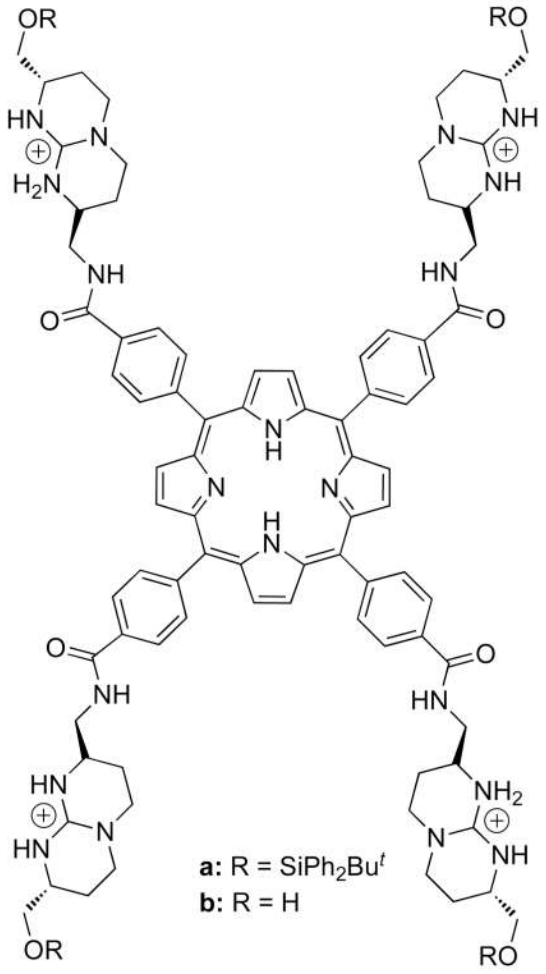

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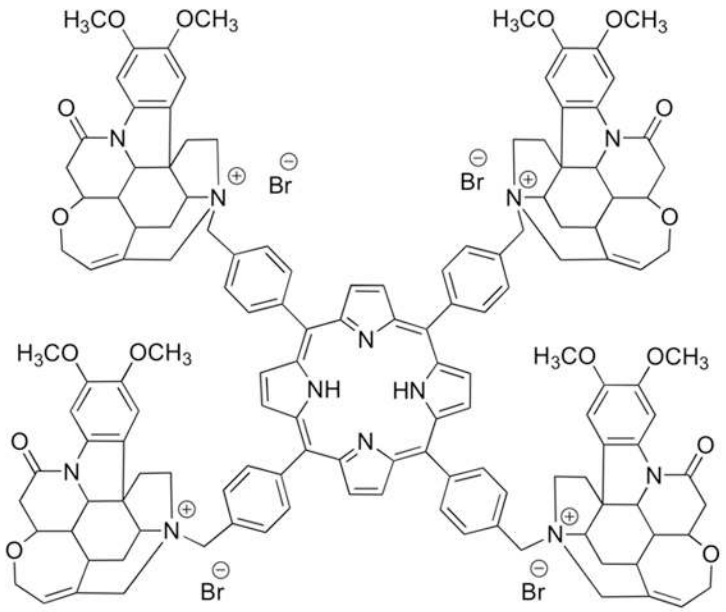

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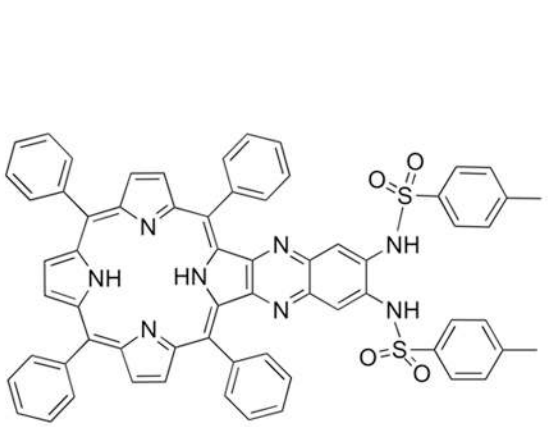

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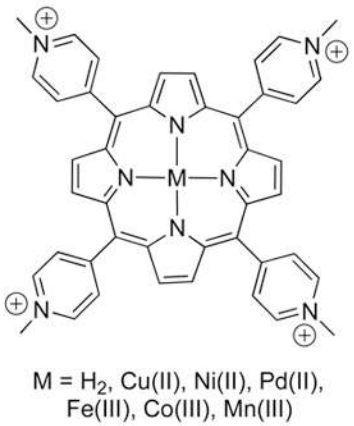

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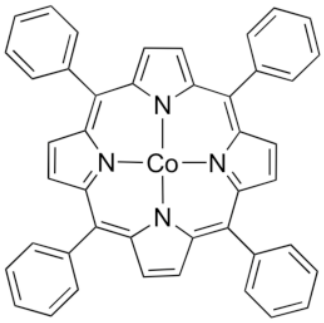

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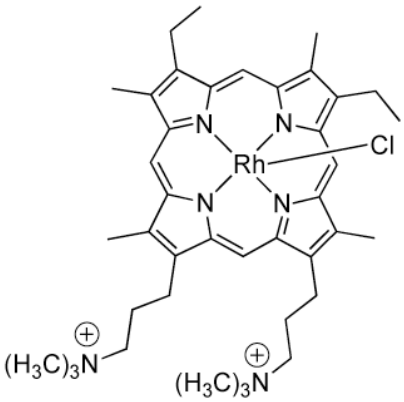

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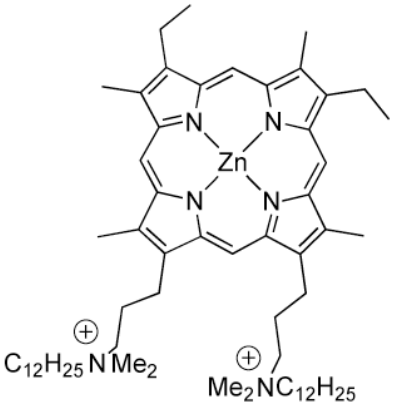

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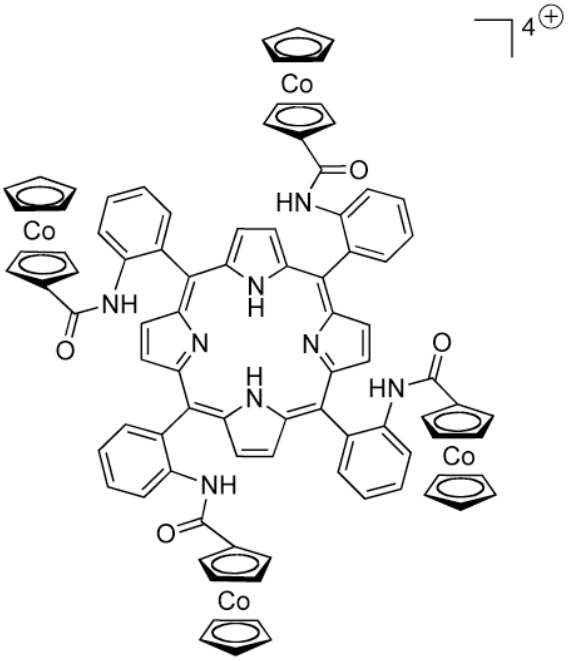

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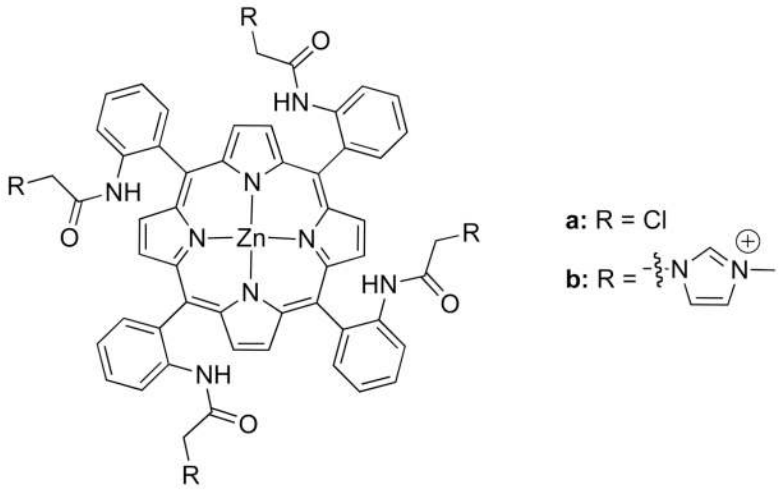

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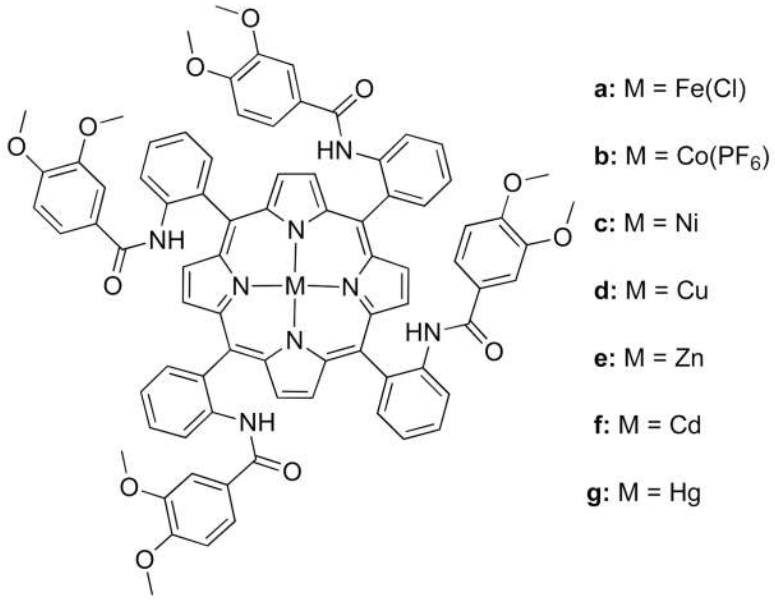

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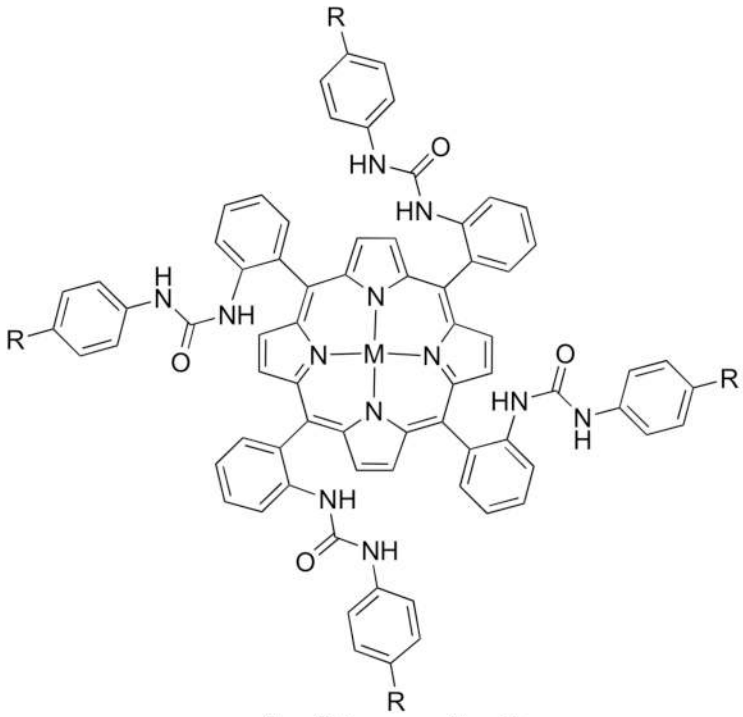
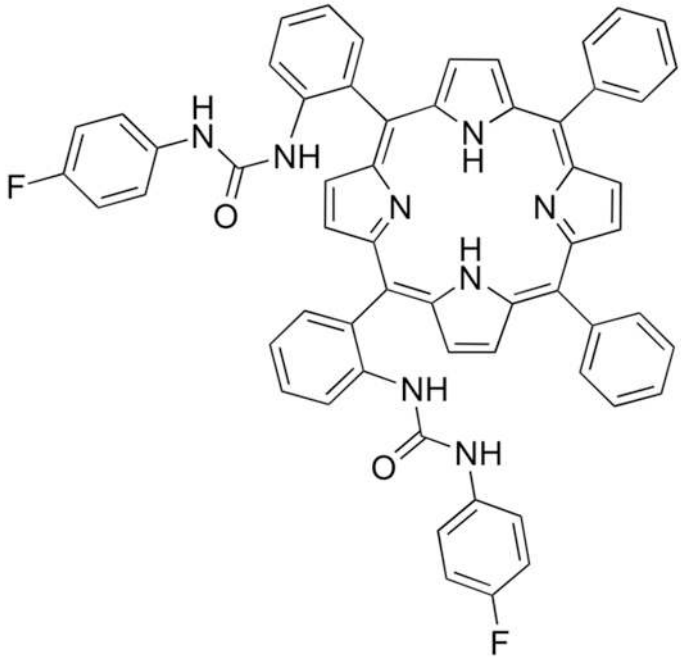


M = 2H M = Zn
a: R = H **e:** R = H
b: R = Cl **f:** R = Cl
c: R = F **g:** R = F
d: R = NO_2

826



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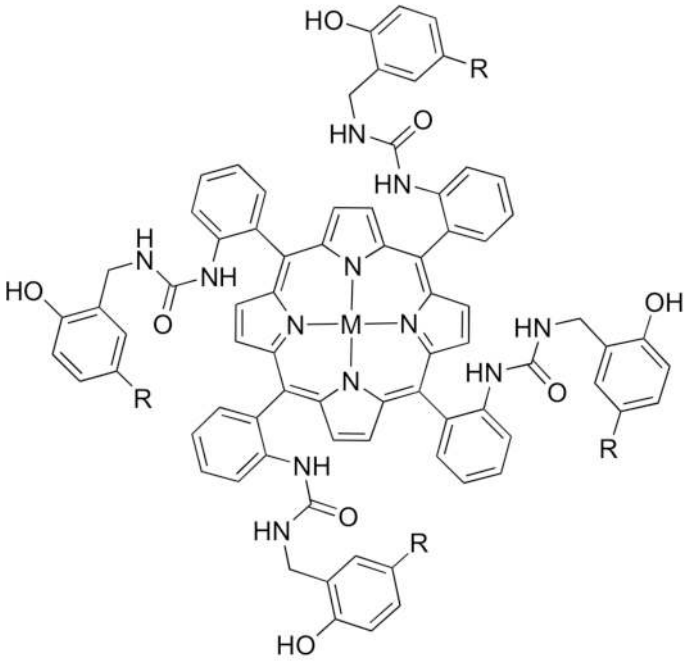
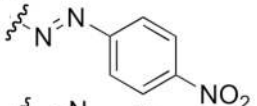
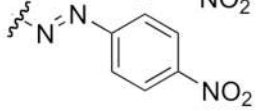


a: M = 2H, R = H
b: M = Zn, R = H
c: M = 2H, R = 

d: M = Zn, R = 

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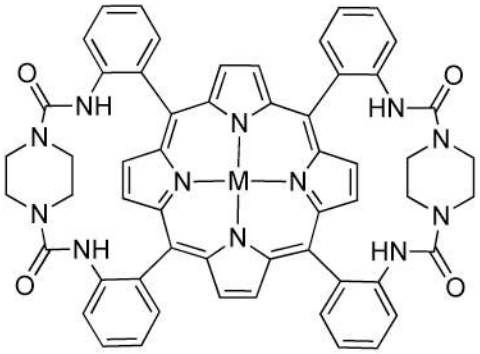
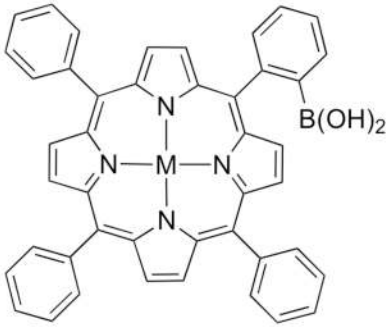


a: M = 2H

b: M = Zn

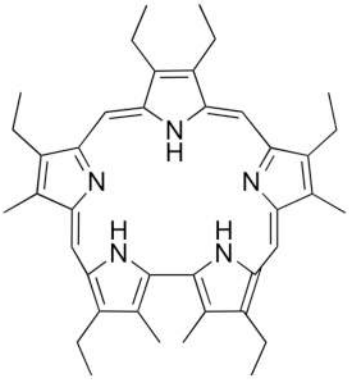
829



a: M = 2H

b: M = Zn

830



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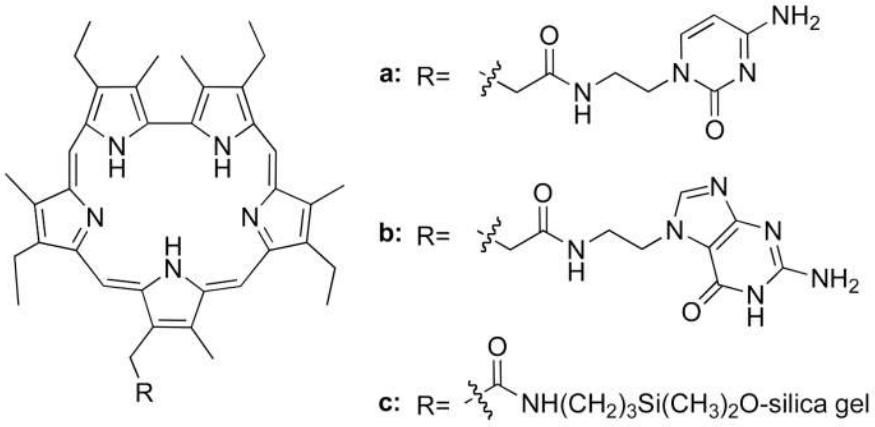

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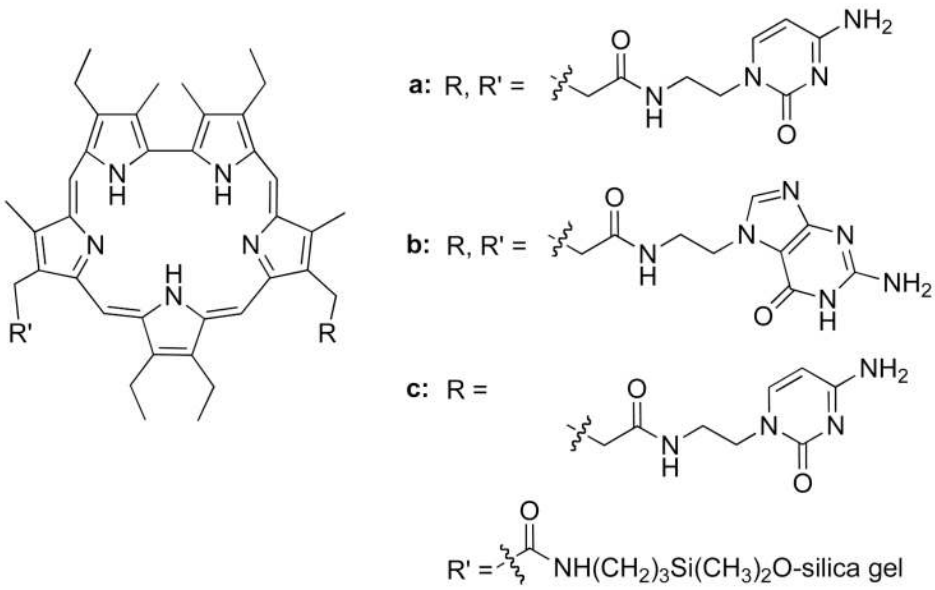

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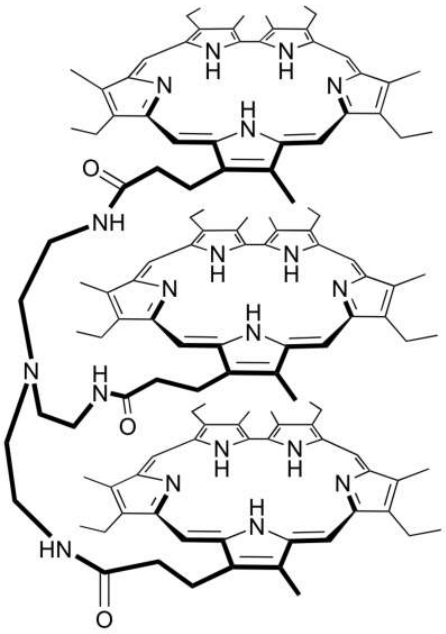

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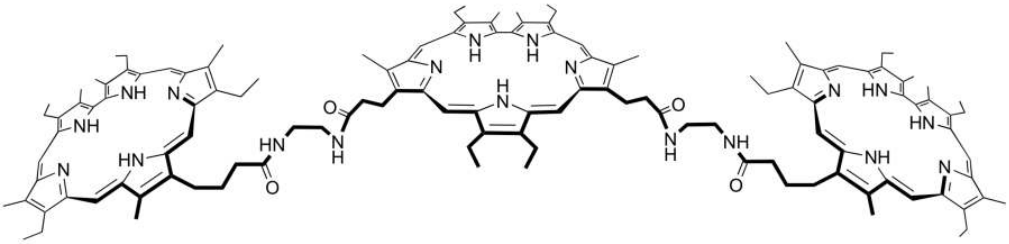

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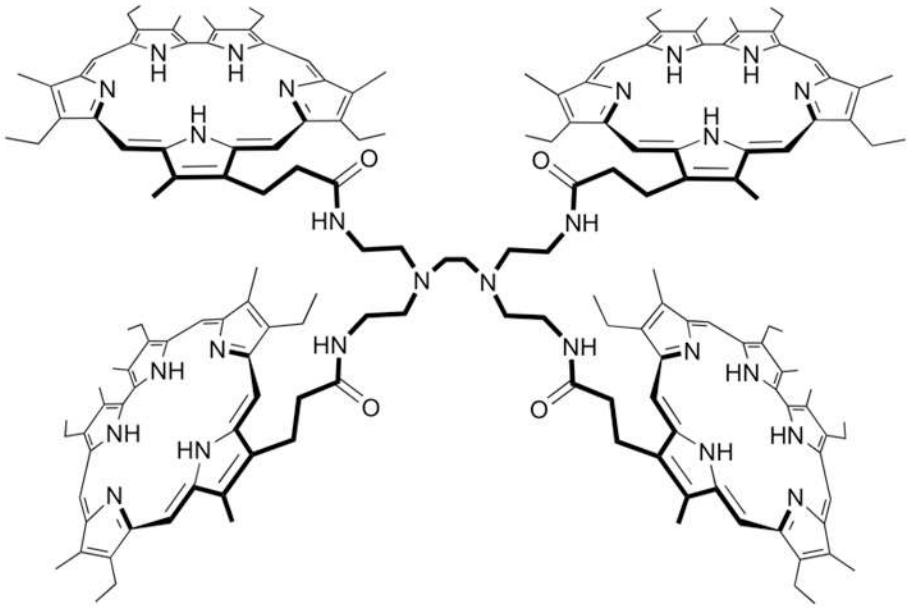

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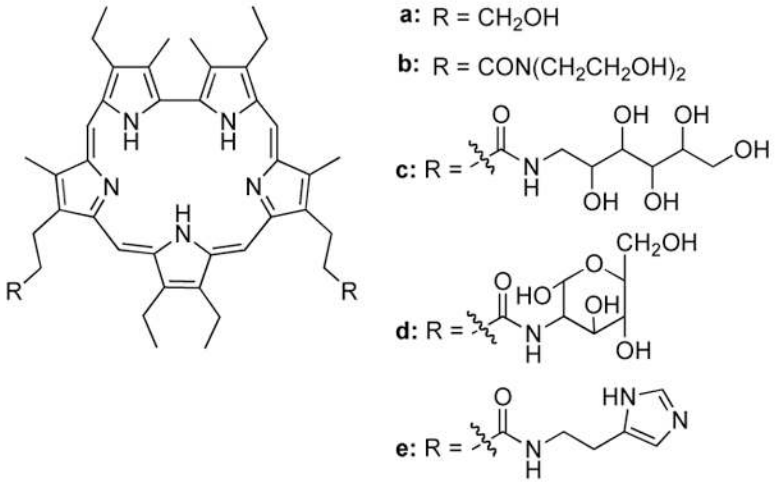

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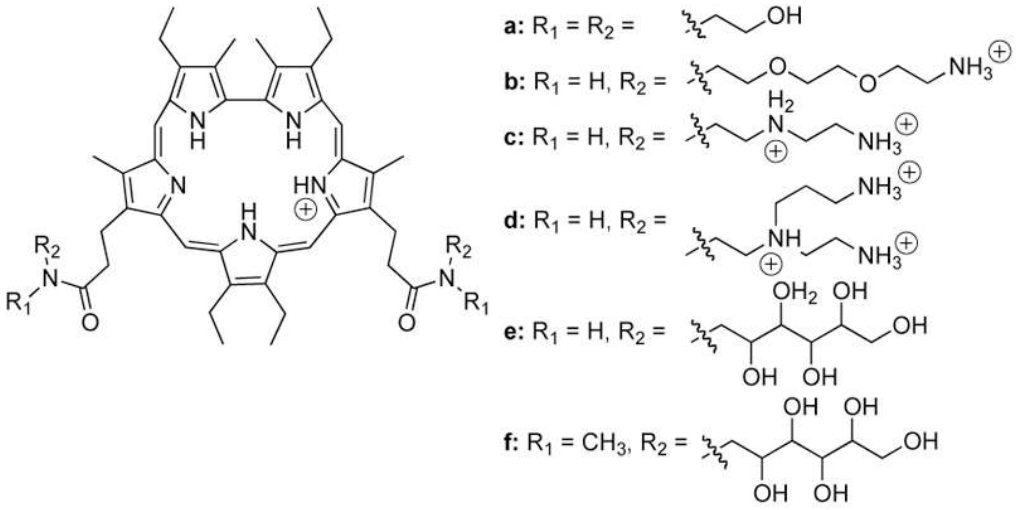

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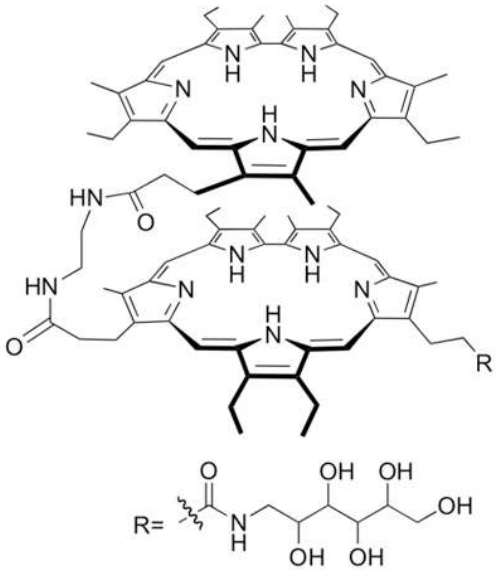

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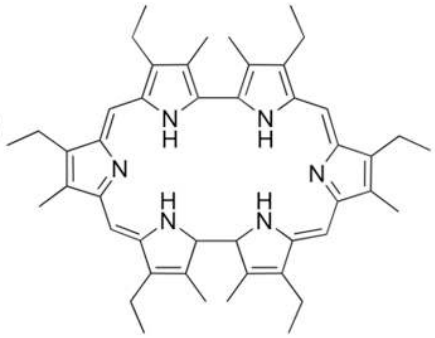

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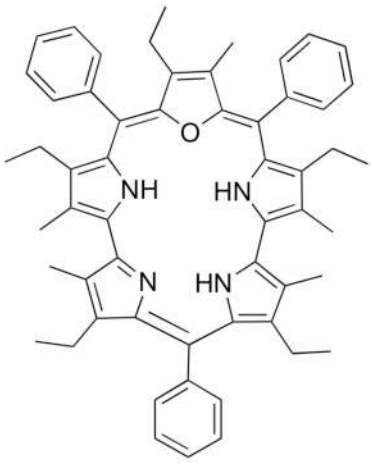

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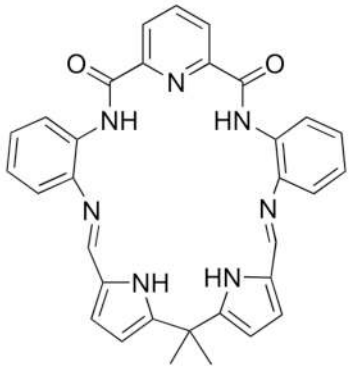

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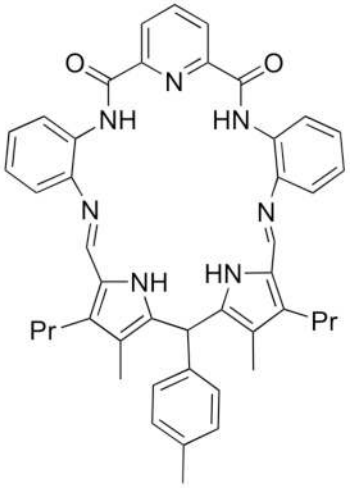

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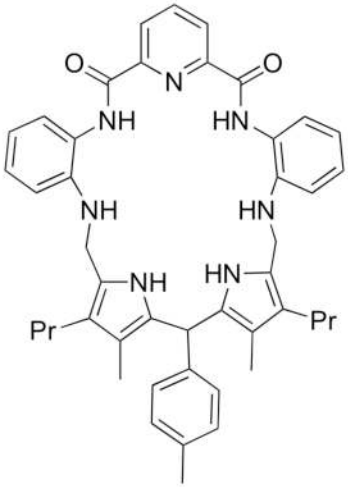

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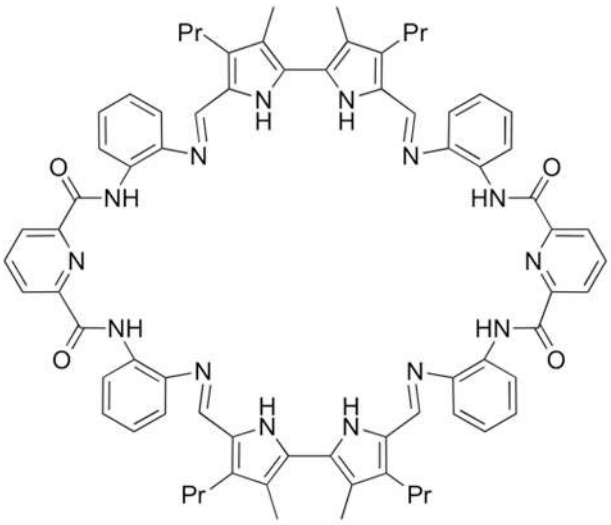

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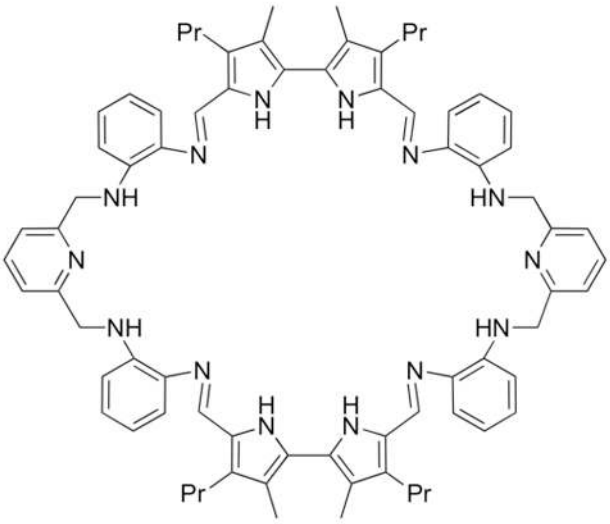

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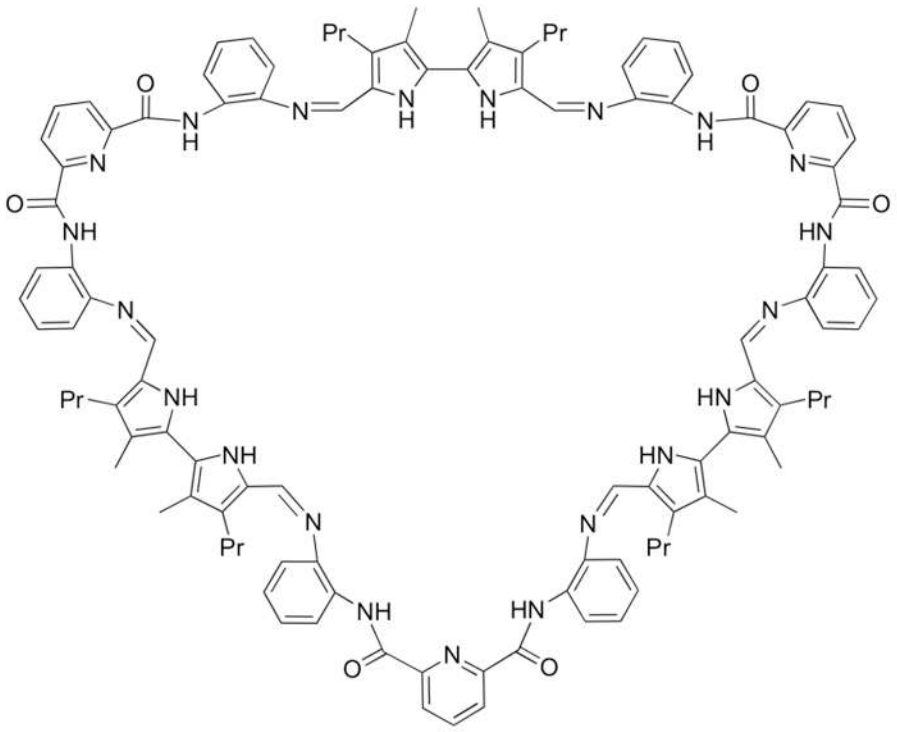

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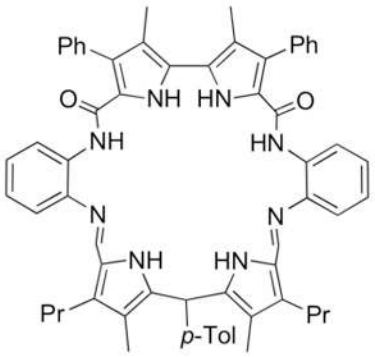

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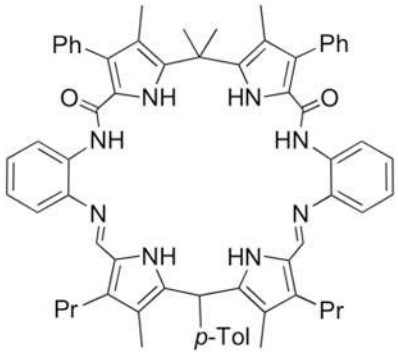

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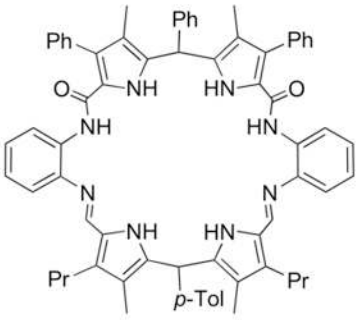

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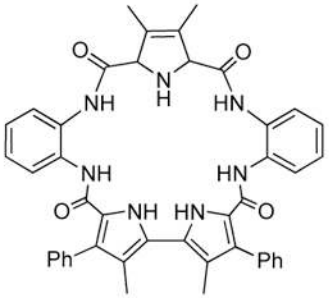

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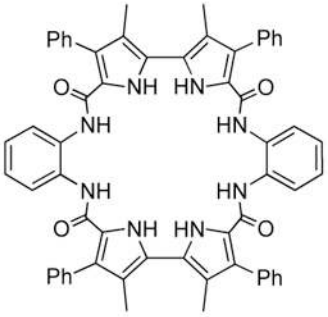

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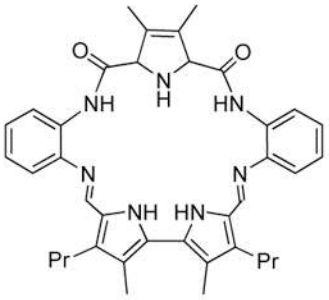

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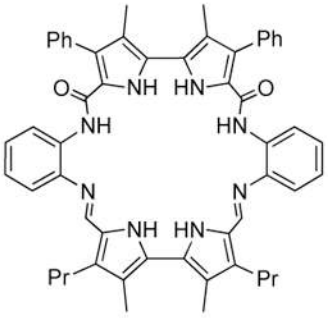

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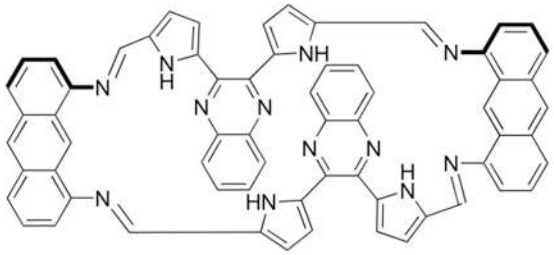

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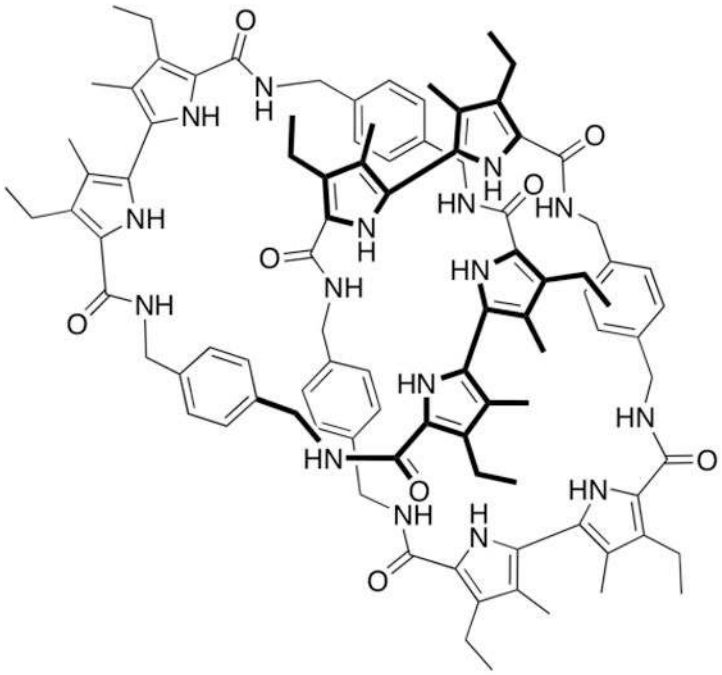

856

Table 1

Structures of commonly targeted phosphates.