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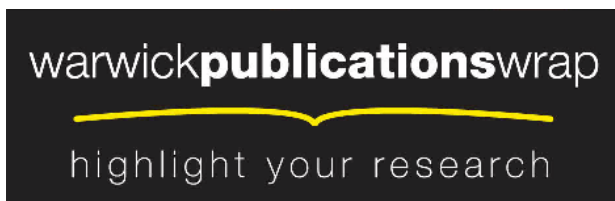
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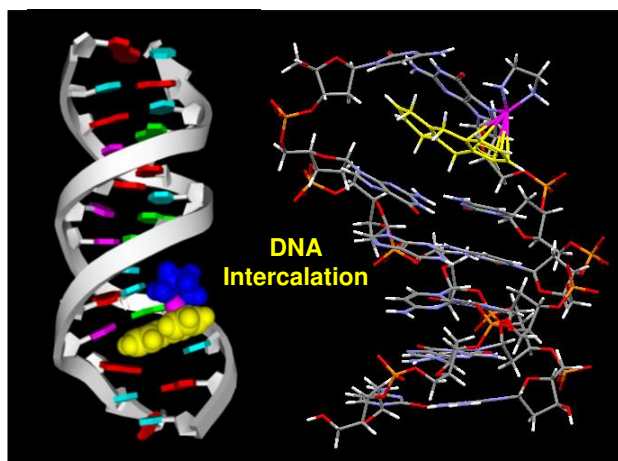
Metal Complexes as DNA Intercalators

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CONSPECTUS



Intercalation was first proposed by Lerman in 1961 to explain the strong affinity of DNA for heterocyclic aromatic dyes and has attracted considerable attention as a mode of DNA binding ever since. Organic intercalators can inhibit nucleic acid synthesis *in vivo* and are common anticancer drugs used in clinical therapy. There are two major intercalation modes: classical and threading intercalation.

We focus here on metallointercalators. The introduction of organic intercalators into transition metal coordination complexes can lead to novel DNA interactions, which can influence biological activity. Metallointercalators possess aromatic ligands

which can intercalate between DNA bases. Metal complexes with σ -bonded aromatic side-arms can act as dual-function complexes, binding to DNA by metal coordination and ligand intercalation. These aromatic side arms introduce new modes of DNA binding involving mutual interactions of functional groups held in close proximity. The biological activity of both *cis*- and *trans*-diamine Pt^{II} complexes with σ -bonded intercalators, is dramatically enhanced.

We have explored a new class of organometallic ‘piano-stool’ Ru^{II} and Os^{II} arene anticancer complexes of the type $[(\eta^6\text{-arene})\text{Ru/Os}(\text{XY})\text{Cl}]^+$, XY = e.g. ethylenediamine (en), and the π -bonded arene ligand which can intercalate into DNA. Arene-nucleobase stacking interactions can have a significant influence on both the kinetics and thermodynamics of DNA binding. In particular, the cytotoxic activity of these complexes, conformational distortions, recognition by DNA-binding proteins and repair mechanisms are dependent on the arene. These complexes are non-cross-resistant with cisplatin towards cancer cells, and constitute a new class of anticancer agents with a different mechanism of action as compared to the anticancer drug cisplatin and its analogs. Cross-resistance is a phenomenon whereby a cell which is resistant to one drug is also resistant to another drug in the same class. Ruthenium-arene complexes with dual functions are more potent towards cancer cells than their non-intercalating analogs.

In this Account, we focus on recent studies of dual function organometallic Ru^{II} and Os^{II} arene complexes and the methods used to detect arene-DNA intercalation. We relate these interactions to the mechanism of anticancer activity and to

structure-activity relationships. The interactions between these complexes and DNA show close similarities to those of covalent polycyclic aromatic carcinogens, especially to N7-alkylating-intercalation compounds. However, Ru-arene complexes exhibit some new features. Classical intercalation and base extrusion next to the metallated base is observed for $\{(\eta^6\text{-biphenyl})\text{Ru}(\text{ethylenediamine})\}^{2+}$ adducts of a 14-mer duplex, while penetrating arene intercalation occurs for adducts of the non-aromatic bulky intercalator $\{(\eta^6\text{-tetrahydroanthracene})\text{Ru}(\text{ethylenediamine})\}^{2+}$ with a 6-mer duplex. The introduction of dual function ruthenium-arene complexes introduces new mechanisms of antitumor activity, novel mechanisms for attack on DNA, and new concepts for developing structure-activity relationships. We hope this discussion will stimulate thoughtful and focused research on the design of anticancer chemotherapeutic agents using these unique approaches.

Introduction

Intercalative binding, as most commonly studied, is the non-covalent stacking interaction resulting from the insertion of a planar heterocyclic aromatic ring between the base pairs of the DNA double helix. This DNA binding mode was first proposed by Lerman¹ to explain the strong affinity for DNA of certain heterocyclic aromatic dyes such as the acridines. Intercalation stabilizes, lengthens, stiffens and unwinds the DNA double helix.¹⁻² The degree of unwinding varies depending on the intercalator. The ethidium cation unwinds DNA by about 26° and proflavine by about 17°.³⁻⁴ These structural modifications can lead to functional changes, often to inhibition of transcription and replication and DNA repair processes, which make intercalators potent mutagens.

There is much interest in the ability of intercalators to inhibit nucleic acid synthesis *in vivo*, leading to activity as mutagens, antibiotics, antibacterials, trypanocides, schistosomicides and antitumor agents.⁵ Intercalative interactions between DNA duplexes and planar polycyclic aromatic organic intercalators,⁵⁻⁶ such as ethidium bromide; acridine and its derivatives, and benzo[a]pyrene (BP),⁷ have been thoroughly studied. Studies of bulky intercalators are rare.⁸ Bis-intercalators⁹⁻¹⁰ have also been reported, such as bis-naphthalimide, which exhibits antitumor activity.⁹ A tetra-intercalator has been reported by Iverson.¹¹ Representative polycyclic aromatic intercalators are shown in Table 1.

There are two major modes of intercalation: classical intercalation^{9,12-14} and threading intercalation.^{11,15-16} Classical intercalators, such as benzo[a]pyrene (BP)⁷

(Figure 1.1), bind to DNA duplexes with essentially all of their aromatic system inserted between GpG base pairs that form the top and bottom of the intercalation site. A threading intercalator occupies and interacts strongly with both the minor and major grooves of DNA simultaneously. For example, the threading intercalation of acridine-4-carboxamides (DACA, Figure 1.2) into the duplex 5'-d(CG(5-BrU)ACG)₂-3' has been reported by Cardin.¹⁶ Many intercalators, including ethidium and acridine, are relatively nonspecific DNA binding agents. Sequence-specific DNA binding is improved when the aromatic ring is substituted with special ancillary groups, or when bis-intercalators are involved. For example acridine-4-carboxamides have a N-(2-dimethylamino)ethyl-4-carboxamide side-chain (Figure 1.2).¹⁶ This type of agent inhibits both topoisomerases I and II.¹⁶

Metal complexes which can have intercalative interactions with DNA form two classes. For relatively inert coordinatively-saturated square-planar Pt(II) complexes and octahedral complexes with aromatic ligands, intercalation into DNA mainly involves the aromatic ligands. Metal complexes containing σ -bonded ligands with aromatic side-arms as intercalators or organometallic complexes with π -bonded arenes as intercalators can be dual-function complexes: the aromatic side-arms or arene ligands can intercalate between DNA bases and while the metal coordinates directly to a DNA base. Intercalative interactions between metal complexes and DNA have novel features which can influence biological activity, and this is the focus of our review.

2. Intercalation by inert square-planar and octahedral metal complexes

Square-planar complexes containing aromatic fragments can bind to DNA by intercalation without direct metal coordination to DNA bases.¹⁷ Lippard et al. have shown that square-planar platinum(II) complexes containing heterocyclic aromatic ligands, such as terpy, quaterpy, phen, bipy and phi, bind to DNA duplexes noncovalently, intercalating between the base pairs (Fig. 2.1).¹⁷ The crystal structure of $[\text{Pt}(\text{terpy})(\text{HET})]^+$ (HET = 2-hydroxyethanethiolato-2,2',2''-terpyridine) bound to $(\text{dCpG})_2$ (Figure 2.2) reveals that the flat metal cation intercalates symmetrically between two GC base pairs.¹⁸

Metallointercalation has been extended into three dimensions by Barton and others using octahedral Rh, Ru or Os complexes containing multi heterocyclic aromatic ligands such as phen, phi or dppz, chrysi, phzi, dpq, dppn and eilatin (Figs. 2.2 and 2.5).¹⁹⁻²¹ Octahedral metallointercalators permit the targeting of specific DNA sites by matching the shape, symmetry, and functionalities of the metal complex to that of the DNA target. There are two kinds of non-covalent interactions between metallo-intercalators and DNA: intercalation and insertion.¹⁹ Metallo-intercalators unwind the DNA and insert their planar ligand between two intact base-pairs, while metallo-insertors eject the bases of a single base-pair, and their planar ligand acts as a π -stacking replacement in the DNA base stack.¹⁹ Complexes with ligands phen, phi or dppz have classic intercalation into duplex DNA, but complexes with ligands chrysi, phzi and eilatin usually insert into duplex DNA.^{19,21} 2D NMR studies²² of $\Delta\text{-}\alpha\text{-}[\text{Rh}(\text{R}, \text{R-Me}_2\text{trien})\text{phi}]^{3+}$ (R, R-Me₂trien = 2R, 9R-diamino-4,7-diazadecane) bound to

5'-d(GTTGCAAC)₂-3' duplex DNA reveal that the phi ligand intercalates classically into the DNA base-pair stack from the major groove at the G⁵pC⁶ site. The crystal structure of the adduct of [Δ -Rh(bpy)₂(chrysi)]³⁺ with an oligonucleotide duplex containing two AC mismatches 5'-d(CGGAAATTCCCG)₂-3' (Fig. 2.4), reveals two binding modes for [Rh(bpy)₂(chrysi)]³⁺:²³ intercalation and insertion. Some of bis-metallointercalators (Fig. 2.5) can undergo threading, which has been extensively studied.^{15,19,24}

The bleomycins (BLMs) are natural products. Cobalt bleomycin probably has the most pharmaceutical relevance of any of its metal adducts studied to date.²⁵ 2D NMR methods and molecular modeling have shown that HOO-CoPLM binds to 5'-d(CCAGGCCTGG)₂-3' in a partial intercalative mode providing a basis for the sequence specificity of binding and chemical specificity of cleavage.²⁶

3. Intercalation by dual-function metal complexes with σ -bonded side-arm intercalators.

In the late 1980's, platinum complexes having a σ -bonded side-arm as an intercalator were designed.^{17,27} Planar aromatic ligands such as acridine orange (AO), 9-aminoacridine (9-AA), and ethidium bromide^{4,28} have been incorporated into Pt complexes as potential intercalators (Figure 3.1). These Pt-intercalator complexes can react with DNA by both coordination and intercalation, and the DNA platination can be sensitive to sequence-dependent local DNA structural modulations such as those accompanying intercalator binding. The reaction between cisplatin and ethidium (Eth)

is promoted by DNA; ethidium binds to platinum via its N8 or N3. Modeling shows that the ethidium is intercalated between the C³/G¹⁰:G⁴/C⁹ base pairs of 5'-d(CGCGCG)₂-3', while the *cis*-{Pt(NH₃)₂}²⁺ fragment coordinates to N7 of G⁴. These complexes introduced new modes of DNA binding involving mutual interactions of functional groups held in close proximity.

Bierbach et al.²⁷ have described a new class of hybrid Pt compounds containing acridinylthiourea as intercalating groups (Fig. 3.2). Platinum-acridinylthiourea (PT-ACRAMTU) and its analogs exhibit promising activity towards several tumor cell lines, and show only partial cross-resistance with cisplatin.^{27,29} The 2D NMR solution structure³⁰ of PT-ACRAMTU with (5'-CCTCGTCC-3')/(3'-GGAGCAGG-5') shows Pt is bound to N7 of G⁵ in the major groove, and ACRAMTU intercalates threadingly into the central 5'-C⁴G⁵/C¹²G¹³ base-pair step on the 5'-face of the platinated nucleobase (Fig. 3.2). Intercalation of ACRAMTU does not cause helical bending, but augments significantly duplex thermal stability, indicating that the PT-ACRAMTU lesion is different from that formed by cisplatin cross-linking.

Planar aromatic ligands have also been introduced into transplatin, *trans*-[PtCl₂(NH₃)₂], for development of a new class of anticancer drug (Figure 3.1).¹⁷ When one ammine is changed to a planar aromatic N-donor ligand such as L = pyridine or quinoline in *trans*-[PtCl₂(NH₃)L], the cytotoxicity of the *trans* complex is dramatically enhanced.³¹ This has been attributed to interaction of the planar aromatic ligand with the DNA duplex, perhaps partially by intercalation.³²

Other dual-function coordination complexes have also been studied (Fig. 3.3).^{14,33}

2D NMR shows combined coordinative and intercalative interactions between the dirhodium(II) complex $cis\text{-}[\text{Rh}_2(\text{dap})(\mu\text{-O}_2\text{CCH}_3)_2(\eta^1\text{-O}_2\text{CCH}_3)(\text{CH}_3\text{OH})](\text{O}_2\text{CCH}_3)^{14}$ (dap=1,12-diazaperylene) and the duplex (5'-GGAAGTTGAGAG-3')/(5'-CTCTCAACTTCC-3'). One rhodium atom binds coordinatively to the N7 of A⁶ base, while the dap ligand intercalates classically into the central A⁶pA⁷ base step.

4. Intercalation by organometallic Ru and Os arene complexes.

Organometallic Ru^{II} arene complexes of the half-sandwich “piano-stool” type $[(\eta^6\text{-arene})\text{Ru}(\text{XY})\text{Cl}]^+$, arene = e.g. tetrahydroanthracene (tha), biphenyl (bip), dihydroanthracene (dha), *p*-cymene (cym), benzene (ben), XY = e.g. ethylenediamine (en), exhibit promising anticancer activity both in vitro and in vivo (Figure 4.1).³⁴⁻⁴⁰ One of the potential targets of these complexes is DNA. The strong binding to N7 of G on DNA is enhanced by H-bonding between the en NH₂ groups and the exocyclic C6O carbonyl oxygen of G. Repulsive interactions with exocyclic amino groups of A and C nucleobases lead to high specificity for G binding (Fig. 4.2).⁴¹ The rates of reaction of $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ complexes with cGMP depend on the nature of the arene, decreasing by over an order of magnitude in the series ($\times 10^{-2} \text{ M}^{-1}\text{s}^{-1}$): tha (14.72) > bip (8.06) > dha (7.08) > cym (2.49) > ben (1.13) (for abbreviations, see above).⁴² Also the cytotoxic activity of these complexes shows a strong dependence on the arene:³⁵⁻³⁶ benzene (Ru-ben, IC₅₀ = 17 μM) < *p*-cymene (Ru-cym, 10 μM), < biphenyl (Ru-bip, 5 μM), < dihydroanthracene (Ru-dha, 2 μM), <

tetrahydroanthracene (Ru-tha, 0.5 μM), the latter complex being as cytotoxic as the clinical platinum drug cisplatin. We have also investigated the chemical and biological activity of half-sandwich osmium arene complexes.^{35,43-45} Their cytotoxicity (towards A2780 cells) also appears to depend on the size of the coordinated arene, benzene (Os-ben, $\text{IC}_{50} = 32.7 \mu\text{M}$) < p-cymene (Os-cym, 7.6 μM), < tetrahydroanthracene (Os-tha, 4.5 μM), < biphenyl (Os-bip, 3.2 μM), the latter two complexes being as cytotoxic as carboplatin.^{35,45-46}

Studies of DNA duplexes singly-modified by Ru-cym (non-intercalating) and Ru-tha (intercalating) reveal that conformational distortions, recognition by DNA-binding proteins, and repair mechanisms are also dependent on the arene.⁴⁷⁻⁴⁸ Distortions induced by Ru-cym and Ru-tha are not recognized by the DNA-binding HMGB1 protein, indicating that the mechanism of antitumour activity of Ru^{II} arene complexes does not involve recognition of their DNA adducts by HMG domain proteins as a crucial step, in contrast to cisplatin and its analogs.^{38-39,46-47,49-52} Adducts of $\{(\eta^6\text{-}p\text{-cymene})\text{Ru}(\text{en})\}^{2+}$ (Ru-cym) are removed from DNA more efficiently than those of Ru-tha, and both adducts are removed from DNA preferentially by mechanisms other than nucleotide excision repair (a major mechanism contributing to cisplatin resistance).^{36,53} DNA binding studies on a series of complexes of the type $[(\eta^6\text{-arene})\text{Os}(\text{XY})\text{Cl}]^{n+}$, where arene = e.g. biphenyl and XY = e.g. ethylenediamine, show that these complexes all bind and distort polymeric DNA with a rate of binding comparable to that of cisplatin.^{35,54} They coordinate mainly to guanine with additional non-coordinative interactions. DNA osmiated with $\{(\eta^6\text{-arene})\text{Os}(\text{XY})\}^+$ (arene =

biphenyl or *p*-cymene, XY = ethylenediamine, picolinate or oxinate), inhibits RNA synthesis like cisplatin and the ruthenium analog Ru-bip.⁵⁴ In contrast to cisplatin, no DNA bending occurs. The unwinding angle induced in supercoiled plasmid DNA by Os-arene complexes is larger (21–27°) than that of ruthenium analogs (7–14°) or cisplatin (6° for monofunctional and 13° for bifunctional adducts).³⁵⁻³⁶ The noncovalent interactions are greater for $[(\eta^6\text{-bip})\text{Os}(\text{en})\text{Cl}]^+$ than those of its Ru analog.³⁵ Remarkably, these Ru^{II} and Os^{II} complexes are non-cross-resistant with cisplatin towards cancer cells, implying promise for tackling the common problem of developed (and intrinsic) drug resistance in chemotherapy.³⁵ These complexes form a new class of novel anticancer agents with a different mechanism of action compared to the anticancer drug cisplatin and its analogs.⁵⁴⁻⁵⁵

Detection of arene intercalation.

DNA intercalation by arene ligands can be detected by several methods. a) Circular dichroism (CD). The presence of the tricyclic tetrahydroanthracene ligand on Ru^{II} (Ru-tha) results in the appearance of a CD band centered around 370-380 nm on interaction with ct-DNA, while binding of the mono-cyclic Ru-cym complex to DNA does not give such a band in this region of the spectrum.⁵⁰ b) Linear dichroism (LD). The complexes Ru-tha, Ru-dha, and Ru-bip cause a significant red shift (ca. 10 nm) of the main DNA band near 260 nm in LD spectra,⁵⁰ whereas complexes Ru-ben and Ru-cym cause no such shift. c) Fluorescence measurements.⁵⁰ Modification of DNA by Ru-bip, Ru-dha, and Ru-tha results in a marked decrease in the fluorescence of EthBr bound to DNA, while the decrease caused by binding to Ru-cym is very small.

These data indicate intercalation by Ru arene complexes with extended arene ligands.

d) Isothermal titration calorimetry (ITC). The intercalation of porphyrins is evident from the enthalpic terms measured by ITC, which are similar to those reported for ethidium bromide with a G4 tetraplex.⁵⁶ e) Viscosity. The binding of $[\text{Pt}(\text{en})(\text{phen})]^{2+}$ to duplex DNA gives rise to an increase in the relative viscosity, indicative of intercalative binding of the phen ligand.⁵⁷ f) Melting temperature (T_m). The T_m of duplex DNA modified by intercalators Ru-bip, Ru-dha, or Ru-tha increases, while T_m decreases for DNA modified by non-intercalators Ru-ben and Ru-cym,⁵⁰. g) NMR. Intercalation between DNA base pairs can often be recognized by distinctive NMR features,¹¹⁻¹⁴ including upfield ^1H NMR shifts of the intercalator, NOE cross-peaks between intercalator and DNA bases at sites of intercalation, and the interruption or weakening of NOE connectivities between sequential DNA nucleotides. 2D NMR studies of the interactions of Ru-bip with 5'-d(CGGCCG)₂-3' reveal that the arene ligand intercalates between G³/C⁴ or C⁵/G⁶ base pairs.¹³ h) X-ray crystallography: Wang et al. have reported a 1.8 Å resolution crystal structure of a macrocyclic-bis-9-aminoacridine complex bound to 5'-(CGTACG)₂-3' by intercalation.¹⁰

Adducts of Ru-arene complexes with nucleobases and DNA

We have determined the structures of monofunctional adducts of the Ru-arene complexes⁴¹⁻⁴² with guanine derivatives by X-ray crystallography and 2D NMR methods. Strong π - π arene-nucleobase stacking (center-to-center distances of 3.45 Å for Ru-tha and 3.31 Å for Ru-dha) is present in the crystal structures of

$[(\eta^6\text{-tha})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ and $[(\eta^6\text{-dha})\text{Ru}(\text{en})-(9\text{EtG-N7})]^{2+}$ (Fig. 4.3). Significant reorientations and conformational changes of the arene ligands in $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{G-N7})]^{2+}$ complexes are observed in the solid state, with respect to those of the parent chlorido-complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$,⁴¹ which maximize intra- or intermolecular stacking of extended arene rings with the purine ring. This flexibility makes simultaneous arene-base stacking and N7-covalent binding compatible.

The tetrahydroanthracene complex Ru-tha is 10x more toxic to cancer cells than the biphenyl complex Ru-bip.³⁶ The extended non-aromatic rings B and C of Ru-tha are more bulky than bip in Ru-bip (Figure 4.1). 2D-NMR experiments,⁵⁸ show the presence of a novel penetrating intercalation of rings B and C of Ru-tha into a DNA hexamer, selectively between two base pairs, $\text{G}^3/\text{C}^{10}:\text{C}^4/\text{G}^9$ or $\text{G}^6/\text{C}^7:\text{C}^5/\text{G}^8$ (Figure 4.4). Large low-field shifts for protons on rings B and C of Ru-tha are observed for the mono-ruthenated DNA adduct. Deshielding of intercalator NMR resonances is rare.

The dinuclear Ru-arene complex $[\{(\eta^6\text{-bip})\text{RuCl}(\text{en})\}_2(\text{CH}_2)_6]^{2+}$ (Fig. 4.5),⁵⁹ can undergo double intercalation into DNA via induced-fit recognition involving epimerization at the dynamic stereogenic centers. DNA cross-linking by $[\{(\eta^6\text{-bip})\text{RuCl}(\text{en})\}_2(\text{CH}_2)_6]^{2+}$ is observed, and the complex generates interstrand cross-links with similar efficiency to cisplatin; 1,3-GG interstrand, 1,2-GG and 1,3-GTG intrastrand cross-links are observed for 20-mer DNA duplexes.⁵⁹ This dinuclear Ru-arene complex blocks intercalation of ethidium considerably more than

mononuclear analogs, and both the free phenyl rings can contribute to the DNA intercalation (Fig. 4.5).

Further work¹² has provided evidence that coordinative and intercalative interactions of Ru-bip can affect the base-pairing of duplex DNA. The fragment $\{(\eta^6\text{-bip})\text{Ru}(\text{en})\}^{2+}$ from Ru-bip is highly specific for G N7 in the 14-mer DNA duplex d(5'-ATACATGGTACATA-3')/(3'-TATGTACCATGTAT-5') but relatively labile - it can migrate to other G residues at high temperature. Ruthenium arene intercalation is dynamic in nature: equilibria can exist between intercalated and non-intercalated conformers, Fig. 4.6. In one conformer, the base T17 is partially extruded from its Watson-Crick base-pair in the helix and the pendent phenyl ring of biphenyl (bip) is involved in π - π stacking with DNA bases either via classical intercalation between the bases, or with the partially extruded T base.^{12,60} Cross-linking of bases by cisplatin can also give rise to extruded bases⁶⁰ and such distortions are thought to affect histone protein and nucleosome recognition. The displacement, or flip-out, of bases near the modified sites is common for DNA modified covalently by aromatic or bulky intercalators, and may act as a trigger for nucleotide excision repair (NER).⁸

5. Comparison with dual-mode organic intercalators

Polycyclic aromatic hydrocarbons,⁶¹ heterocyclic amines,⁶² their nitro derivatives and food mutagens,⁶¹⁻⁶² are well known classes of environmental pollutants that cause the formation of bulky DNA lesions in vivo. They are dual-function organic intercalators which can modify DNA bases covalently and exhibit intercalative

interactions. Oligonucleotides containing these groups have been studied extensively.^{8,61,63} The modification of DNA bases by polycyclic aromatic carcinogen induces various structural distortions, including double-helix bending, diminished base-stacking, classical or threading intercalation and impaired Watson–Crick pairing that can lead to flip-out of the nucleotide opposite the modified site. The enhanced lesion dynamics may play an important role in facilitating NER.

Interactions between dual function transition metal complexes and DNA show some similarities to those of covalent polycyclic aromatic carcinogen-DNA adducts,⁶¹ especially to the N7-alkylating-intercalation compounds. However, Ru-arene complexes exhibit some new features. For Ru-bip, classical intercalation causes a large distortion of the DNA helix and can lead to base extrusion next to the modification site. Such structural distortions and lower stability have also been observed for the formamidopyrimidine AFB- α -FAPY DNA adduct.⁶⁴ For the DNA adduct with the non-aromatic bulky tetrahydroanthracene intercalator Ru-tha, a novel penetrating intercalation is observed in which Ru-tha coordinates N7 to guanine. C-H---X (X = O or N) hydrogen bonds between protons of ring C of tha and O or N atoms of the base opposite the ruthenated nucleotides are observed but no displacement of the base opposite or next to the modified nucleotide. However, the local structure of the ruthenated double helix is highly distorted, which is similar to that observed for polycyclic-aromatic-carcinogen-modified DNA adducts. Such a highly distorted double helix is not observed for the DNA adducts of platinum complexes containing ethidium, acridine or acridinylthiourea derivatives ACRAMTU

as side-arm intercalators, where threading intercalation occurs but little helical bending. Furthermore, sequence-dependent modification is observed for the Ru-arene complexes and polycyclic-aromatic-carcinogen-modified DNA adducts, but not for platinum complexes with different side-arm intercalators. The high structural similarity between the dual function Ru-arene lesions and N7-alkylation-intercalation compounds might provide insight into structure-activity relationships for these complexes. These findings help to explain why ruthenium arene complexes have a different mechanism of antitumor activity as compared to cisplatin and are recognized differently by nucleotide repair enzymes.

6. Conclusion

This review has focused on new dual function metal complexes which can interact with DNA by both coordination and intercalation modes of binding. This produces unusual DNA distortions and novel mechanisms of biological activity. Ru-arene complexes with such dual functions (e.g. Ru-bip or Ru-tha) are more potent towards cancer cells than their non-intercalating analogs (e.g. Ru-*p*-cym or Ru-ben),³⁶ or less intercalating isomers (*p*-terphenyl arene complexes are more potent than *o*- or *m*-terphenyl complexes). This design concept can be applied to structure-activity relationships for other organometallic anticancer complexes, including cyclopentadienyl complexes (Sadler, P. J. et al., unpublished) and introduces novel mechanisms of action. The unusual nature of such DNA distortions affects recognition by DNA binding proteins and repair enzymes and hence the downstream processes related to apoptosis and cancer cell death.

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BIOGRAPHICAL INFORMATION

Hongke Liu is a Professor in the College of Chemistry and Materials Science at Nanjing Normal University. He obtained his BA, MA at Northwestern University, China and gained his Ph.D. in Chemistry from Nanjing University. From 2003-2005, he spent two years as a Wellcome Trust Travelling Research Fellow in Peter Sadler's laboratory at the University of Edinburgh. His current research focuses on bioinorganic chemistry and biofunctional materials.

Peter Sadler holds a Chair of Chemistry at the University of Warwick and is also an European Research Council Advanced Investigator. He obtained his BA, MA and DPhil at the University of Oxford, spent two years as a Medical Research Council Research Fellow at the University of Cambridge and National Institute for Medical Research before being appointed as a Lecturer at Birkbeck College, University of London, where he subsequently became Reader in Biological Inorganic Chemistry, and Professor of Chemistry. From 1996-2007 he was Crum Brown Chair of Chemistry at the University of

Edinburgh. His research interests are centered on the chemistry of metals in medicine.

FOOTNOTES

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Table 1. Some polycyclic aromatic and bulky intercalators.

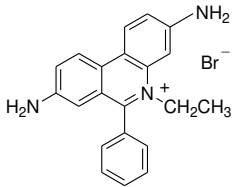
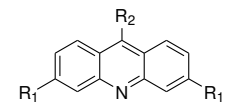
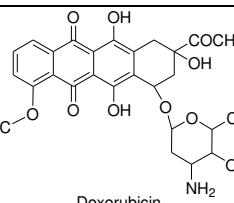
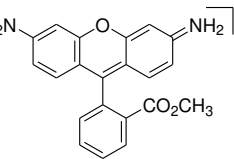
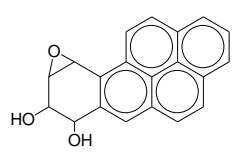
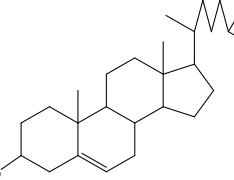
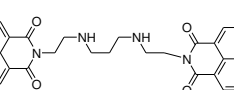
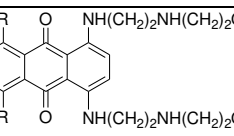
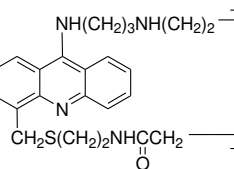
Intercalator	structure	comments	reference
Ethidium bromide		Fluorescent tag, nucleic acid stain for agarose gel electrophoresis.	6
Proflavine Acridine Orange Acridine derivatives	 Proflavin: R ₁ = NH ₂ , R ₂ = H Acridine Orange: R ₁ = N(Me) ₂ , R ₂ = H 9-Aminoacridine: R ₁ = H, R ₂ = NH ₂	Antitumor drugs. Can be attached to abasic sites on synthetic oligonucleotides or as side-arms on Pt and Ru anticancer complexes.	5-6
4'-Epiadriamycin Adriamycin Daunomycin Hedamycin Doxorubicin	 Doxorubicin	Cancer chemotherapy, threading and classical DNA binding modes.	5
Rhodamine	 Rhodamine 123	Dye for fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA.	6
Benzo[a]pyrene (BP)		Can be attached to abasic sites on synthetic oligonucleotides.	7
Cholesterol		Example of bulky intercalator.	8
Bis-naphthalimide		Antitumor activity Elinafide (LU 79553) on clinical trial.	9
Miltioxantrone (R = OH) Ametantrone (R = H) Macrocyclic-bis-9-aminoacridine	 	Mono or bis(inter-duplex) intercalation.	10

Figure Captions

Figure 1.1 NMR solution structure⁷ of the d(C⁴A⁵C⁶)-d(G¹³G¹⁴G¹⁵) segment of the 10S-[BP]dA-dG 9-mer duplex (5'-G¹G²T³C⁴[BP]A⁵C⁶G⁷A⁸G⁹-3') / (5'-C¹⁰T¹¹C¹²G¹³G¹⁴G¹⁵A¹⁶C¹⁷C¹⁸-3'), where the BP group attached to A⁵ base intercalates classically between G¹³G¹⁴ base step of the opposite strand.

Figure 1.2 X-ray structure showing threading intercalation of 9-amino-6-bromo-DACA¹⁶ (space-filling model) into the DNA duplex 5'-d(C|G(5-BrU)AC|G)₂-3' (wireframe), where | indicates intercalation.

Figure 2.1 Platinum metallointercalators. quaterpy = quaterpyridine, phen = phenanthroline, bipy = bipyridine, phi = phenanthrenequinone diimine.

Figure 2.2 a) Molecular structure of [Pt(terpy)(HET)]⁺. b) Crystal structure of [Pt(terpy)(HET)]⁺•d(CpG)₂ showing HET intercalated between two GC base pairs.¹⁸

Figure 2.3 Metallointercalators containing phen, phi, dppz, phzi and eilatin ligands.^{19,21} dppz = dipyrido[3,2-*a*:2,3-*c*]phenazine, phzi = benzo[*a*]phenazin-5,6-quinone diimine.

Figure 2.4 X-ray crystal structure of [Δ-Rh(bpy)₂(chrysi)]³⁺ bound to 5'-d(CGGAATTCCCG)₂-3' duplex.²³ chrysi = 5,6-chrysene quinone diimine.

Figure 2.5 Bis Ru^{II} and Ir^{III}-metallointercalators.^{15,24} pp = dipyrido[3,2-*d*:2',3'-*f*]quinoxaline (dpq), dppz or benzo[*i*]dipyrido[3,2-*a*:2',3'-*c*]phenazine (dppn).

Figure 3.1 Platinum complexes covalently linked to organic intercalators.

Figure 3.2 Structures of ACRAMTU (left), Pt-ACRAMTU (center) and model of a DNA duplex containing the Pt-ACRAMTU adduct (right, the platinum fragment, thiourea linker, and acridine chromophore are depicted in blue, green, and red, respectively).³⁰

Figure 3.3 $cis-[Rh_2(dap)(\mu-O_2CCH_3)_2(\eta^1-O_2CCH_3)(CH_3OH)]^+$ and $[(\eta^6-C_6Me_6)RuCl(dppz)]^+$ (dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine) dual function complexes.

Figure 4.1 Structures of Ru^{II} and Os^{II} arene ‘piano-stool’ complexes.

Figure 4.2 H-bonding and steric interactions which give rise to strong binding of $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ to guanine but very weak binding to adenine.⁴¹

Figure 4.3 Crystal structures of $[(\eta^6\text{-dha})Ru(en)(9EtG)]^{2+}$ (left) and $[(\eta^6\text{-tha})Ru(en)(9EtG)]^{2+}$ (right), showing the arene-purine π - π stacking and hydrogen bonding between en NH and G C6O.⁴¹

Figure 4.4 Model of duplex DNA ruthenated at N7 of G3 (a-b) or N7 of G6 (c-d) with $\{(\eta^6\text{-tha})Ru(en)\}^{2+}$,⁵⁸ showing a novel penetrative intercalation. The tetrahydroanthracene ligand (tha) is in green space filling form. a), c) top views; b), d) side views.

Figure 4.5 Left: mono- and dinuclear-Ru(II) complexes.⁵⁹ Right: model of a 1,3-interstrand cross-link formed by a dinuclear Ru(II) complex on the DNA duplex (5'-AATGTCTAA-3')/(3'-TTACAGATT-5'), showing bis-intercalation. DNA bases,

one strand green, second strand yellow; backbone sugars and phosphate groups, sticks; BisRu(bip), Ru purple, N blue, C black, H gray.

Figure 4.6 Molecular models of two conformers of duplex (5'-ATACATGGTACATA-3')/(3'-TATGTACCATGTAT-5') ruthenated at N7 of G18 with $\{(\eta^6\text{-bip})\text{Ru}(\text{en})\}^+$. In a) the biphenyl arene is intercalated, and in b) stacked on T17 as a flipped-out base.¹²

Figure 1.1

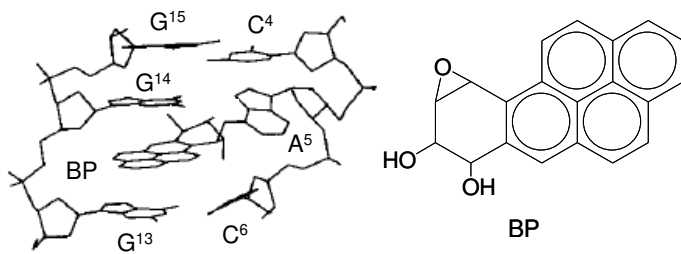


Figure 1.2

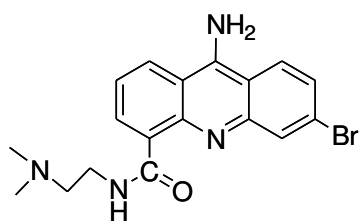
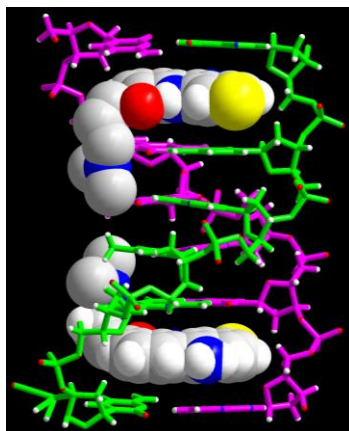


Figure 2.1

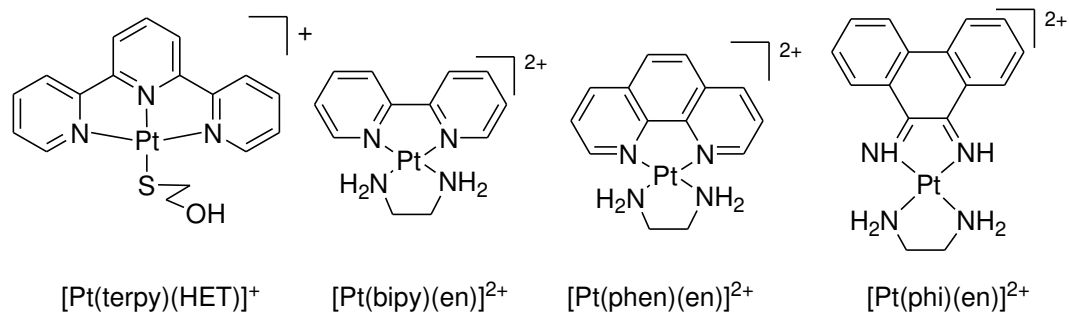


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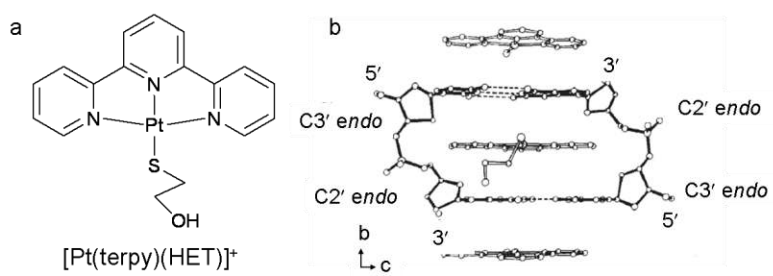


Figure 2.3

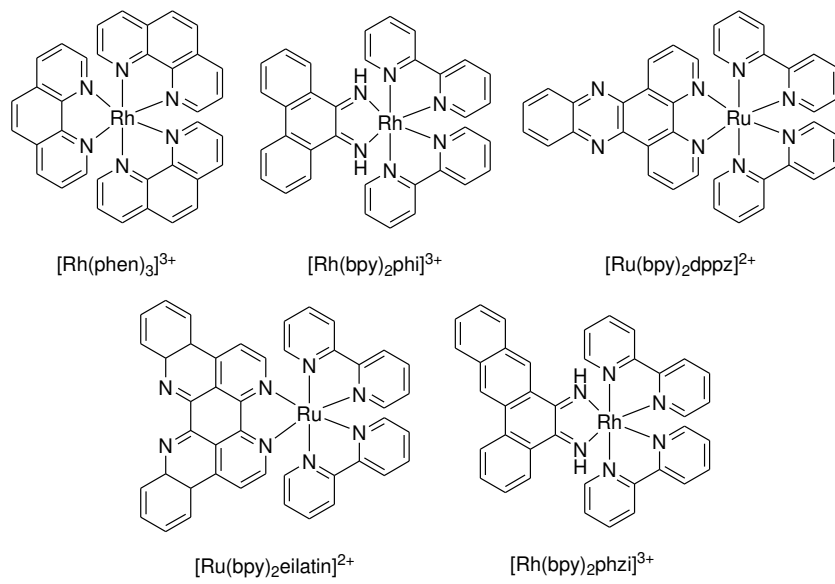


Figure 2.4

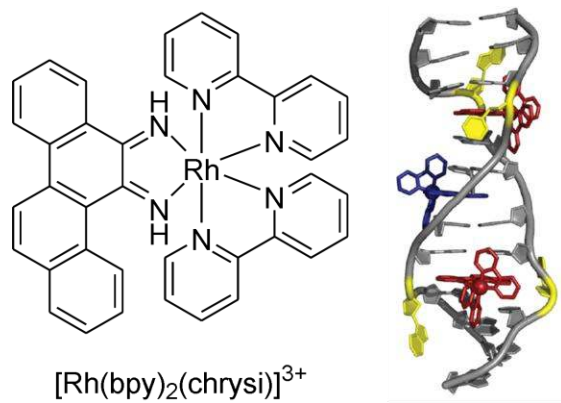


Figure 2.5

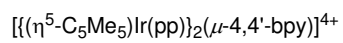
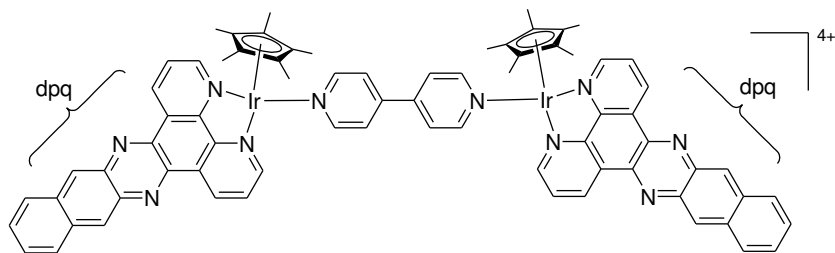
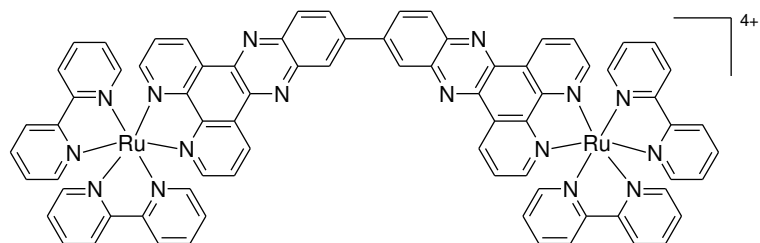


Figure 3.1

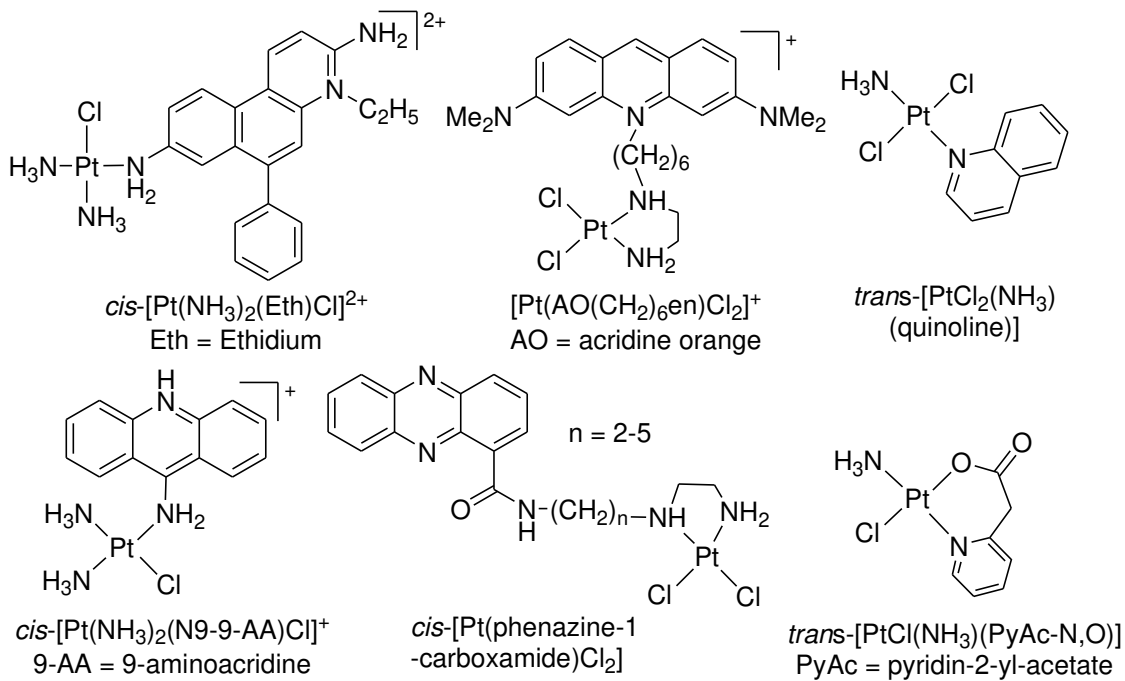


Figure 3.2

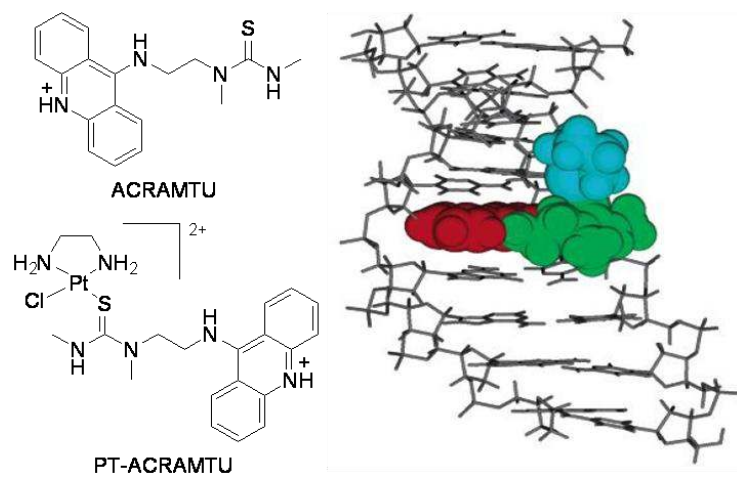


Figure 3.3

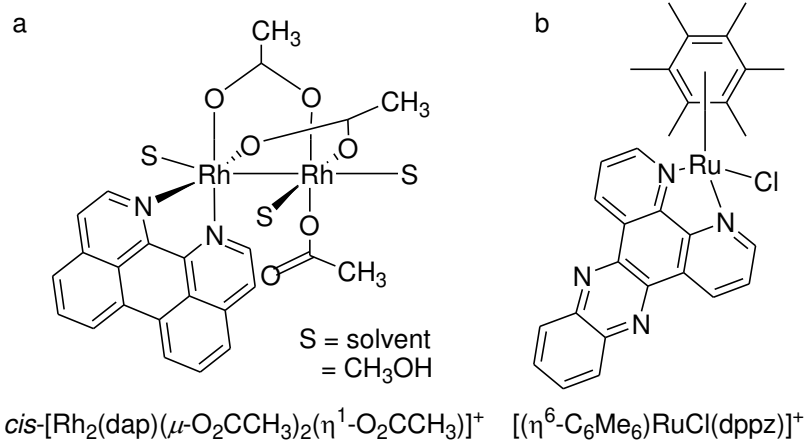


Figure 4.1

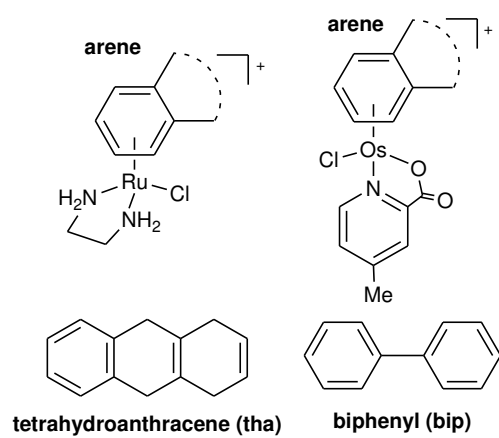


Figure 4.2

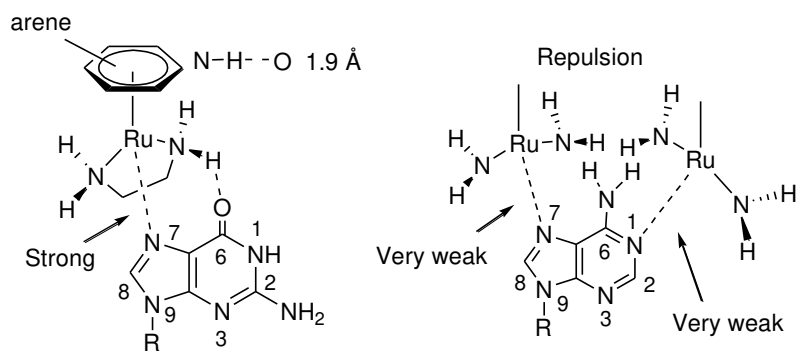


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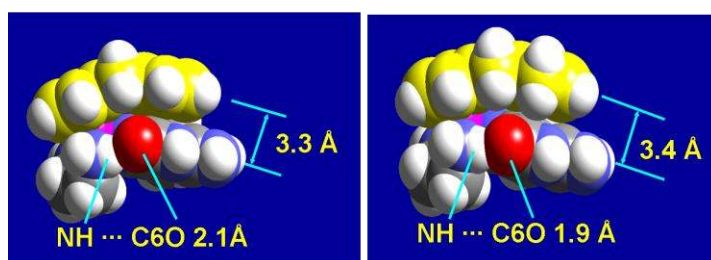


Figure 4.4

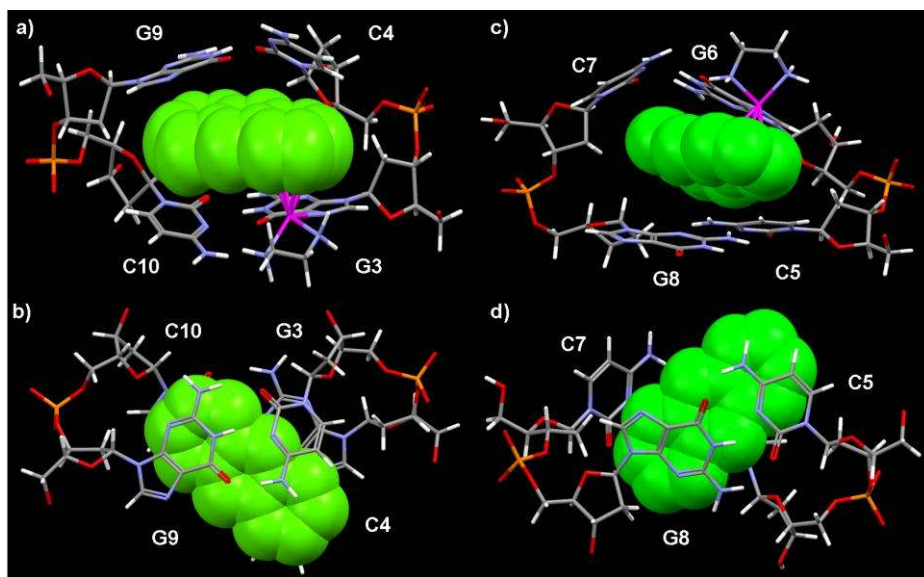


Figure 4.5

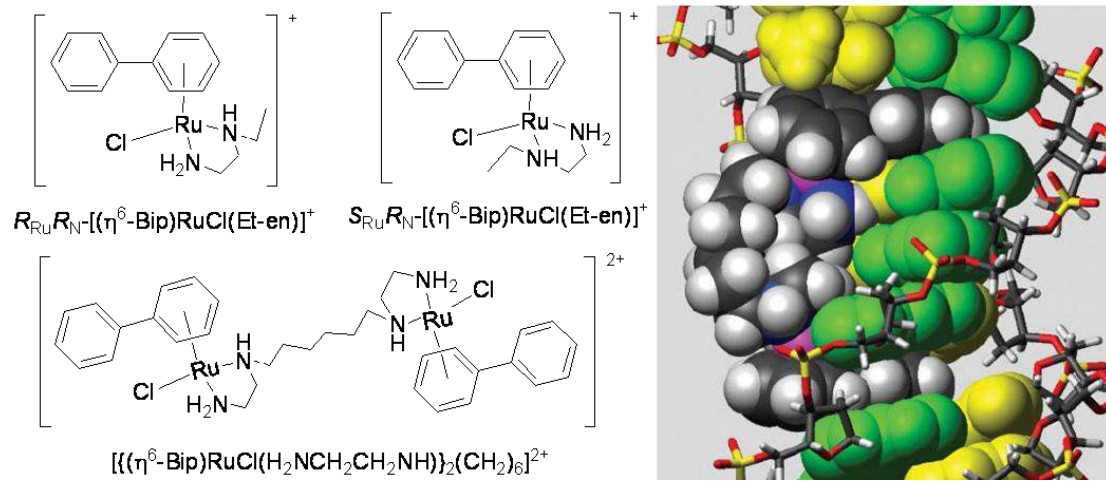
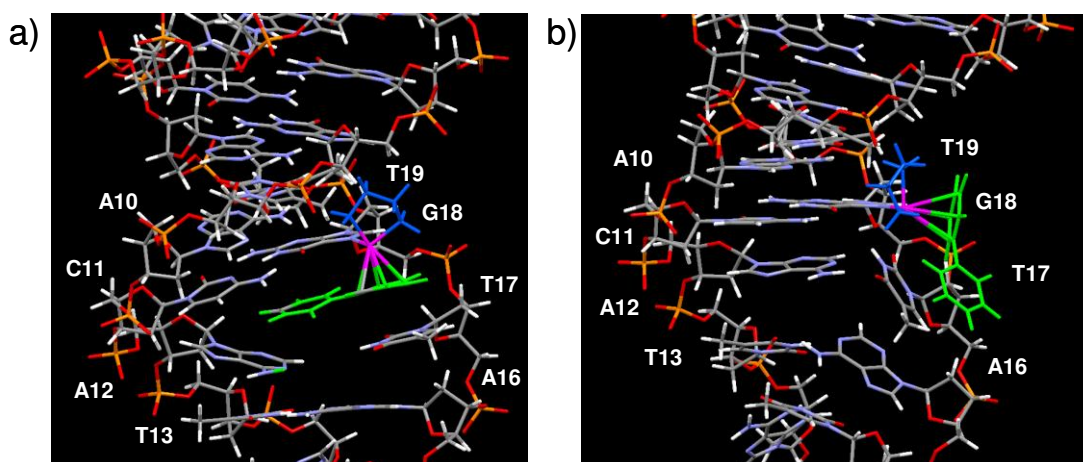


Figure 4.6



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