

perfect fit between enzyme and inhibitor is not as important as the rigidity of the peptide structure in the binding region. This rigidity is an absolute requirement for the proper function of the inhibitor as a permanent inhibitor.

Experimental Procedure

Ava-HCl was produced by acid hydrolysis of 2-piperidone. Aeg was synthesized from 1,2-diaminoethane and chloroacetic acid according to [9]. Ale-HCl was purchased from Merck. The addition of Boc or Z protecting groups and the esterification with hydroxysuccinimide were carried out according to standard procedures [10]. All derivatives and intermediates were characterized by ^1H NMR spectroscopy and mass spectrometry.

A general procedure for the synthesis of BPTI derivatives: De(Ala¹⁶-Arg¹⁷)-[*seco*-15/18]BPTI (25 mg) (preparation see refs.[2,3]) was dissolved in bidistilled water (5 mL) and treated with a 30-fold molar excess of activated ester, dissolved in 1 mL of dioxane or dimethyl sulfoxide. The pH was maintained at 4.75 by addition of NaOH. After 4–5 h the solution was gel filtered on Sephadex G-25, and the monoacylated product was separated by ion-exchange chromatography on CM Sepharose FF at pH 8.6 (NaCl gradient). The corresponding fractions were desalted on Sephadex G-25, and the protecting groups were cleaved with trifluoroacetic acid (15 min for Boc; 10 h for Z). The acid was removed under vacuum, and the residue taken up with the equivalent amount of bovine trypsin in Tris buffer pH 8. After 10 min the pH was lowered to 1.7 with dilute HCl, and the solution was subjected to gel filtration on Sephadex G-50 at pH 1.7. Finally, the inhibitor-containing fractions were repurified on CM Sepharose FF by ion-exchange chromatography, desalted on Sephadex G-25, and lyophilized. Yield: 0.5–2.5 mg, 2–10%. Ion-spray mass spectra of the synthesized inhibitor variants gave the expected molecular weights. The identity of the Aeg derivative was also confirmed by complete sequencing with the Edman degradation method. [Gly¹⁶-Gly¹⁷]BPTI was synthesized as described in [3].

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Synthesis and Complexation Studies of Neutral Anion Receptors

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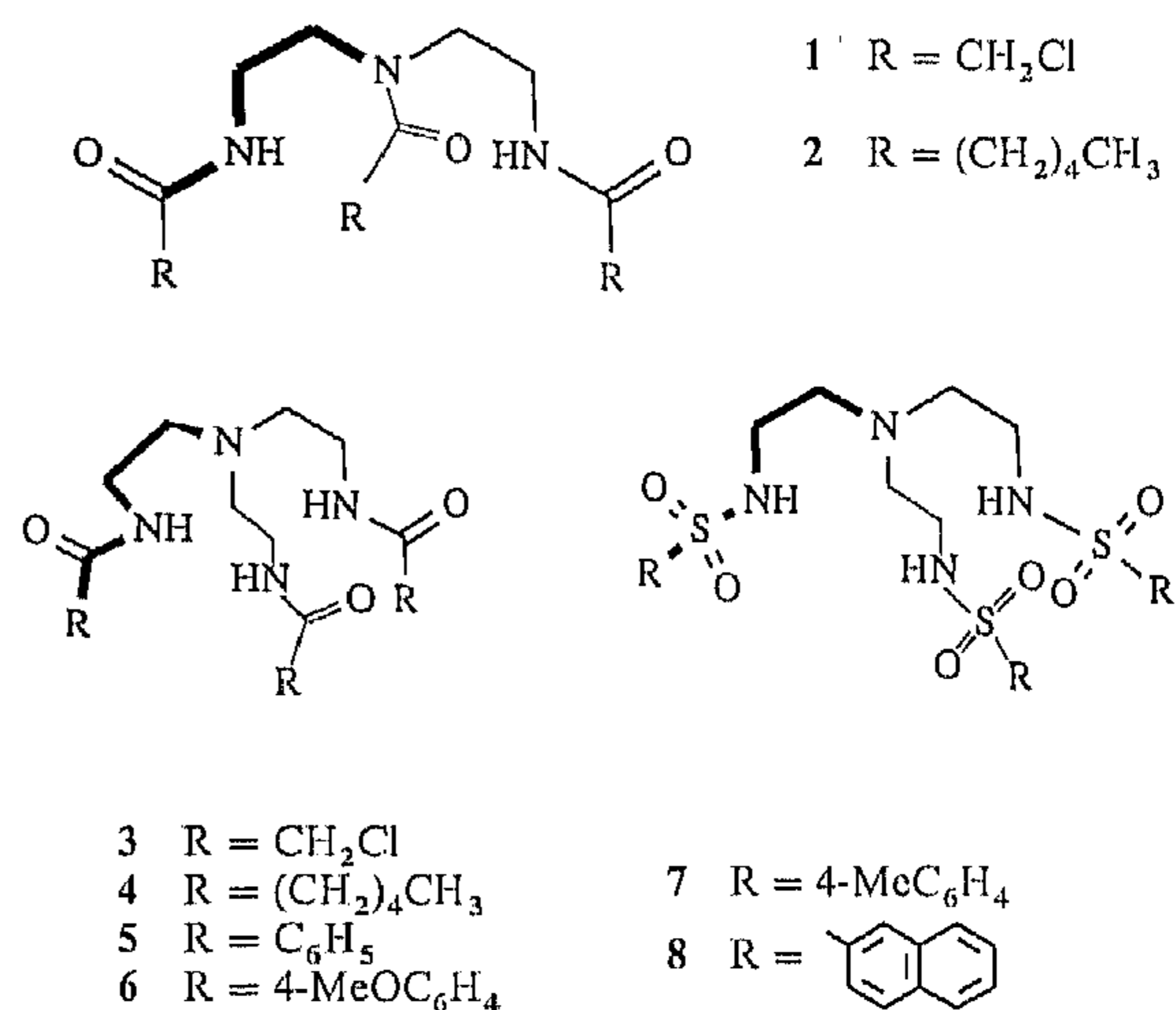
The design and synthesis of receptor molecules for the selective complexation of ions has attracted much attention during the past two decades.^[1] However, the number of host molecules for anions is very low compared to the number for cations. The reported host molecules contain either positive-

ly charged sites^[2] or Lewis acid metal centers^[3] to accomplish anion binding.^[4]

In nature, the selective ion flow to and from the cell is regulated by ion-binding proteins that act as ion carriers and channels across the cell membrane. Quioco et al.^[5] have shown that the high specificity of phosphate transport by proteins is determined by extensive hydrogen bonding in the binding site. The phosphate binding site is formed by two similarly folded globular protein domains and is located in a cleft approximately 8 Å inside from the protein surface. Phosphate binding involves the formation of twelve hydrogen bonds, five from the main chain and seven from the side-chain residues.^[5a]

In our attempts to mimic nature in the recognition of anions by the formation of multiple hydrogen bonds in three-dimensional arrangements, we have designed a new series of ligands with hydrogen-bond donor as well as acceptor sites. This strategy was based on the known structures of phosphate binding sites of proteins. In this communication we demonstrate that surprisingly strong anion binding can be achieved with relatively simple, neutral host molecules in which the H-bond donor and H-bond acceptor sites are present in a tetrahedral arrangement and interact with complementary groups on the anions. Since these receptor molecules are uncharged they are of particular interest as ionophores in membrane transport and for the introduction of anion selectivity in potentiometric membrane sensors.

The host molecules 1, 2, and 3–8 were synthesized starting from diethylenetriamine and tris(aminoethyl)amine, respectively, by reaction with the appropriate acid chlorides in the presence of triethylamine as base.^[6] Compounds 1–8 were isolated in 70–90% yield after recrystallization from methanol, and were characterized by ^1H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis.



In ^1H NMR titration experiments of the ligands with $\text{Bu}_4\text{N}^+\text{A}^-$ ($\text{A}^- = \text{H}_2\text{PO}_4^-, \text{HSO}_4^-, \text{Cl}^-$) in chloroform, the NH signal of the ligands shifted $\Delta\delta \approx 1.5$ –2.0 to lower field until a host–guest ratio of 1:1 was reached. No shift was observed in the NMR signal when the concentration of the anion was increased; however, in the titration with H_2PO_4^- the NH signal shifted until a host–guest ratio of 1:2 was attained. These stoichiometries were confirmed by the characteristic $\delta(^{31}\text{P})$ values for all host–guest complexes in acetonitrile upon addition of two equivalents of $\text{Bu}_4\text{N}^+\text{H}_2\text{PO}_4^-$. For example, the signal for ligand 8 showed a shift of $\Delta\delta = 0.345$ with one equivalent of H_2PO_4^- and a further shift

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of $\Delta\delta = 0.374$ with two equivalents. This host-guest complexation ratio of 1:2 was also reported by others and is attributed to dimerization of the H_2PO_4^- ion by the formation of intermolecular hydrogen bonds.^[7] The observed chemical shifts do not arise from proton transfer from H_2PO_4^- and HSO_4^- to the ligands, since no shifts in the position of the CH_2N signal were observed in the ^1H NMR spectra of the complexes. Moreover, when ligands 1–8 were protonated by addition of *p*-toluenesulfonic acid or picric acid, a distinct downfield shift of the CH_2N signal of $\Delta\delta = 1.0$ – 1.1 was observed. The FAB⁺ mass spectra of the complexes of all the host molecules H with $\text{Bu}_4\text{N}^+\text{A}^-$ exhibit strong signals corresponding to $[\text{HA}^-]^-$ and $[\text{HBu}_4\text{N}^+\text{A}^-]^-$. The association constants (Table 1) for the complexation of ligands 1–8 with anions A^- (Bu_4N^+ as the counterion) were determined in acetonitrile by conductometry.^[8]

Table 1. Association constants K [M^{-1}] for the reaction of 1–8 with the anions H_2PO_4^- , HSO_4^- , and Cl^- determined by conductometry [a].

	H_2PO_4^-	HSO_4^-	Cl^-
1	4700	36	96
2	870	65	63
3	6100	170	1740
4	280	31	290
5	870	56	100
6	510	73	190
7	3500	79	540
8	14200	38	1600

[a] Reactions in acetonitrile. The counterion in all cases was Bu_4N^+ . Measurements were conducted at constant ionic strength with starting concentrations of 1–8 of 1–3 mM and of the salt of 0.9–1.1 mM. The concentrations of the salt and ligand were varied by dilution of the sample solution with a stock solution of the free salt. Initial concentrations were prepared by adding the appropriate amount of free ligand to 10 mL of the stock solution. The K values were determined with curve-fitting methods. The error is 5% for $K > 10^2 \text{ M}^{-1}$ and 10% for $K < 10^2 \text{ M}^{-1}$.

From this data it is clear that all ligands bind phosphate ions preferentially ($\text{H}_2\text{PO}_4^- > \text{Cl}^- > \text{HSO}_4^-$). The reference compound chloroacetamide was not complexed in these conductometric titrations. Ligand 8 shows highest affinity towards the phosphate ion, probably because of the increased electrophilicity of the sulfonamide NH group and preorganization of the binding sites by π -stacking interactions of the naphthyl groups.^[9] The chloroacetamide derivatives 1 and 3 have a larger binding affinity than the caproamides 2 and 4, which may be attributed to the higher polarization of the amide moieties in 1 and 3 caused by the inductive electron-withdrawing effect of the α -chloro substituents.

In conclusion, host molecules 1–8 show selective binding affinity towards anions exclusively by hydrogen-bond formation, and are thereby a unique mimic for anion-binding proteins. Although the association constants are not as high as those of the natural proteins, the simple structure of the models offers the possibility for synthetic manipulation. To achieve higher association constants and selectivities we are currently examining calixarenes and crown ether templates for organizing H-bond donor and acceptor binding sites for anions.

Experimental Procedure

The synthesis of 8 described is representative for the preparation of 1–8: To a solution of tris(aminomethyl)amine (1.0 g, 6.8 mmol) and triethylamine (3.5 mL) in CH_2Cl_2 (100 mL) at 10 °C was added 2-naphthalenesulfonyl chloride (5.4 g, 23.9 mmol). The reaction mixture was stirred for 3 h, allowed to warm up to room temperature, stirred for another 3 h, and poured into water (200 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The

combined organic layers were concentrated, and the resulting solid was recrystallized from methanol to give 8 in 84% yield. M.p. 127–128 °C; ^1H NMR (250 MHz, CDCl_3 , 25 °C): $\delta = 8.48$ (s, 3H, ArH), 7.9–7.7 (m, 12H, ArH), 7.5–7.6 (m, 6H, ArH), 6.28 (s, 3H, NH), 2.98 (t, 6H, CH_2), 2.56 (t, 6H, CH_2); ^{13}C NMR: $\delta = 136.5$, 134.8, 132.1, 129.7, 129.4, 128.7, 128.5, 127.9, 127.4, 122.5 (ArC), 54.4 (CH_2NH), 40.9 (CH_2N); FABMS (NBA matrix): m/z 717.1 ($M + 1$); correct C,H,N analysis.

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Electron Transfer-Catalyzed Diels-Alder Reactions with 2-Vinylindoles**

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Electron Transfer (ET) is one of the most important elementary processes in chemistry.^[1] During the last 15 years, mechanistic-theoretical investigations of ET reactions have made a great comeback, culminating in the award of the Nobel Prize for Chemistry to R. A. Marcus in 1992. In certain cases, reactions which proceed too slowly or not at all with neutral molecules can be effected by electron transfer. Thus, the [4 + 2] cycloaddition between two electron-rich

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