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Review Article

Recognition of Anions by Synthetic Receptors in Aqueous Solution

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Abstract

Natural anion binding systems achieve high substrate affinity and selectivity most often by arranging converging binding sites inside a cavity or cleft that is well shielded from surrounding solvent molecules by the folded peptide chain. Types of interactions employed for anion recognition are electrostatic interactions, hydrogen-bonding, and coordination to a Lewis-acidic metal center. In this review, successful strategies aimed at the development of synthetic receptors active in water or aqueous solvent mixtures are described. It is shown that considerable progress has been made during recent years in the development of potent anion receptors and that for every type of interaction used in nature for anion binding, corresponding synthetic models exist today. Representative examples of these systems are presented with a special emphasis on synthetic receptors whose characterization involved a detailed thermodynamic analysis of complex formation to demonstrate the important interplay between enthalpy and entropy for anion recognition in water.

1. Introduction

The chemistry of life mainly takes place in water and involves the finely tuned interplay between molecular species ranging from simple inorganic salts to organic compounds of widely varying complexity and molecular weight. Supramolecular chemistry, whose origin can be precisely dated to the first account by Pedersen on crown ethers in 1967 [1], takes a lot of its inspiration from nature, and one of its central aspects has always been the development of new compounds, termed either hosts or synthetic receptors, that mimic natural systems in their ability to bind with high affinity and selectivity to a given substrate. The tremendous research activity that followed shortly after the publication of Pedersen's paper was, however, mainly aimed at the development of crown ether-type hosts for cationic substrates such as alkali or alkaline-earth metal ions, or ammonium ions. Anion recognition, in contrast, took much longer to develop despite the facts that the majority of substrates and cofactors involved in biochemical processes are negatively charged [2], and the first host for an anion was described by Park and Simmons only one year after crown ethers appeared in the literature for the first time [3]. Today, anion coordination chemistry can be considered a 'full member of the field of supramolecular chemistry' [4], which is clearly reflected in the number of reviews [5–11] and the monograph [12] published on this topic in recent years. As the majority of contributions only came during the last 20 years, anion coordination is, however, also still a young member and, as a consequence, quite vital.

The slow start of the field of anion coordination chemistry is usually explained by some intrinsic properties of anions that make them more difficult to bind than cations. Anions are relatively large. Simple halides, for example, are significantly larger than the corresponding isoelectronic alkali metal ions (Table 1). They therefore not only require a larger host cavity for efficient inclusion, the smaller charge over size ratio of halides also causes their electrostatic interactions with a host to be weaker than those between a host and an isoelectronic cation. Table 1 shows that anions usually also have higher free energies of solvation than cations with the same absolute charge and comparable size. It is therefore generally believed that strong interactions are required in water for a host to efficiently compete with the water molecules tightly bound in the solvation sphere of an anion. Many anions are involved in protonation equilibria in aqueous solution and host molecules therefore have to be active in a pH window in which the anion is not fully protonated. Finally, simple inorganic anions come in different geometries such as spherical, linear, trigonal planar, tetrahedral, or octahedral, and (organic) polyanions can have even more complex structures, which has also to be considered in receptor design. Although these properties may indeed have caused problems in the development of anion receptors initially, the wealth of hosts described recently with high affinity and selectivity toward anionic

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Table 1. Ionic radii, enthalpies of solvation, and free energies of solvation of selected ions [13]

Ion	r	$-H_{hydr}$	$-G_{hydr}$
Li^+	59	531	481
F^-	133	510	472
Na^+	102	416	375
Cl^-	181	367	347
K^+	138	334	304
NH_4^+	148	329	292
Br^-	196	336	321
Rb^+	149	308	281
I^-	220	291	283
NO_3^-	179	312	306
CO_3^{2-}	178	1397	1315
$H_2PO_4^-$	200	522	473
SO_4^{2-}	230	1035	1090
PO_4^{3-}	238	2879	2773
ClO_4^-	240	246	214

Ionic radii r in ppm; enthalpies of solvation H_{hydr} and free energies of solvation G_{hydr} in kJ mol^{-1}.

substrates clearly shows that these problems have been overcome. Today, certain anion properties are even considered advantageous. The different shapes of anions, for example, can be used as a starting point for the design of hosts selective for one anion over structurally different others.

For the purpose of this review we divide anion hosts into two classes: polar ones that act in water or aqueous solution and lipophilic ones that act in organic solvents, and we will concentrate on mainly the former type here. This should not imply that hosts interacting with anions in aprotic media are less relevant. Both types of anion hosts can potentially be used in a number of interesting applications. Lipophilic hosts may be able to transport anions across membranes and could thus possess biological activity, they could serve as sensing devices such as ion-selective electrodes, or as phase transfer catalysts. Hydrophilic hosts, on the other hand, could be used in medicinal diagnostics, in the analysis of biological systems, or in environmental monitoring [14]. Receptors such as the ones developed in the Hamilton groups that target negatively charged groups on the surface of proteins thus participating in biological processes and predictably changing their outcome might even be of pharmaceutical value [15]. It must be emphasized, however, that besides having potential applications anion hosts active in aqueous solution are also working models for natural anion binding systems, and it is this property that interests us here.

In this review, we give an overview on possible strategies with which anion binding by synthetic hosts in aqueous solution can be achieved and we will point out the similarities of these systems with natural ones. The review therefore starts with a chapter, in which representative examples of natural anion binding systems are presented. We then describe synthetic hosts, grouped by the type of functional group responsible for anion binding, that have successfully been used to bind anions in water or aqueous solvent mixtures. This discussion especially highlights investigations containing thermodynamic analyses of the interaction between synthetic hosts and anions by means of, for example, microcalorimetry, as they give important information on the enthalpic and entropic contributions to complex formation. We hope that by this approach we are able to reveal some general principles governing anion recognition in water. As our intention is to center this review around these general principles, only a selection of representative anion hosts is presented. This selection is, of course, entirely subjective and we wish to apologize to everyone whose work, although relevant, we did not include. We should also point out that this review does not contain references to the elegant steroid based anion hosts introduced by Davis and mercuraborands introduced by Hawthorne or to other systems for which, to the best of our knowledge, interactions with anions are only reported in aprotic solvents. Interested readers are referred to recently published reviews [16, 17]. Also cyclodextrin complexes of organic anions are not mentioned, whose driving force of complex formation is often the inclusion of the substrate's organic part into the cavity of the cyclodextrin ring and not specific interactions of the host with the anionic moiety of the guest.

2. Anion recognition in natural systems

Synthetic anion hosts are either positively charged or neutral. In the first case, anion binding is due to electrostatic interactions often in combination with hydrogen-bonding. Functional groups involved in this kind of interactions are mainly NH groups of pyrroles or protonated amines. Amidinium and guanidinium moieties combine attractive coulombic interaction with the ability to form two strong parallel hydrogen bonds to, for example, carboxylates, phosphates, or phosphonates. They have thus received special attention as anion binding sites. No hydrogen bond formation is possible in hosts containing quaternary ammonium groups where anion complexation relies solely on electrostatic interactions. Uncharged receptors use ion–dipole interactions for anion recognition, which often involve hydrogen bond formation between the substrate and NH groups of amide, thioamide, urea, or thiourea moieties of the host. Finally, metal containing hosts bind anions coordinatively to a Lewis-acidic center. Each of this basic principle of anion coordination has its counterpart in natural anion binding systems and the following representative examples of natural systems should demonstrate this close relationship.

Spermine/spermidine

Spermidine (4-azaoctane-1,8-diamine; $H_2N(CH_2)_3NH(CH_2)_4NH_2$) and spermine (4,9-diazadodecane-1,12-diamine; $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) are ubiquitous naturally occurring polyamines that seem to fulfill several functions in a cell not all of which are fully understood yet [18]. At physiological pH, both compounds are polycationic thus possessing high affinity for anions. *In vitro* studies have, for example, demonstrated that spermidine and spermine precipitate DNA and protect this polyanion from denaturation by heat or damage by shearing [19]. These stabilizing effects were attributed to neutralization of the negative charges on the DNA phosphate groups. When dilute solutions of DNA are treated with spermidine or spermine under controlled conditions of ionic strength, both compounds interact with individual DNA molecules rather than by bridging two or more [20]. The fact that structurally unrelated trivalent cations such as $[Co(NH_3)_6]^{3+}$ possess similar properties indicates, however, that the interactions are mainly electrostatic in nature without a specific structural component [21]. Also effects of spermidine and spermine on RNA biosynthesis and protein kinase activity have been demonstrated both of which involve interactions of the polycations with anionic substructures of the binding partners [18]. Dissociation constants of the interaction between spermidine and DNA, 16S rRNA, phospholipids, and ATP are reported to be 15.6, 2.66, 5.56, and 2.24 mM, respectively [22], and binding of the fully protonated forms of spermidine and spermine to ATP^{4-} and PP_i^{4-} (pyrophosphate, $P_2O_7^{4-}$) was shown to be entropy driven (Table 2) [23, 24]. An increase in helicity from 11 to 24% of a tetra-anionic peptide has been observed upon addition of spermine that was ascribed to interactions between the spermine ammonium groups and glutamate side chains [25]. Finally, conformational studies were carried out recently showing that, when interacting with triphosphate, spermidine adopts a bent conformation [26].

Carboxypeptidase A

This enzyme is responsible for the hydrolytic release of a C-terminal hydrophobic (aromatic) amino acid from a polypeptide chain. The active center of carboxypeptidase A can be subdivided into three regions: the catalytic site itself that contains a zinc ion which is not involved in anion binding, a hydrophobic binding pocket for the recognition of the side chain of the terminal amino acid, and an anion binding site that enables the enzyme to distinguish both ends of the peptide chain [27, 28]. The latter contains the guanidinium group of an arginine (Arg145) that forms two strong hydrogen bonds to the substrate's terminal carboxylate group (Figure 1). The guanidinium group itself is held in place by two further hydrogen bonds to an asparagine (Asn144) and to a tyrosine (Tyr248) side chain. Similar guanidinium/carboxylate interactions also occur in the active centers of the enzymes thrombine and trypsin both of which cleave peptides directly behind an arginine residue. [29, 30] In these cases, however, the guanidinium group is part of the substrate and the carboxylate part of the protein.

Phosphate binding protein

There are several natural systems, in which guanidinium/phosphate interactions occur. An interesting binding motif, the so-called arginine fork (Figure 1) has, for example, been proposed to participate in interactions between RNA-binding proteins such as the human immunodeficiency virus (HIV) Tat protein and an RNA stem-loop structure termed TAR [31]. Here, we present the binding mechanism of the phosphate binding protein (PBP) in more detail because of its direct relevance for the development of hydrophilic synthetic anion hosts. PBP is a periplasmatic protein that acts as a high-affinity transport system for orthophosphate in bacteria once this anion has passed the outer cell membrane [32]. X-ray crystallography revealed that strong phosphate binding of PBP, the dissociation constant K_d of the PBP–phosphate complex amounts to 0.31×10^{-6} M at pH 8.5 [33], is achieved by the formation of altogether 12 hydrogen bonds to a fully desolvated HPO_4^{2-} anion residing inside a deep cleft of the protein [34]. Eleven of these hydrogen bonds are formed between oxygens of the substrate and hydrogen bond donor groups of the protein (Figure 2). Only one hydrogen bond involves the substrate's proton and a hydrogen bond acceptor group of PBP, more specifically the carboxylate group in the side chain of Asp56, but this bond is crucial for substrate selectivity. If a fully deprotonated tetrahedral oxoanion such as sulfate lacking a hydrogen bond donor is included into the binding pocket of PBP, repulsive

Table 2. Thermodynamic parameters in kJ mol^{-1} for the complexes of spermidine trihydrochloride and spermine tetrahydrochloride with ATP^{4-} and PP_i^{4-} (pyrophosphate, $P_2O_7^{4-}$) (both anions as their sodium salts) at $T = 298$ K [23, 24]

Amine	Anion	ΔG	ΔH	$T\Delta S$
Spermidine	ATP^{4-}	−32.5	−0.5	32.0
Spermine	ATP^{4-}	−46.2	4.0	50.2
Spermidine	PP_i^{4-}	−15.4	19.7	35.1
Spermine	PP_i^{4-}	−20.0	26.5	46.5

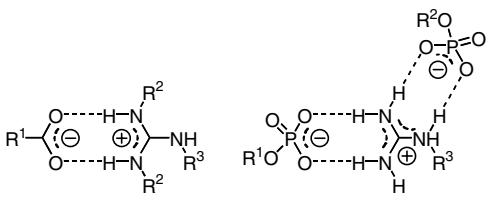


Figure 1. Schematic representation of a guanidinium/carboxylate salt bridge (left), and of an arginine fork (right).

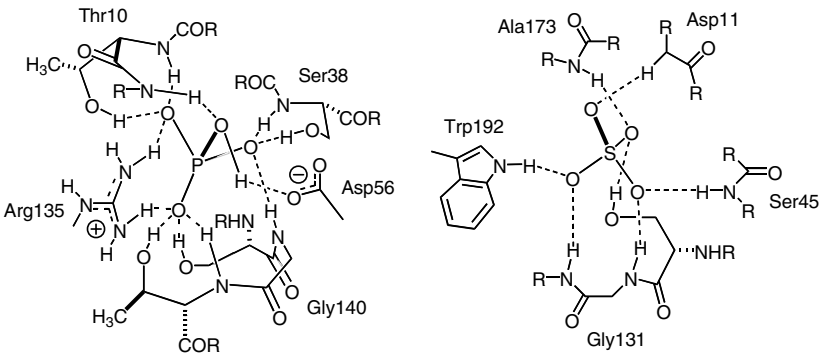


Figure 2. Schematic representation of the array of hydrogen bonds with which, respectively, HPO_4^- and SO_4^{2-} are bound inside the active centers of the phosphate binding protein (left) and the sulfate binding protein (right).

interactions with the carboxylate group of Asp56 occur causing binding to be significantly less tightly. There exists a salt bridge inside the binding pocket of PBP to the carboxylate group of Asp137 that also involves the guanidinium moiety of Arg135 and it was speculated that removal of the negative charge at position 137 would eliminate possible repulsive effects on phosphate binding, at the same time increasing attractive electrostatic interactions with the Arg135 guanidinium group. Studies with mutant proteins in which Arg135 was replaced by Asn showed, however, no dramatic effect on anion binding, which led to the conclusion that the precise arrangement of hydrogen bonds in the PBP–phosphate complex is more important for substrate affinity (and selectivity) than charge interactions [33]. This interpretation is supported by other site-directed mutagenesis studies showing that the carboxylate/guanidinium interaction outmatches the interaction of a carboxylate group with the amino group in the side chain of Lys by as much as -21 kJ mol^{-1} in the protein environment [35, 36], and by calculations that revealed a negative hence non-complementary electrostatic potential of the protein surface surrounding the substrate in the active center of PBP [37]. It therefore seems that electrostatics are no decisive element in the anion affinity of this and possibly also other proteins.

Sulfate binding protein

The sulfate binding protein (SBP), which is also responsible for anion transport in bacteria, has not only a similar biological function as PBP, several aspects of the mechanism with which it binds its substrate are also closely related to the one of PBP. Complexation of sulfate inside the active center of SBP relies on a network of seven hydrogen bonds most of which are formed between the oxygens of the substrate and NH groups of the protein backbone. In addition, a tryptophane NH and a serine OH group are also involved in binding (Figure 2) [38–40]. The binding pocket of SBP contains no guanidinium moiety or other positively charged amino acid and anion binding therefore relies primarily on hydrogen bond formation. An additional electrostatic component could arise from four α-helices converging with their N-termini to the binding pocket but it was shown that only the first turns of these helices contribute to electrostatic ion–dipole stabilization whereas the contribution of the whole helix macrodipoles appears to be insignificant [41]. One might expect that because of the smaller number of hydrogen bonds inside the active center and the lack of a salt bridge, anion affinity of SBP is smaller than that of PBP. The opposite is, however, true as SBP binds to sulfate 10–20 times more tightly ($K_d = 0.12 \times 10^{-6}$ M) than PBP does to phosphate [42]. Anion affinity is independent of pH over the range pH 5 to pH 8.1 which has been attributed to the lack of hydrogen bond acceptors inside the binding pocket of SBP. This lack also explains the weak affinity of SBP toward protonated anions. HPO_4^{2-} is, for example, bound with a 50,000 fold larger dissociation constant of 6×10^{-2} M [42]. A systematic analysis showed that sulfate and phosphate recognition of other proteins is based on similar principles [43].

Vancomycin

The antibiotic activity of vancomycin and related glycopeptides is due to the inhibitory effect of these compounds on the crosslinking of peptidoglycan precursors involved in bacterial cell wall biosynthesis [44, 45]. This crosslinking, which leads to a mechanical stabilization of the cell wall, is normally achieved by cleavage of the terminal D-Ala residue from a pentapeptide portion of one precursor molecule and coupling of the following amino acid residue, which is also D-Ala, to an L-Lys residue of another. Vancomycin blocks the action of the enzyme thus causing an insufficient stabilization of the cell wall and ultimately the death of the bacteria by binding to the same D-Ala–D-Ala dipeptide fragment recognized by the transpeptidase that catalyzes these two steps. The crystal structure of vancomycin complexing a peptide ligand shows five intermolecular hydrogen bonds, three of which are formed between vancomycin NH groups and the carboxylate of the substrate's terminal D-Ala residue

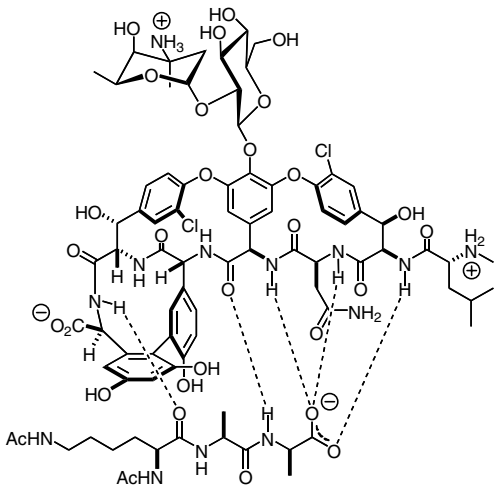


Figure 3. Schematic representation of the interaction between vancomycin and the dipeptide Ac-L-Lys(Ac)–D-Ala–D-Ala.

(Figure 3) [46–49]. Although vancomycin contains basic amino groups that are protonated under physiological conditions they seemed to be not involved in binding.

In aqueous solution, the stability constant K_a of the vancomycin Ac-L-Lys(Ac)–D-Ala–D-Ala complex amounts to $1.5 \times 10^6\ M^{-1}$ [50]. A microcalorimetric investigation showed that the interaction between vancomycin and Ac-D-Ala–D-Ala is exothermic with an adverse entropic contribution [51]. Binding strength increases with increasing chain length of the substrate, while replacement of one alanine subunit in the substrate by glycine leads to a reduction of complex stability (Table 3).

In a series of papers, the group around Williams dissected the free energy released during binding of vancomycin to its substrate into favorable attractive contributions, unfavorable entropic contributions, and contributions from the hydrophobic effect [52]. It was shown that the dominant contribution to complex stability comes from the interactions involving the carboxylate group of the substrate [53, 54]. The additional hydrogen bonds between the ligand and vancomycin strengthen carboxylate binding, decrease the flexibility of the substrate, improve its orientation inside the binding pocket of vancomycin, and ultimately also optimize hydrophobic interactions [55]. A quantitative estimation of the share of hydrogen bonding and hydrophobic interactions to the overall free energy change of binding is reported [56].

Table 3. Thermodynamic parameters in kJ mol^{-1} for the complexes of vancomycin with Ac-D-Alanine and various dipeptides at $T = 298$ K [51]

Substrate	ΔG	ΔH	$T\Delta S$
Ac-D-Ala	−14.0	−30.6	−16.6
Ac-D-Ala–D-Ala	−25.8	−30.4	−4.6
Ac-D-Gly–D-Ala	−23.8	−29.2	−5.4
Ac-D-Ala–D-Gly	−19.7	−25.6	−5.9

Prodigiosins

Prodigiosins are pyrrol alkaloids of a characteristic red color produced by microorganisms such as *Streptomyces* and *Serratia* [57]. These compounds are structurally quite diverse but the pyrrolylpyrromethene skeleton bearing a methoxy group at C4 is the same for all members of the prodigiosin family (Figure 4). Prodigiosins possess a number of interesting biological activities. They have, for example, high immunosuppressive activity [58], they lead to apoptosis (programmed cell death) in several cancer cell lines, while sparing non-malignant cells [59], they are able to bind to DNA and assist oxidative cleavage in the presence of Cu(II) [60–62], and they uncouple proton translocation thus inhibiting vacuolar acidification. The latter property has been attributed to the ability of prodigiosins to act as a symporter for H^+ and Cl^- across liposomal membranes [63–65]. This proton-coupled transmembrane transport of halides is somewhat comparable to phase transfer catalysis because protonation at the weakly basic nitrogen of the azafulvene subunit converts the prodigiosin molecule into a large lipophilic cation that pulls the counterion across the membrane. Chloride binding is believed to be mainly due to electrostatic interaction but additional stabilizing effects of hydrogen bonds and/or charge transfer interactions cannot be excluded [64].

It should be mentioned that another important mechanism for chloride transport across biological membranes are chloride channels. The crystal structures of two chloride channels from *E. coli* and *S. typhimurium* have recently been solved [66]. These structures show that chloride selectivity is accomplished at the narrowest part of the ion channels, the so-called ion filter, where chloride is selectively coordinated to hydrogen bond donors located at the N-termini of α-helices, namely two backbone NH groups and two OH groups, one from Ser107 and one from Tyr445 (Figure 4). Anion recognition thus somewhat resembles that of SBP.

Carbonic anhydrase

There are many examples of metalloenzymes in which, at some stage of the catalytic cycle, the metal center

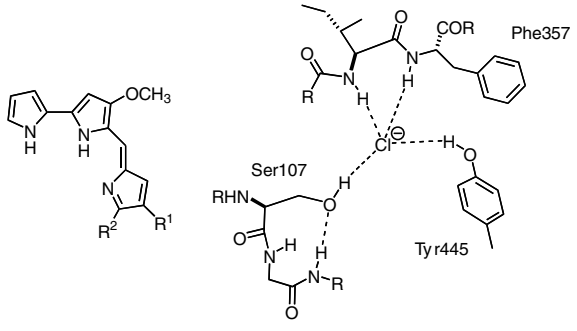


Figure 4. General structure of prodigiosins (left) and schematic representation of the interaction between chloride and the narrowest pore region of the chloride ion channel (right).

interacts with an anionic substrate or coenzyme. We have only selected two examples to illustrate this important type of anion binding. Carbonic anhydrase catalyzes the conversion of CO_2 to HCO_3^- with a turnover number of 1.4×10^6 s^{-1}, which makes it one of the most efficient enzymes known to date [67]. The crystal structure of carbonic anhydrase shows that the active center contains a Zn^{2+} ion coordinated to three histidines. Also 7–10 water molecules have been detected within 10 Å distance from the zinc in the crystal structure and in the fully solvated enzyme there may be even more, but most probably only one water molecule is directly bound to the metal ion completing its tetrahedral geometry [68]. The pK_a of this water molecule is significantly reduced by the interaction with the Lewis acid and after deprotonation, a zinc-hydroxide complex is formed whose strongly nucleophilic ligand can add to a CO_2 molecule simultaneously residing inside the active center (Figure 5) [67]. This view of the reaction mechanism is supported by the fact that several anions inhibit carbonic anhydrase most probably because they compete with water for the coordination site at the metal center. Several investigations indicate, however, that the situation may be somewhat more complicated. Although spectroscopic studies suggested that, for example, cyanide binds to the zinc [69], a crystal structure of the cyanide/carbonic anhydrase showed that the cyanide is non-covalently associated with the zinc–water or zinc-hydroxide form of the enzyme [70]. In the thiocyanate/carbonic anhydrase complex, on the other hand, the zinc is pentacoordinated with the anion, the water molecule, and the three histidine residues in its first coordination sphere [71]. Binding of hydroxide to zinc during the catalytic cycle of carbonic anhydrase is undoubted, however.

Cytochrome c *oxidase*

The main reason for the high toxicity of cyanide is the inhibitory effect of this anion on the enzyme cytochrome *c* oxidase also known as complex IV. This enzyme is the last in the respiratory chains of mitochondria and aerobic bacteria and catalyzes the reduction of molecular oxygen to water [72]. The energy made available by this reaction is then used to translocate protons across the membrane, resulting in the proton and charge gradient required for the synthesis of ATP. Cytochrome *c* oxidase contains two copper centers, Cu_A and Cu_B, as well as two heme A subunits, *a* and a_3. Cu_A and heme *a* serve to conduct electrons from the electron donor, cytochrome *c*, to the catalytic center itself, which consists of the two other metal sites. In the oxidized enzyme, heme a_3 and Cu_B are 4.5 Å apart thus composing a binuclear center in which oxygen and its reduced intermediates are held until the catalytic cycle is completed [72]. The enzyme is inhibited by carbon monoxide but also by azide, thiocyanate, and cyanide, which is ascribed to a coordination of these anions to the metals at the enzyme's binuclear catalytic center (Figure 5). Although there is some debate of whether the anions bridge the two metal ions or are bound to only one [73, 74], strong metal-anion coordination at the active site is the generally accepted inhibition mechanism. This interaction is the principal reason for the toxicity of cyanide whereas that of CO arises from the affinity of this molecule to hemoglobin. Because organisms contain less cytochrome *c* oxidase than hemoglobin, exposure to much smaller amounts of cyanide is lethal.

3. Hosts containing ammonium groups

Ammonium based anion receptors are usually macrocyclic or polymacrocyclic and contain several protonated amino groups or quaternary ammonium groups in the periphery of the cavity. The first ever reported anion host, a bicyclic diazacryptand [3], belongs to this class of receptors, for instance, and many other examples have been described since then. Because of strong Coulomb attraction, complex formation between ammonium based hosts and anions in water is rather the rule than the exception [6, 9, 75, 76] but to fully understand the underlying principles, several issues have to be addressed.

Protonation of a monocyclic or polycyclic polyamine increases the positive charge density around the cavity and, as a consequence, anion affinity. Strongest anion binding can therefore be expected from a fully protonated host with a maximum number of ammonium

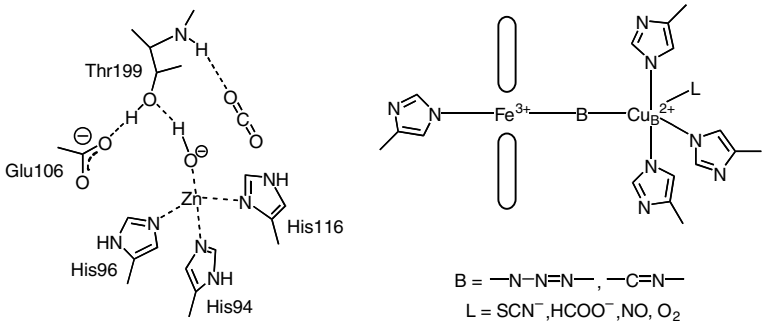
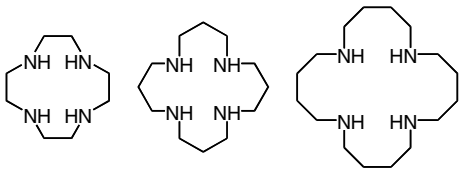


Figure 5. Schematic representation of the metal coordination inside the active centers of carbonic anhydrase (left) and cytochrome *c* oxidase (right); B is a bridging ligand between the heme a_3 subunit and Cu_B while L is a terminal ligand on Cu_B.



pK_{a1}	10.7	10.9	11.8
pK_{a2}	9.7	9.8	11.4
pK_{a3}	1.7	7.2	10.6
pK_{a4}	0.9	5.7	8.9

Figure 6. Dependence of pK_a of the individual protonation steps of a monocyclic tetramine on the distance between the amino groups [77, 78].

groups. The degree of protonation is, however, a function of pH and although a significant degree of protonation can already be expected at neutral pH, complete protonation usually requires an acidic medium which could be detrimental for the complexation of weakly basic anions such as carboxylates. The pH required for full protonation of the host depends, among other things, on its structure. Reducing the distance between the amino groups in a macrocyclic polyaza host, for example, causes protonation to become more and more difficult because of unfavorable charge accumulation (Figure 6) [77, 78]. To avoid strongly acidic solutions in anion recognition, the amino groups in many ammonium based hosts are therefore separated by at least a propylene residue or linkers of similar length.

At a pH where the host is not completely protonated, multiple equilibria have to be considered and, as a consequence, several host species exist simultaneously in solution all behaving differently. For a quantitative assignment of the concentration of each compound present at a defined pH and its contribution to binding, potentiometric measurements during which the increase in the protonation constants of the host caused by the presence of an anionic guest is determined have been proven to be useful. This method requires the use of a supporting electrolyte to control the ionic strength of the solution and for this purpose, sodium tosylate is frequently used because the tosylate anion does not or only weakly interact with most ammonium based hosts [79].

Not only the degree of protonation controls the binding properties of a macrocyclic polyamine, however, but also the position where protonation occurs particularly when hydrogen bonds contribute to complex stabilization. In general, non-adjacent amino groups along the host are protonated first and the least basic ones such as tertiary amines last if at all. Finally, protonation has an effect on host conformation because charge repulsion causes ammonium groups to move away from each other causing the molecular framework of a polyamine to expand upon protonation. In addition, ammonium groups along macrocyclic or polymacrocyclic systems preferentially adopt *out* configurations, an effect that has already been realized by Park and Simmons who showed that protonated bicyclic diaza hosts favor the *out–out* conformation while the less favorable *in–in* conformation is optimal for anion recognition (Figure 7) [3].

Despite a possible negative influence of these effects on complex stability it seems reasonable to assume that strong electrostatic interactions between charged hosts and guests would cause complex formation to be an exothermic process. According to a simple electrostatic model for ion pairing [80], this is strictly true only for electrostatic interactions in vacuum, however. In solution, energy is required to remove solvent molecules from the solvation spheres of the binding partners, which could cause the overall enthalpic gain in complex formation to be negligible. In this case, complex formation can only occur if the release of solvent molecules increases the overall entropy of the system. Such entropically driven processes have indeed been encountered with polyammonium hosts (*vide infra*) but because binding often involves additional cooperative interactions, for example hydrogen bonding or aromatic interactions, which are not considered in the simple electrostatic model for ion pairing and which produce favorable enthalpic contributions to binding, exothermic complex formation is possible. By comparing the association constants of the complexes between cyclophane **3/1** and various non-charged and anionic naphthalene derivatives, Schneider and co-workers determined a contribution of $\Delta G = 5 \pm 1$ kJ mol^{-1} per salt bridge in water (at an ionic strength of around 0.02 M) to complex stability [81–86]. This value agrees well with association constants reported in the literature for various other host guest complexes stabilized by electrostatic interactions despite the fact that ions different in size and particularly in polarizability are involved (COO^-, SO_3^-, $OPO(OH)O^-$, $OPOO_2^{2-}$, phenolate O^-, $R_2NH_2^+$, R_4N^+, pyridinium N^+, R_4P^+ etc.) [81, 83]. Extrapolation to an ionic strength of zero causes the increment in ΔG for an anion–cation interaction to increase to 8 ± 1.7 kJ mol^{-1} [87]. Another important result reported by the Schneider group that concerns electrostatic interactions in water is the surprisingly small effect of flexible bonds on the strength of the interaction between linear α,ω-dianions and α,ω-dications [87]. The fairly linear correlation between

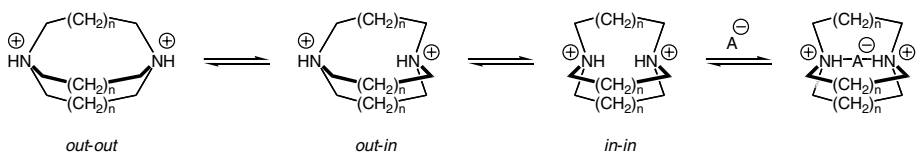
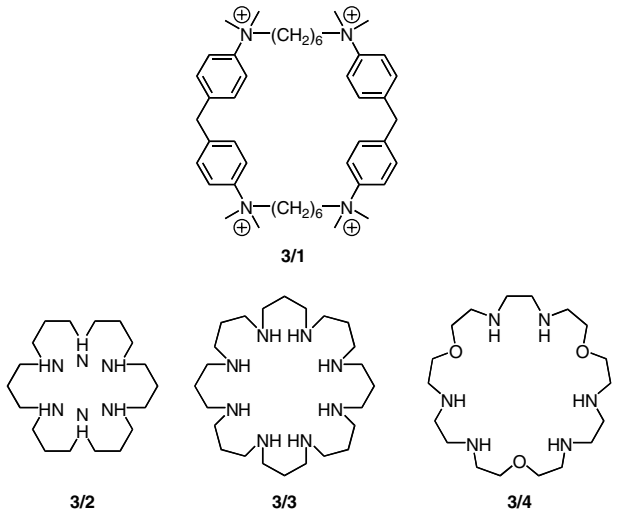


Figure 7. Equilibria between the different conformers of a diprotonated bicyclic diaza host. The *in–in* conformation is optimal for anion binding.



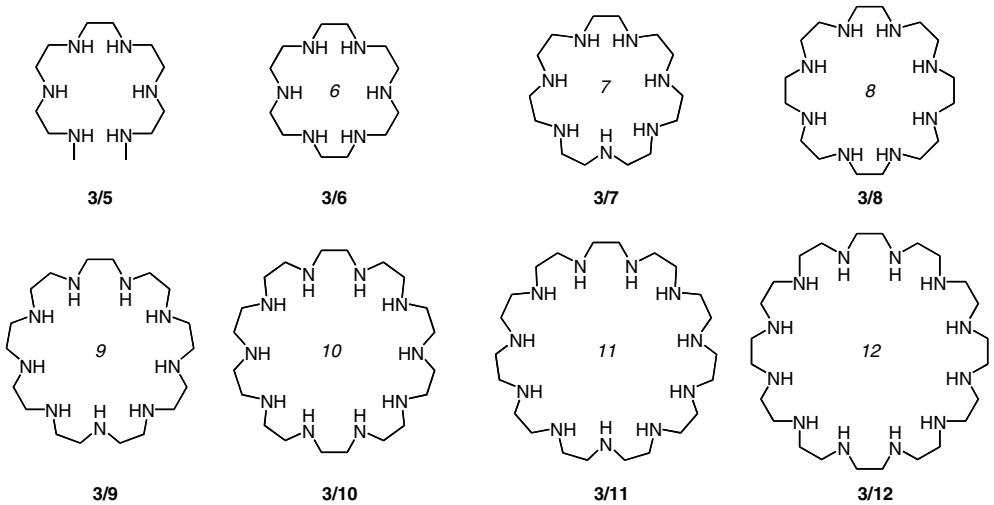
Scheme 1.

the free energies of association ΔG and the number of flexible bonds n yielded an energetic disadvantage of only $\Delta\Delta G = 0.5$ kJ mol^{-1} for one single bond, indicating that a conformational flexibility of the host (or the guest) has probably no large effect on complex stability in systems stabilized by electrostatic interactions.

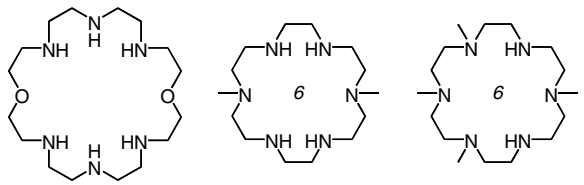
One final point that affects anion binding of charged ammonium based anion hosts is the (almost) unavoidable presence of counterions competing with the actual guests for free binding sites. All of the above mentioned issues have to be addressed in the design of polyaza hosts, and the following examples represent a selection of instructive systems.

Monocyclic polyamines are probably among the most simple anion host structures one can conceive. That such compounds, for example the three polyamines **3/2**–**3/4**, do possess affinity toward a variety of anions including sulfate, di- and tricarboxylates, nucleotides, and anionic transition metal complexes was demonstrated at an early stage of anion coordination chemistry by Lehn and co-workers [88]. Complex stability was determined by regression analysis of the pH metric titration curves recorded in water in the presence of the anionic guests. The results indicate that electrostatic interactions play a major role in binding strength. Thus, the smallest most highly charged anions are usually the ones bound best by a given host. Structural effects and size complementarity do affect complex stability but these effects often do not translate into significant differences in anion selectivity which is most probably due to the conformational flexibility of the hosts that allows them to easily adapt to different anion geometries. Although hosts **3/2**–**3/4** do possess different affinities toward various dicarboxylates, for examples, the association constants of the complexes between a given dicarboxylate and the three hosts differ by a factor of 16 at most. Appreciable binding selectivity was only observed in the complexation of citrate, 1,3,5-benzenetricarboxylate, $[Co(CN)_6]^{3-}$, and $[Fe(CN)_6]^{4-}$ each of which is bound by **3/3** up to 500 times more strongly than by the other two hosts [88].

Systematic investigations by Bencini, Bianchi, and García-España involving a series of linear and macrocyclic polyamines were carried out with the aim to elucidate the effects of the degree of protonation and of ring size on binding selectivity [89, 90]. A comparison of the affinity of macrocyclic hosts **3/6**–**3/12** toward the octahedral anionic transition metal complexes $[Fe(CN)_6]^{4-}$ and $[Co(CN)_6]^{3-}$, and the planar complex $[Pt(CN)_4]^{2-}$ showed that complexation is detectable when at least four amino groups of the macrocycles are protonated [91]. Only in the case of the smaller hosts **3/6** and **3/7**, binding of anions to the triprotonated form can be observed. Complex stability increases with increasing number of positive charges on the host up to the maximum degree of protonation, which is usually one or two below the total number of amino groups in the ring, indicating that Coulombic interactions are the driving force in complex formation. The linear host **3/5** forms less stable complexes than the corresponding cyclic one **3/6** [92], complex stability increases in the



Scheme 2.



3/13 3/14 3/15

Scheme 3.

order $[Pt(CN)_4]^{2-} < [Co(CN)_6]^{3-} < [Fe(CN)_6]^{4-}$ i.e. with increasing charge of the anion, and finally, complex stability decreases when the rings become larger. Deviations from the latter trend are only observed when enlargement of the host causes a change in complex geometry. Thus, a host that is able to include an anion into its macrocyclic cavity forms more stable complexes than a smaller one that can bind the anion only externally [93]. The threshold where ring enlargement allows anion inclusion thus causing a rise in complex stability is marked in $[Co(CN)_6]^{3-}$ and $[Pt(CN)_4]^{2-}$ recognition by host **3/11** [91]. In the case of $[PdCl_4]^{2-}$, the increase in complex stability starts with host **3/9** and reaches a maximum with host **3/10** [94], while no such dependence is observed in $[Fe(CN)_6]^{4-}$ binding [91]. Molecular modeling studies showed that the lower stability of the smaller hosts is not due to a principal inability of these compounds to accommodate the anions inside their cavity. Also the smaller hosts are large enough for guest inclusion but optimization of electrostatic and hydrogen bonding interactions can cause complexes of the smaller hosts to be more stable when the anion is externally bound. That hydrogen bonding is important for complex stabilization is evident in a number of crystal structures [91, 94, 95]. Solution evidence for hydrogen bonding interactions is discussed below.

High affinity for biologically relevant phosphate and nucleotide anions of the polyaza hosts **3/6**–**3/12** has also been demonstrated [90, 96, 97]. Whereas the phosphate complexes of **3/11** are more stable than those of **3/10**, an effect similar to the one observed in the recognition of anionic complexes of transition metals, no such dependence was observed in diphosphate or ATP binding. NAD and NADP recognition has been studied with host **3/7** [98]. The observed higher affinity of **3/7** toward NADP was rationalized by an interaction of the extra phosphate moiety in this guest with two adjacent ammonium groups of the host. Recognition of nucleotides by polyaza hosts has also been demonstrated by Kimura and co-workers [99].

One of the most interesting properties of macrocyclic polyamines that goes beyond simple phosphate recognition is their ability to catalyze both the dephosphorylation of ATP and, under suitable conditions, the phosphorylation of ADP [100, 101]. The catalytic cycle proposed for ATP hydrolysis in the presence of polyamine **3/13**, the host best studied in this respect, is schematically depicted in Figure 8. The first step consists in the binding of ATP to **3/13**. Due to the structure of the macrocycle, in particular the distances between the six amino groups, at pH 7 only four to five of these groups are protonated and can act as binding sites for the oxoanion. When appropriately positioned, the remaining unprotonated amine can then help to cleave the terminal phosphate group from the triphosphate resulting in the formation of a phosphoramidate intermediate. The final steps in the catalytic cycle are the dissociation of the ADP complex and the hydrolysis of the phosphoramidate, not necessarily in this order. The action of **3/13** in ATP hydrolysis is truly catalytic [102, 103]. No product inhibition is observed, for instance, because the affinity of **3/13** for ADP is smaller than for ATP. As a consequence, ADP hydrolysis in the

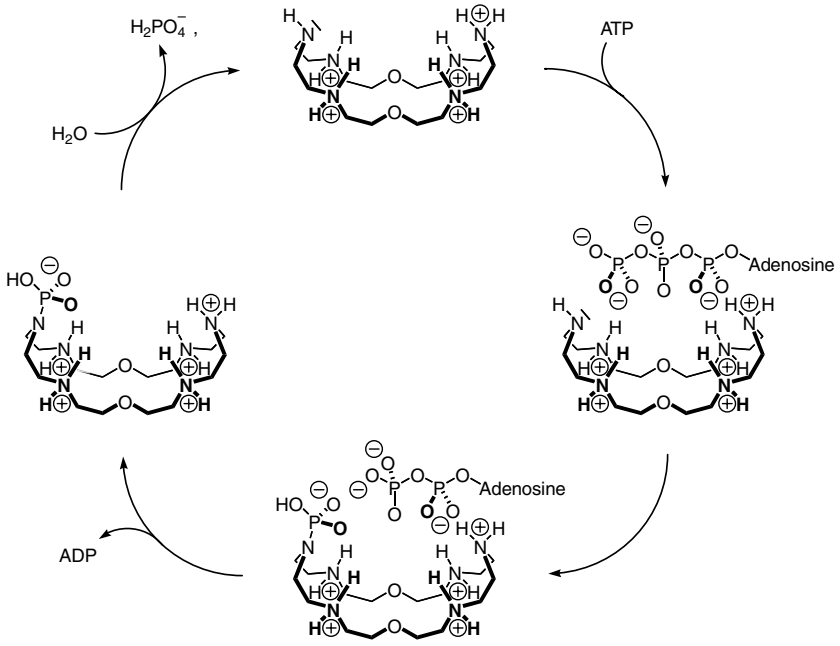
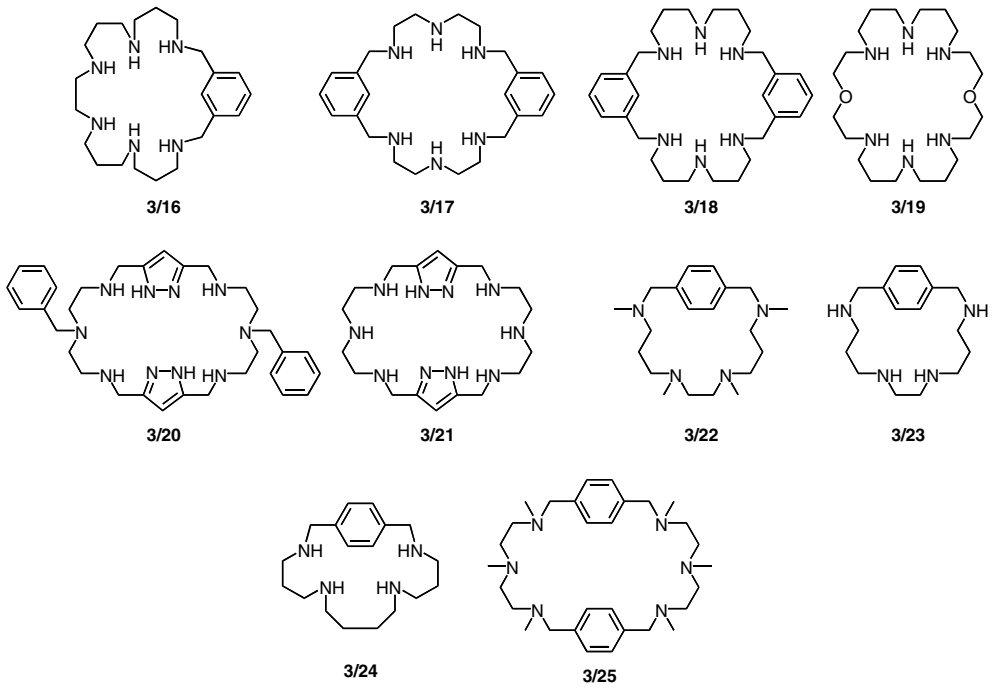


Figure 8. Catalytic cycle for ATP dephosphorylation by polyamine **3/13**.



Scheme 4.

presence of **3/13** is ca. 3 times slower than ATP hydrolysis. The phosphoramidate intermediate formed during ATP dephosphorylation can also act as a phosphorylation agent for suitable substrates. Biomimetic ATP synthesis can be achieved, for example, by phosphorylation of ADP in the presence of divalent cations such as Mg^{2+} or Ca^{2+} [104].

The hydrolytic activity of macrocyclic polyamines is not correlated with their affinity toward ATP since hosts displaying large ATP affinity often do not produce any rate enhancement in dephosphorylation [105, 106]. Systematic investigations have revealed that size complementarity of the ATP triphosphate group and the host is much more important. In this context it was shown, for example, that the catalytic efficiency of macrocyclic polyamines with 21-membered rings is superior to that of larger macrocycles [107]. Moreover, the rates of dephosphorylation were found to increase with increasing number of nitrogen atoms in the ring. Thus, **3/7** is also a potent catalyst for ATP hydrolysis [107]. Aromatic rings in polyaza hosts can be beneficial if, by π-stacking interactions, they cause a better mutual orientation of the triphosphate chain of ATP and the polyammonium bridge of the receptor in the complex [106]. Also *N*-methylation can have an effect on the rate of ATP dephosphorylation [105]. The catalytic efficiencies of hosts **3/6**, **3/14**, and **3/15**, for example, increase in the order **3/14** < **3/6** < **3/15**. Thus, di- and tetramethylation of **3/6** produces opposite effects on catalytic activity, which was explained by an unfavorable effect of the methyl groups on complex geometry in the case of **3/14** which causes the nucleophilic amino group of the host to be too far away from the terminal phosphate group of ATP while the reverse is true for **3/15** [105]. Finally, it should be mentioned that also a mimic for N^{10}-formyltetrahydrofolate synthetase was devised on the basis of **3/13** [108, 109].

Many efforts were directed toward a control over anion affinity and selectivity of polyaza macrocycles. Methyl groups on ring nitrogens have been shown, for example, to direct protonation to secondary nitrogens giving rise to defined protonation patterns in the host and, as a consequence, increase in anion selectivity [105]. Thus, the tetraprotonated form of **3/14** in which the protons are preferentially located on the secondary nitrogens binds ATP^{4-} in 0.15 M $NaClO_4$ at 298 K more than one order of magnitude more strongly than the tetraprotonated form of **3/6** ($\log K_a$ (**3/14**) = 7.39, $\log K_a$ (**3/6**) = 5.91) [92, 105]. **3/15**, a polyaza host with four methyl groups, binds ATP under the same conditions slightly more efficiently than **3/14** ($\log K_a$ (**3/15**) = 7.48) while no large difference is observed in the affinity of the three hosts toward $P_2O_7^{4-}$ demonstrating that the effect of methylation can sometimes be small ($\log K_a$ (**3/15**) = 7.48, $\log K_a$ (**3/14**) = 7.84, $\log K_a$ (**3/6**) = 7.22).

Another approach to control the receptor properties of polyaza macrocycles involves the introduction of aromatic subunits. These subunits not only impose a higher rigidity on the macrocycles, they also allow for π-stacking interactions with suitable guests thus cooperatively contributing to complex stability. Complex formation between **3/16** and ATP, ADP, and AMP, for example was found to not only involve electrostatic and hydrogen bonding interactions between the ammonium groups of the host and the guest's phosphate moiety, but also aromatic interactions the latter of which induce characteristic shifts of guest and host signals in the ^1H NMR spectra of the complexes with respect to the spectra of the free components demonstrating that the

adenine group of the guests and the aromatic subunit of the host are in close contact [110, 111]. These aromatic interactions cause the nucleotide complexes of **3/16** to be considerably more stable than those of a non-cyclic host lacking the aromatic subunit.

The structurally closely related hosts **3/17** and **3/18** also interact strongly with ATP, ADP, AMP, and with various phosphate anions such as HPO_4^{2-}, $P_2O_7^{4-}$, and $P_3O_{10}^{5-}$ [112, 113]. Anion affinity follows the usual trend as it increases with increasing charge of the guest and the host. Thus, the most stable complexes are formed between the fully protonated hosts and the fully deprotonated guests. More important is, however, the comparison of the nucleotide affinity of **3/17** and **3/18** with that of hosts **3/13** and **3/19** lacking the aromatic subunits. Whereas the most basic host **3/19** forms more stable complexes with phosphate than the less basic host **3/18**, the opposite is true for nucleotide binding which demonstrates a stabilizing effect of π–π interactions in complex formation. Spectroscopic evidence for a close contact in the complexes between the aromatic subunits of **3/18** and the adenine moiety of the nucleotides came again from NMR investigations [113]. Host **3/17** also interacts with sulfate (0.1 M aqueous NaOTs) with an association constant log K_a of, for example, 4.36 for the hexaprotonated form [114].

The pyrazole containing macrocyclic polyamine **3/20** has recently been shown to interact with L-glutamate in water [115]. NMR investigations suggested that, in addition to electrostatic and hydrogen bonding interactions, cation-π interactions between the benzyl groups of **3/20** and the L-glutamate ammonium group contribute to complex stability. This assumption is supported by the fact that host **3/21**, without appended benzyl groups, forms less stable complexes under the same conditions.

Host **3/22** was used to investigate the effect of the presence of an aromatic moiety in a polyaza host in combination with *N*-methylation on anion affinity [116]. It was shown that **3/22** is very selective for ATP over other phosphate anions such as ADP, AMP, $P_2O_7^{4-}$, or $P_3O_{10}^{5-}$ with conditional constants measured in water being one to two orders of magnitude larger for ATP. Moreover, comparison of the behavior of **3/22** and **3/23** showed that *N*-methylation causes a significant increase in binding strength of at least two orders of magnitude. Molecular modeling studies ruled out the possibility of a participation of π-stacking interactions in the complex between ATP and **3/22**, however.

Microcalorimetric characterization of the binding of phosphate and sulfate to a number of macrocyclic polyaza hosts, among them **3/6**, **3/7**, **3/14**, **3/15**, **3/23**, **3/24**, and **3/25**, revealed interesting information on the enthalpic and entropic contributions to complex formation [97, 117]. First, both anions are bound by all of the hosts investigated in a defined 1:1 stoichiometry. Second, not all reactions investigated follow the usual trend of increasing binding strength with increasing charge of the anion or the host. For instance, the stability of the complexes formed by HPO_4^{2-} with the mono-, di-, and triprotonated forms of **3/24** decrease with increasing charge on the ligand, while the stability of the complexes formed by **3/25** with $HP_2O_7^{3-}$, $H_2P_2O_7^{2-}$, and $H_3P_2O_7^{-}$ increases with decreasing charge of the anion. Third, binding of phosphate to the hosts is often athermic or endothermic and promoted by favorable entropic contributions ($T\Delta S > 0$) in agreement with the ideal electrostatic model. A considerable number of reactions is also exothermic, however, and accompanied by entropy loss ($T\Delta S < 0$). These observations have been rationalized by considering the contributions of hydrogen bonding interactions between hosts and anions to complex stability [97].

There are four possible types of hydrogen bonds **a**–**d** between an amine or ammonium ion and an oxoanion involving the NH group as donor (Figure 9). Taking into account that deprotonation of an amino group is endothermic while protonation of a phosphate is almost athermic, partial proton transfer in these types of hydrogen bonds is expected to give unfavorable enthalpic contributions. Only hydrogen bonding mode **e** in which the amine acts as an acceptor should furnish favorable enthalpic changes (Figure 9). Thus, the stability decrease of the complexes formed by HPO_4^{2-} with the mono-, di-, and triprotonated forms of **3/24** can be interpreted in terms of increasing hydrogen bond donor properties of the host, leading to an increasingly unfavorable enthalpic contribution to binding. On the other hand, the stability increase of the complexes formed by pyrophosphate and **3/25** as the charge decreases on the anion from $HP_2O_7^{3-}$ to $H_3P_2O_7^{-}$ can be attributed to the greater donor ability of the more protonated anions causing more favorable enthalpic changes in complex formation. In contrast to phosphate anions, no protonation of sulfate has to be considered in the pH range used for the investigations. Thus, a binding mode **e** in which sulfate acts as hydrogen bond donor (Figure 9) is not possible. Moreover, binding modes with sulfate anions acting as hydrogen bond acceptors should be enthalpically more unfavorable than those of phosphate anions because protonation of sulfate is more endothermic. As a consequence, most reactions between polyaza hosts and sulfate were found to be endothermic or almost athermic and the stability of the complexes formed is therefore largely determined

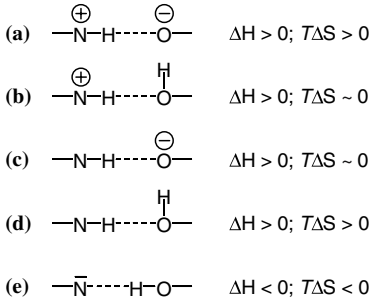
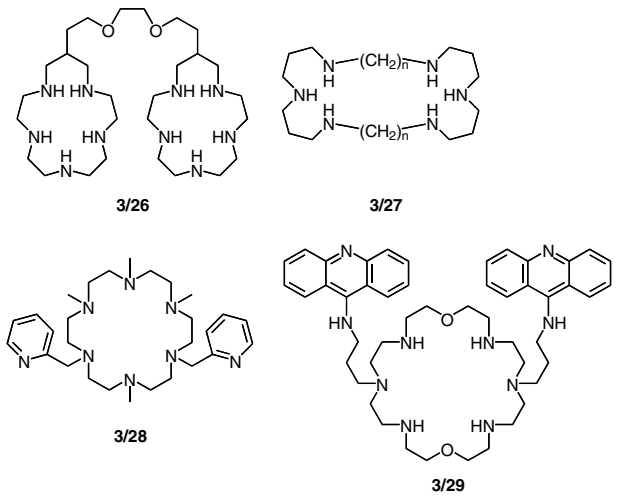


Figure 9. Possible types of hydrogen bonding interactions between an amine or ammonium ion and an oxoanion.



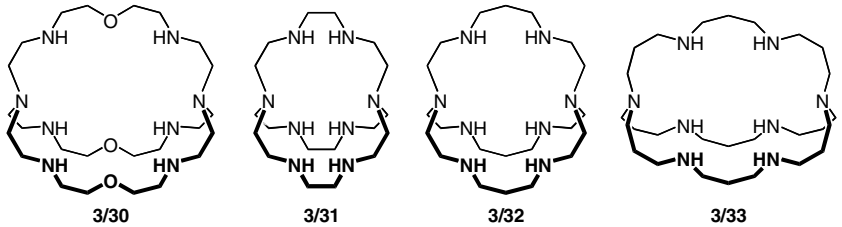
Scheme 5.

by the favorable entropic terms produced by the desolvation of the interacting species [117]. Interestingly, at pH > 8.55 sulfate is recognized by **3/6** in preference to phosphate, but a selectivity inversion occurs on lowering the solution pH when phosphate complexes become more predominant. Because lowering the pH causes phosphate to be protonated, the hosts selectively recognizes a protonated tetrahedral oxoanion over a non-protonated one in this pH range thus mimicking the behavior of the phosphate-binding protein (*vide supra*). Another impertant conclusion of the authors is that, although the insertion of aromatic subunits into macrocyclic polyamines gives rise to more rigid systems, the anion affinity of these hosts is determined not by their rigidity but by their ability in organizing hydrogen bonds and salt bridges in the complex.

Strategies that have also been tested to deliberately control the binding properties of macrocyclic polyamines involve the introduction of additional binding sites in the periphery of the macrocyclic cavity or the use of hosts with topologies matching those of the potential substrate. **3/26** was designed, for example, to form sandwich-type complexes with anions, in which the anions are bound between the two polyamine rings [118]. It was shown that the tetraprotonated form of this ditopic host with two protons on each subunit binds various anions such as $[Fe(CN)_6]^{4-}$, $[Fe(CN)_6]^{3-}$, citrate, AMP, ATP, or HPO_4^{2-} ca. one order of magnitude better than the triprotonated monotopic parent compound demonstrating the cooperative effect of the two receptor subunits in anion recognition. Also host **3/27** can be regarded as a ditopic receptor. Only in this case, both triamine binding sites are located in the same ring and a linker of variable length controls binding selectivity [119]. Success of this concept was demonstrated for dicarboxylate recognition: while the smaller host with $n = 7$ has the highest affinity for the shorter succinic acid ($^-OOC(CH_2)_2COO^-$: log $K_a = 4.30$) and glutaric acid ($^-OOC(CH_2)_3COO^-$: log $K_a = 4.40$), the longer dicarboxylic acids pimelic acid and suberic acid are bound best by the larger host with $n = 10$ ($^-OOC(CH_2)_5COO^-$: log $K_a = 4.40$; $^-OOC(CH_2)_6COO^-$: log $K_a = 4.25$).

Cooperative effects to binding of substituents appended to a polyaza host were shown to cause an increase in glutamate affinity of **3/20** (*vide supra*). Other examples of polyaza hosts whose receptor properties are affected by substituents in the periphery of the cavity are **3/28** and **3/29**. The pyridine groups on **3/28**, for example, enhance ATP binding at acidic pH [120]. More important is, however, their influence on metal complexation. The polyamine **3/29** bearing two acridine moieties makes use of combined electrostatic and stacking interactions for the binding of ATP, NADPH, and NADP [121]. **3/29** exhibits a remarkable selectivity for NADPH which is bound by a factor of ca. 10^3 better than NADP and by a factor of $>10^6$ better than NAD(H). Moreover, binding can be followed by optical methods as complex formation causes an enhancement of the acridine fluorescence.

The most significant improvement in anion affinity and, more importantly, selectivity of ammonium based anion hosts came from changing the receptor topology from macrocyclic to macrobicyclic or polycyclic. Pioneering work in this respect was carried out in the Lehn group where it was shown that the azacryptands **3/30**–**3/33**, for example, not only possess high affinity for sulfate and phosphate but also for anions such as singly charged halides, azide, or nitrate all of which are not or only very weakly bound by monocyclic polyaza hosts, a result attributed to the macrobicyclic effect [122, 123]. Moreover, anion selectivity of **3/30**–**3/33** depends sensitively on the structural complementarity between host and guest. Thus, **3/30** has a higher affinity for azide than for any other singly charged anion tested, most probably because of the perfect fit of the linear azide anion into the ellipsoidal cavity of the host [122, 123]. Spherical halides are less well bound with decreasing affinity from fluoride to iodide (Table 4). The smallest azacryptand

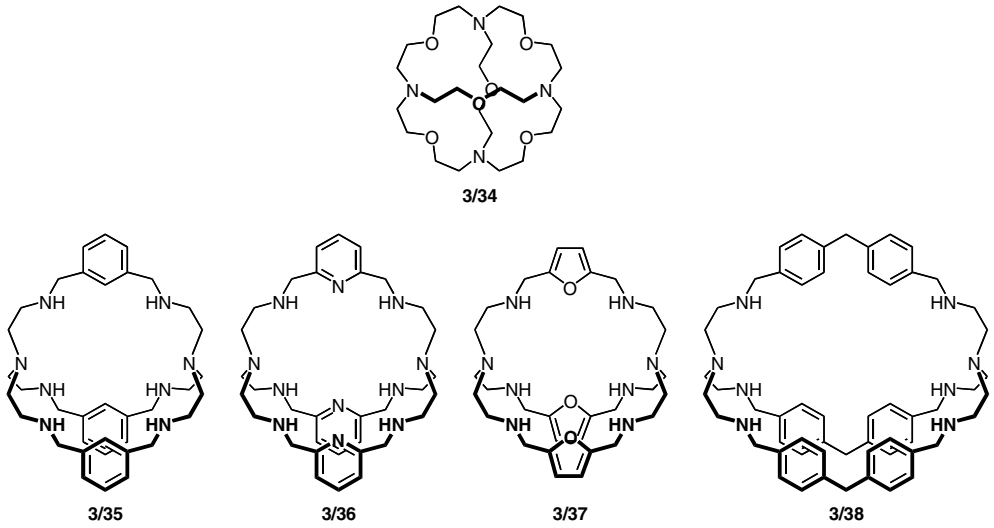


Scheme 6.

Table 4. Stability constants log K_a of various anion complexes of the hexaprotonated forms of azacryptands **3/30**–**3/33**

Anion	log K_a (**3/30**) [123][a]	log K_a (**3/31**) [125][b]	log K_a (**3/32**) [125][b]	log K_a (**3/33**) [127][a]
F^-	4.10	10.55	6.10	–
Cl^-	3.00	< 2	5.75	1.70
Br^-	2.60	–	4.40	2.20
I^-	2.15	–	2.25	2.40
N_3^-	4.30			
NO_3^-	2.80			
SO_4^{2-}	4.90			
HPO_4^{2-}	5.50			
$P_2O_7^{4-}$	10.30			

[a]Measured in water at 25 °C in the presence of 0.1 M NaOTs as supporting electrolyte.
[b]Measured in water at 25 °C in the presence of 0.1 M NMe_4OTs as supporting electrolyte.

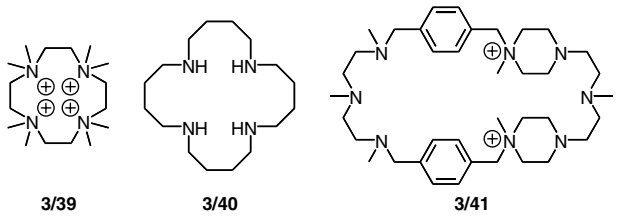


Scheme 7.

3/31 practically only interacts with fluoride with high affinity [124, 125], while complexation of chloride occurs only after addition of the sixth proton to the host [126]. This property explains the impressive selectivity of **3/31** for fluoride over chloride $K_a(F^-)/K_a(Cl^-)$ of ca. 10^8. Increasing the cavity size causes the halide complexes of the azacryptands to become less stable (Table 4). Interestingly, the decrease in affinity in the order $F^- > Cl^- > Br^- > I^-$, still observed for **3/32**, reverses for **3/33**, most probably because iodide fits into the cavity of the largest host best [127]. That complexation of the anions indeed occurs inside the cavity of the azacryptands was demonstrated by a number of crystal structures [123–125].

The macrotricyclic host **3/34** was originally designed as a receptor for alkali metal ions. It turned out, however, that the tetraprotonated form of this so-called 'soccer ball' is also an efficient host for chloride [128]. Complexation of chloride occurs in the center of the cavity of **3/34** where the anion interacts efficiently with four converging NH protons [129]. The affinity of **3/34** toward chloride, a stability constant of log $K_a > 4$ has been determined for the complex is significantly larger than that of other azacryptands reflecting the ideal size and shape complementarity of **3/34** toward this guest. Non-spherical or significantly larger anions such as NO_3^-, ClO_4^-, and F_3CCOO^- are not bound at all while the bromide complex has a ca. three orders of magnitude smaller association constant [128].

More recently, also the anion affinity of a number of azacryptands containing rigid aromatic subunits has been investigated, examples of which are hosts **3/35**–**3/38**. Receptors **3/35**–**3/37** interact with oxoanions such as NO_3^-, SO_4^{2-}, SeO_4^{2-}, or $S_2O_4^{2-}$ and carboxylates or dicarboxylates such as acetate, lactate, oxalate and malonate in aqueous solution [114, 130, 131]. Complexation of perchlorate has been detected in the solid state. Interestingly, whereas **3/35** and **3/37** form 1:1 complexes with the anion residing inside the cavity of the bicyclic systems, **3/36** prefers cleft-like arrangements in its complexes with the guest bound between the straps of the host. Especially noteworthy is the exceptionally high association constant of the oxalate complex of **3/35** (log $K_a = 10.71$ for the hexaprotonated form of **3/35** and



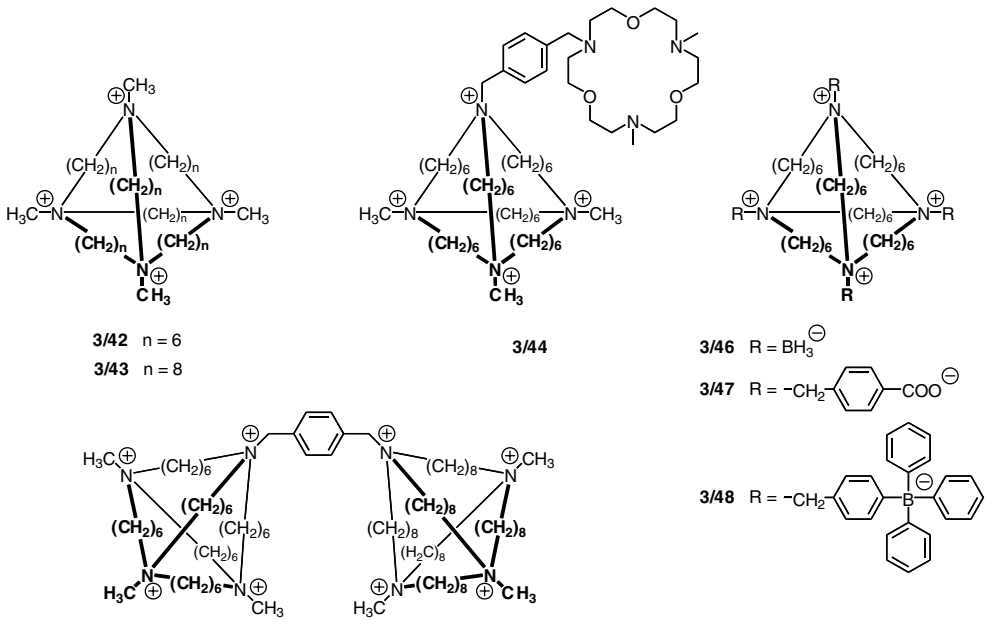
3/39 3/40 3/41

Scheme 8.

even 13.83 for the heptaprotonated form) that has been rationalized by additional stabilizing effects caused by π–π stacking interactions between a carbonyl-type carboxylate group of the guest and an aromatic ring in the linker [132]. **3/35** also interacts with fluoride in aqueous solution [133, 134]. A pH dependent ^{19}F NMR study recently revealed that complex formation is detectable even at a pH as high as 8. Decreasing the pH causes the signal in the NMR assigned to complexed fluoride to become stronger until at a pH of ca. 6, at which the azacryptand is essentially hexaprotonated, no free fluoride can be detected in solution anymore. In the solid state, an additional water molecule resides inside the cavity of the fluoride complex of **3/35** [134]. The largest azacryptand in this series, **3/38**, binds dicarboxylates in aqueous solution at pH 6 [135]. Highest affinity was observed for adipate ($K_a = 2.6 \times 10^3$ M^{-1}) while shorter or longer dicarboxylates are complexed less efficiently. Similar to **3/27**, this behavior was attributed to an optimal size and shape complementarity of the cavity of **3/38** to adipate. A very stable complex is also formed between **3/38** and terephtalate ($K_a = 2.5 \times 10^4$ M^{-1}) and for this anion, a crystal structure confirmed that binding occurs inside the cavity of the host [135].

Crystal structures of complexes between macrocyclic or macrobicyclic ammonium based anion hosts often indicate the presence of hydrogen bonds and there is evidence that this type of interaction can contribute to complex stability even in aqueous solution. The results of the microcalorimetric investigations described above, for example, were interpreted by considering contributions of hydrogen bonding to complex formation. Additional evidence for the importance of hydrogen bond formation in aqueous solution was obtained by comparing the binding properties of cyclic polyamines with those of hosts in which quaternization of the amino groups prevents the formation of hydrogen bonds. The tetraammonium ion **3/39**, for instance, is unable to interact appreciably even with highly charged anions such as ATP or $[Co(CN)_6]^{3-}$ both in solution and in the solid state. On the other hand, the tetraprotonated form of **3/40** possesses high affinity toward the same anions despite the fact that the charge density of **3/40** is even lower than that of **3/39** [136, 137]. Thus, a major contribution to complex stability must arise from hydrogen bonding interactions. In a related study it was shown that the stability of the chloride complex of **3/34** is reduced by ca. three orders of magnitude upon quaternization of the nitrogens [138]. Finally, whereas **3/25** forms complexes with ATP and ADP whose stability constants log K_a range between 3 and 4.8, no such complexes are observed for **3/41** [139, 140]. This result was ascribed to a divergent orientation of the acidic protons in the pentaprotonated form of **3/41** while the acidic protons in the tetraprotonated form of **3/25** converge.

Removal of hydrogen bond donor sites in a polyaza host by quaternization obviously causes a reduction in anion affinity because hydrogen bonding interactions



3/42 n = 6
3/43 n = 8

3/44

3/46 R = BH_3^-
3/47 R = $-CH_2-C_6H_4-COO^-$
3/48 R = $-CH_2-B^-(C_6H_5)_3$

3/45

Scheme 9.

Table 5. Stability constants log K_a of various anion complexes of Schmidtchen's macrotricyclic hosts in water

Host	Cl^-	Br^-	I^-
3/42 [141]	1.7	3.0	2.7
3/43 [138, 141]	< 0.5	2.0	2.46
3/47 [143]	2.43	3.33	3.81

are prevented and binding has to rely solely on electrostatic interactions. It has to be considered, however, that the overall charge of hosts containing quaternized ammonium groups is independent of the pH and, as a consequence, no acidic medium is required for complex formation to take place. This clear advantage motivated Schmidtchen to systematically pursue the development of anion receptors derived from quaternary ammonium salts. In a series of elegant papers, he showed that potent anion receptors can be devised without the need to include hydrogen bond donor sites in the framework of a polyammonium host provided that the receptor has an appropriate topology to allow for specific and strong interactions with the guest. The hosts introduced by Schmidtchen in this context consist of the macrotricyclic quaternary ammonium salts **3/42** and **3/43** both of which structurally somewhat resemble **3/34**. These hosts form complexes with halides in water with stability constants log K_a ranging between 0.5 and 2.5 [138, 141]. Binding selectivity correlates with the size of the cavity with the smaller host **3/42** forming the most stable complex with bromide and the larger host **3/43** with iodide (Table 5). That complexation of the halides takes place inside the host cavities was confirmed by the crystal structure of the iodide complex of **3/42** [142]. Also other anions such as carboxylates or phosphates including nucleotides interact with **3/42** and **3/43**. Complex stability increases with increasing charge of the anion, for example in the order $CO_3^{2-} > HCO_3^-$ or $HPO_4^{2-} > H_2PO_4^-$, consistent with the binding mechanism that is based on electrostatic interactions [141].

Schmidtchen was also able to demonstrate that the tricyclic quaternary ammonium salt **3/43** can act as a catalyst for reactions involving anionic substrates and transition states [144]. The decarboxylation of 6-nitrobenzisoxazole-3-carboxylate in water at 25 °C is, for example, accelerated by a factor of 110 (k_{cat}/k_{un}) in the presence of **3/43** with respect to the rate of reaction in the absence of the host (Figure 10a) [145]. This effect was rationalized in terms of an inclusion of the nitroaromatic moiety of the substrate into the hydrophobic cavity of **3/43** which causes a release of 'high energy' solvent molecules into the bulk solution. Thus, an energetically costly rearrangement of solvent structure around the substrate upon reaching the transition state is avoided leading to a greater activation entropy and consequently to a rate enhancement [145]. The intramolecular cyclization of 2-(3-halopropyl)-4-nitrophenols is also catalyzed by **3/43** (Figure 10b) [146] as are intermolecular reactions such as nucleophilic aromatic substitutions of 2,4-dinitrofluorobenzene, 4-chloro-3,5-dinitrobenzenesulfonate, or 4-chloro-3,5-dinitrobenzoate with nitrite, hydroxide, azide, or other nucleophiles (Figure 10c) [147–150]. The largest rate enhancement amounting to $k_{cat}/k_{un} = 1700$ was observed for the reaction of 4-chloro-3,5-dinitrobenzenesulfonate with azide [150]. In the case of nucleophilic aromatic substitutions, the catalytic effect of **3/43** is explained by a co-inclusion of the substrate and the nucleophile into the cavity of the host. This assumption is supported by the fact that the smaller host **3/42** is not able to act as a catalyst in these or in the other reactions studied.

The versatility of hosts **3/42** and **3/43** was further demonstrated by constructing the ditopic derivatives **3/44** and **3/45**. Host **3/44**, also termed Tetrazac, has been shown to bind ω-aminocarboxylates such as 4-aminobutyric acid (GABA) in 90% water/methanol [151]. Although the stability constants of various anion complexes of **3/44** are smaller than those of an analog of **3/44**, in which the tricyclic residue is replaced by a triethylamine group, binding selectivity of **3/44** is larger for hydrophobic or zwitterionic ammonium salts by a factor of, respectively, 3 and 2.5. No difference in the complexation of 4-aminobutyric acid or 6-aminohexanoic acid by **3/44** was found, however, (log $K_a = 2.4$)

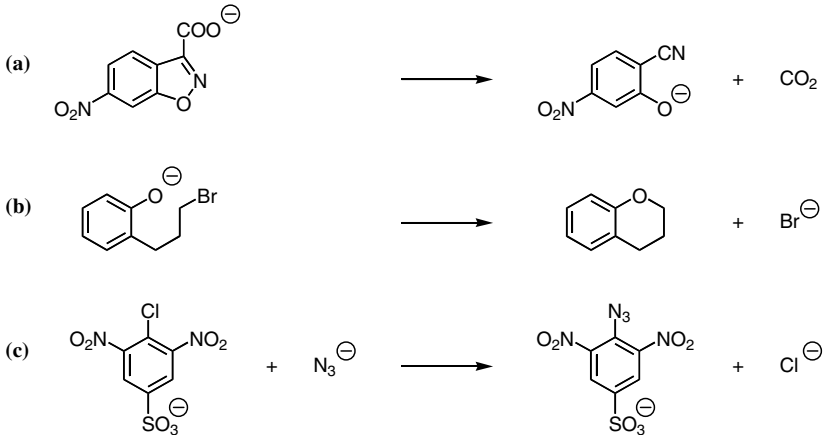
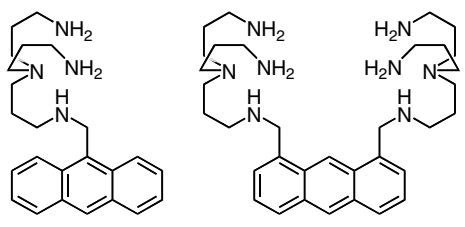


Figure 10. Examples for reactions catalyzed by host **3/43**.

indicating that the ditopic host is flexible enough to adapt to substrates of different geometries [151]. **3/45** was devised as a host for dianionic guests [152, 153]. A comparison of the affinities of **3/45** and **3/43** toward mixed dianionic substrates containing a phenolate moiety and a carboxylate moiety connected via a linker of variable length showed that the binding constants of the monotopic receptor are generally smaller than the corresponding values of the ditopic host which is, however, essentially an effect of the higher charge of **3/45**. The superiority of **3/45** over its monotopic counterpart **3/43** only becomes evident when the selectivity ratios K_a(**3/45**)/K_a(**3/43**) characterizing the advantage of the ditopic over the monotopic receptor are compared which clearly show a significant boost in affinity of **3/45** when the linker between the two anionic groups of the substrate exceeds a certain length. This observation is consistent with the idea that binding of both anionic groups of the substrate at each binding site of the host becomes possible when the substrate's linker is large enough to span the distance between the two receptor subunits. In quantitative terms, the superiority of **3/45** over **3/43** amounts to a factor of 3 [152, 153]. More recently, also porphyrin derivatives with two or four appended residues of **3/42** were synthesized and shown to bind with binding constants log K_a ranging from 5.35 to 6.02 to nucleotides in aqueous buffer solution (HEPES, pH 7.4) [154].

One principal disadvantage of the use of charged anion receptors is the presence of their counterions in solution that compete with the substrate for vacant binding sites. Schmidtchen was able to circumvent this problem by designing the overall neutral zwitterionic hosts **3/46**–**3/48** [143, 155, 156]. Because of the rigid molecular framework of these compounds, internal collapse caused by intramolecular ion pair formation can be avoided. As a consequence, host **3/47**, for example, exhibits improved affinity with respect to **3/42** for halides in water (Table 5) [143]. The temperature dependence of the stability constants showed that bromide and iodide binding are enthalpically driven processes (ΔH(bromide) = −31.82 kJ mol^{-1}; ΔH(iodide) = −69.92 kJ mol^{-1}) counterbalanced to some extent by negative entropy contributions (ΔS(bromide) = −33.49 J K^{-1} mol^{-1}; ΔS(iodide) = −141.93 J K^{-1} mol^{-1}). This result was explained in terms of a larger energy required for desolvation of bromide with respect to iodide which leads to a smaller enthalpic gain in bromide complexation. The release of water molecules from the bromide anion is, on the other hand, entropically more favorable resulting in a less unfavorable overall entropic contribution in the binding of **3/47** to bromide than to iodide [143]. Anion binding of the other two zwitterionic hosts **3/46** and **3/48** has so far only been studied in organic solvents [155, 156].



3/49 **3/50**

Scheme 10.

Optical sensors for anions on the basis of polyammonium derived receptors were developed in the Czarnik group [157]. Addition of anions such as phosphate, ATP, citrate, sulfate, or acetate to an aqueous solution of, for example, **3/49** at pH 6 causes an increase in the fluorescence intensity of this host thus allowing a straightforward detection of the binding event by optical spectroscopy [158]. The use of such systems to monitor enzymatic ATP hydrolysis has been demonstrated, although not at a desirable pH [159]. Host **3/50** containing two binding sites separated by an anthracene residue is highly selective for pyrophosphate. Its 2200 times higher affinity for pyrophosphate with respect to phosphate provided the basis for monitoring the action of the enzyme inorganic pyrophosphatase in real-time [160].

4. Hosts containing guanidinium or amidinium groups

The use of guanidinium and amidinium groups as binding sites in synthetic receptors is a strategy to mimic the anion binding properties of the side chain of arginine. The advantage of guanidinium and amidinium moieties lies in their ability to combine Coulomb attraction in the binding of oxoanions such as carboxylates or phosphates with the formation of two strong parallel hydrogen bonds (Figure 1). Moreover, both groups are strongly basic with pK_a values typically ranging between 11 and 13, ensuring that they remain protonated over a wide pH range (Figure 11). There are, however, also disadvantages associated with the use of amidinium or guanidinium based anion receptors. Disubstituted amidinium groups, for example, are conformationally flexible and only one conformation is optimal for complex formation (Figure 11). The like is true for substituted guanidinium ions, whose interaction with anions is further complicated by the fact that binding is not restricted to one

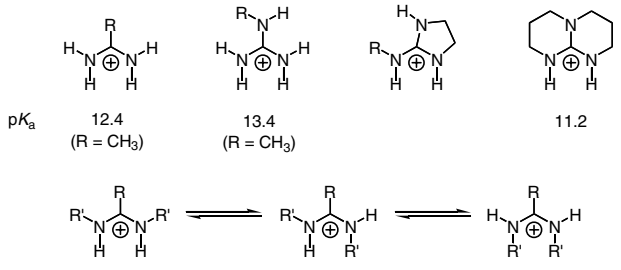
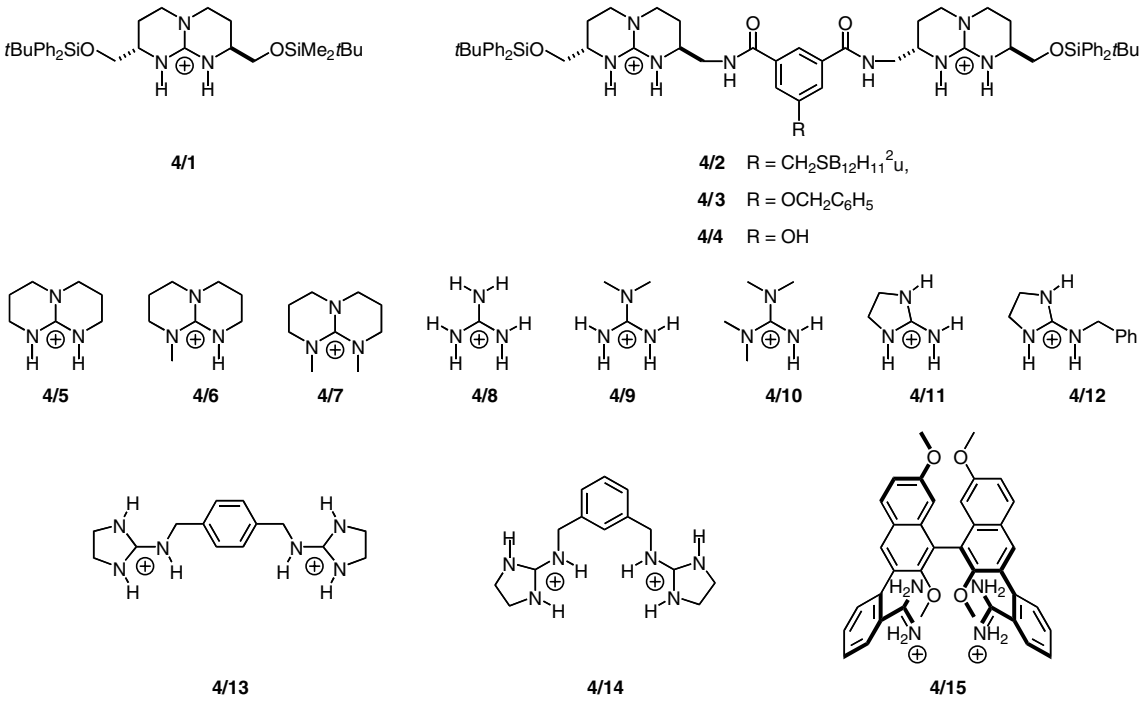


Figure 11. Amidinium and guanidinium based binding sites employed in synthetic anion receptors with their corresponding pK_a values [161, 162] and possible conformations of disubstituted amidinium ions.



Scheme 11.

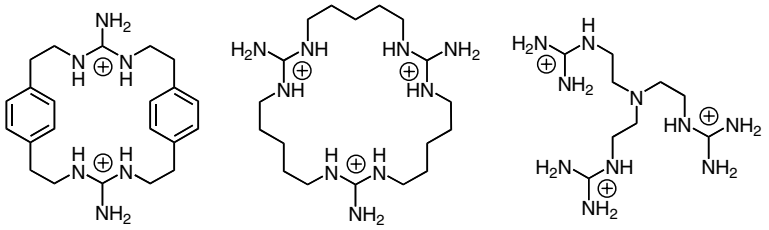
Table 6. Thermodynamic parameters in kJ mol^{-1} for the anion complexes of various guanidinium based hosts

Host	Anion	Solvent	T [K]	K_a [M^{-1}]	ΔH	$T\Delta S$
4/1 [164]	Acetate	CH_3CN	303	2.0×10^5	−15.5	15.2
4/1 [164]	Acetate	DMSO	303	6.5×10^3	−14.2	7.9
4/2 [164]	*p*-Nitrophenyl phosphate	CH_3CN	303	1.1×10^5	−33.5	−4.3
4/3 [164]	*p*-Nitrophenyl phosphate	CH_3CN	303	1.1×10^5	−28.4	0.7
4/4 [164]	*p*-Nitrophenyl phosphate	CH_3CN	303	1.6×10^5	−37.7	−7.5
4/3 [166]	Sulfate	CH_3OH	303	6.8×10^6	32.3	71.9
4/4 [166]	Sulfate	CH_3OH	303	4.9×10^6	29.6	68.4
4/5 [167]	Acetate	DMSO	298	5.6×10^3	−15.1	6.2
4/5 [167]	Acetate	CH_3OH	298	1.0×10^2	4.2	15.6
4/13 [168]	Glutarate	CH_3OH	298	2.7×10^3	15.5	34.9
4/13 [168]	1,3-Adamantane dicarboxylate	CH_3OH	298	9.5×10^3	16.7	39.9
4/14 [168]	Glutarate	CH_3OH	298	7.5×10^3	16.7	38.7
4/14 [168]	1,3-Adamantane dicarboxylate	CH_3OH	298	1.2×10^4	18.4	42.4

side of the planar guanidinium moiety. Nature solves this problem by using secondary interactions to restrict the orientation of a guanidinium group in the active center of a protein. In synthetic receptors, on the other hand, the use of conformationally restrained amino imidazoline or bicyclic guanidinium residues has been proven to be useful.

An additional disadvantage of guanidinium ions is their strong solvation, which is so efficient that despite the favorable binding pattern, ion pairing with carboxylates or phosphates in aqueous solution is practically negligible ($K_a < 5\ M^{-1}$) [163]. Hydrophilic anion hosts therefore usually make use of two or more guanidinium moieties or the cooperative effect of other anion binding sites such as pyrroles to be effective in water.

Thermodynamic data are mainly available for the interaction between guanidinium based receptors and anions in organic solvents. Complex formation between **4/1** and acetate in acetonitrile or DMSO is, for example, strongly entropy driven with a favorable enthalpic contribution (Table 6). Almost no heat effects could be observed by microcalorimetry in methanol thus allowing no decision of whether no interactions occur or whether only the enthalpy of complex formation is zero in this solvent [164]. Binding affinity is affected by the counterion and by the structure of the substituents on the bicyclic guanidinium moiety, the latter of which has been attributed to effects of the substituents on solvation [165]. Phosphate binding to **4/2**–**4/4** proved to be exothermic in acetonitrile and DMSO with an



4/16 4/17 4/18

Scheme 12.

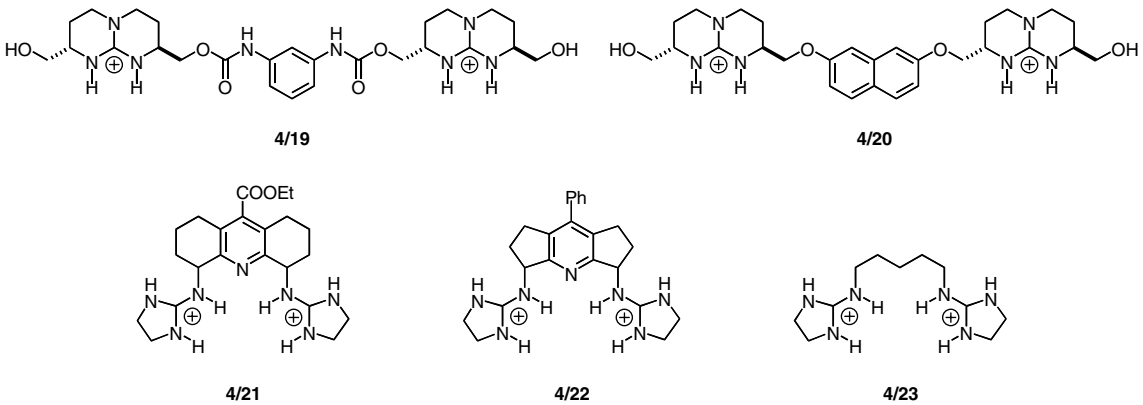
unfavorable or weakly favorable entropy of complex formation [164], while the interaction of the same hosts with sulfate in methanol is entirely driven by entropy [166].

Similar results are reported by the Hamilton group on the interaction between a series of monotopic hosts (**4/5**–**4/12**) with acetate [167]. It was shown that, in general, anion affinity is higher of guanidinium derivatives that can form bidentate hydrogen bonds. Moreover, complex formation is exothermic in DMSO and endothermic in methanol. These investigations have recently been extended to include bis(guanidinium) hosts **4/13** and **4/14** [168]. Binding of glutarate and 1,3-adamantane dicarboxylate in DMSO proved to be too strong to be followed quantitatively. In water/DMSO mixtures complex stability decreases but interactions are clearly visible even in 75% D_2O/d_6-DMSO. Qualitative microcalorimetric studies of the solvent dependence of complex formation indicated that the favorable enthalpic contribution observed in DMSO decreases upon increasing the solvent polarity until in pure methanol, binding becomes weakly endothermic and driven by entropy (Table 6). It is thus concluded that increasing the solvent polarity causes the association to change from one promoted primarily by hydrogen bond formation to one that is driven by solvent liberation [168]. This interpretation agrees with the results reported by Schmidtchen on complex formation of hosts **4/1**–**4/4** [164–166] and by Diederich on carboxylate binding of host **4/15** the latter of which is also endothermic in methanol but favored by entropy [169]. The thermodynamic characterization of the interaction between a tris(guanidinium) host with citrate in water, recently been carried out by the Anslyn group, is summarized further below [170].

The first examples of hydrophilic guanidinium based anion receptors were described by Lehn and co-workers [171, 172]. Complex formation was followed by means of pH titrations between a number of cyclic and non-cyclic hosts such as **4/16**–**4/18** and phosphates (PO_4^{3-}, HPO_4^{2-}, $P_2O_7^{4-}$, $HP_2O_7^{3-}$, $H_2P_2O_7^{2-}$) in water, or carboxylates in methanol/water 9:1. Stability constants log K_a typically range between 1 and 3 and increase with increasing charge on host or guest. This and the fact that the investigated guanidinium based hosts form less stable complexes than ammonium based hosts of equivalent structure and charge, a result that is consistent with the lower charge density of the guanidinium moiety, led to the conclusion that electrostatic interactions dominate anion binding.

Non-cyclic hosts containing two bicyclic guanidinium moieties were introduced by Schmidtchen. Weak binding of thymidine-5′-phosphate to **4/19** was detected in water ($K_a = 10.6\ M^{-1}$) [173]. More stable complexes are formed between the somewhat more rigid derivatives **4/20** and phosphate or several nucleotides approaching stability constants of up to 1000 M^{-1} [174]. A comparison of complex stability in DMSO, methanol, and water revealed that, again, electrostatics dominate binding interactions.

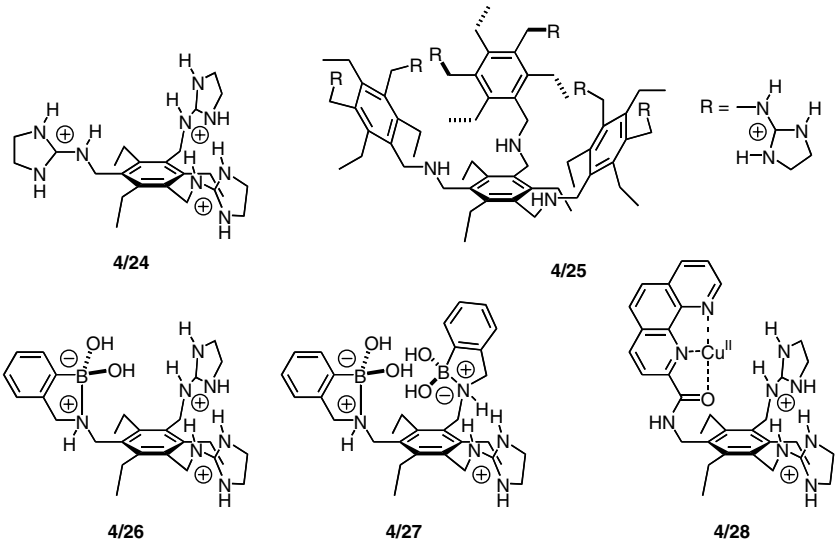
Cleft-like anion receptors containing guanidinium groups have also been used by other groups for



4/19 4/20

4/21 4/22 4/23

Scheme 13.



Scheme 14.

phosphate or carboxylate recognition. Examples are hosts **4/21** and **4/22** containing a rigid octahydroacridine linker with two amino imidazoline binding sites that were designed by Anslyn and co-workers to mimic phosphate binding inside the active center of the staphylococcal nuclease [175–177]. Initial binding studies using a mixture of all stereoisomers of **4/21** showed that this host can bind up to two dibenzyl phosphate ions in DMSO [175]. Formation of complexes of higher stoichiometry can be suppressed by increasing the solvent polarity and in 2:1 d_6-DMSO/D_2O, only the more stable 1:1 complexes are formed. Not surprisingly, complex stability is reduced in more polar solvent mixtures with respect to DMSO but the increase in binding efficiency observed upon increasing the ionic strength of the solution by addition of chloride was unexpected. The origin of this effect was elucidated by characterizing the anion affinity of the individual stereoisomers of **4/21** and **4/22** independently, an investigation that revealed that in the presence of tetraphenylborate as counterion, the *meso* forms of the hosts are the best receptors due to preorganization of the imidazoline moieties on the same face of the linker [176]. When the counterion is chloride, however, higher anion affinity is observed for the *d,l* receptors because the complexes of these stereoisomers are structurally stabilized by the cooperative action of a chloride ion. While phosphodiesters are bound only weakly in polar solvents by **4/21**, phosphomonoesters such as uridine-5′-monophosphate associate with the same host even in water (3 mM AMPS, pH 9.4) with a binding constants of 9.6×10^2 M^{-1} [177]. To test the contribution of preorganization to binding, the flexible bis(guanidinium) host **4/23** was synthesized whose affinity to uridine-5′-monophosphate amounts to only 41 M^{-1} under the same conditions. The use of the octahydroacridine scaffold thus results in a 7.5 kJ mol^{-1} advantage in binding. It should also be mentioned that, in accordance with the design principle, **4/21** has been shown to catalyze the cleavage of mRNA with a 20-fold increase over the uncatalyzed reaction [178, 179].

More recent work of the Anslyn group demonstrated that attachment of amino imidazoline moieties to a 1,3,5-trisubstituted 2,4,6-triethylbenzene scaffold provides access to another class of potent hydrophilic anion receptors. Hexasubstituted benzenes have several advantages when used as scaffolds for synthetic hosts the most important one being their restricted conformational freedom with every other substituent oriented on the same face of the benzene ring which is due to steric repulsion between adjacent aromatic substituents [180]. Amino imidazoline binding sites such as the ones in host **4/24** attached to a triethylbenzene scaffold are thus well preorganized for anion binding because they surround a defined cavity with the scaffold's benzene ring at its bottom. As a consequence, interactions between **4/24** and tricarboxylates such as citrate and tricarballate could be observed even in pure water with a binding constant of the citrate complex of 6.9×10^3 M^{-1}, for example [181]. Dicarboxylates, carboxylates or phosphates are bound with considerably reduced affinity. Moreover, derivatives of **4/24** lacking the ethyl groups or containing ammonium instead of

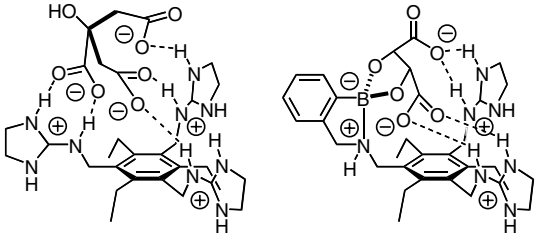


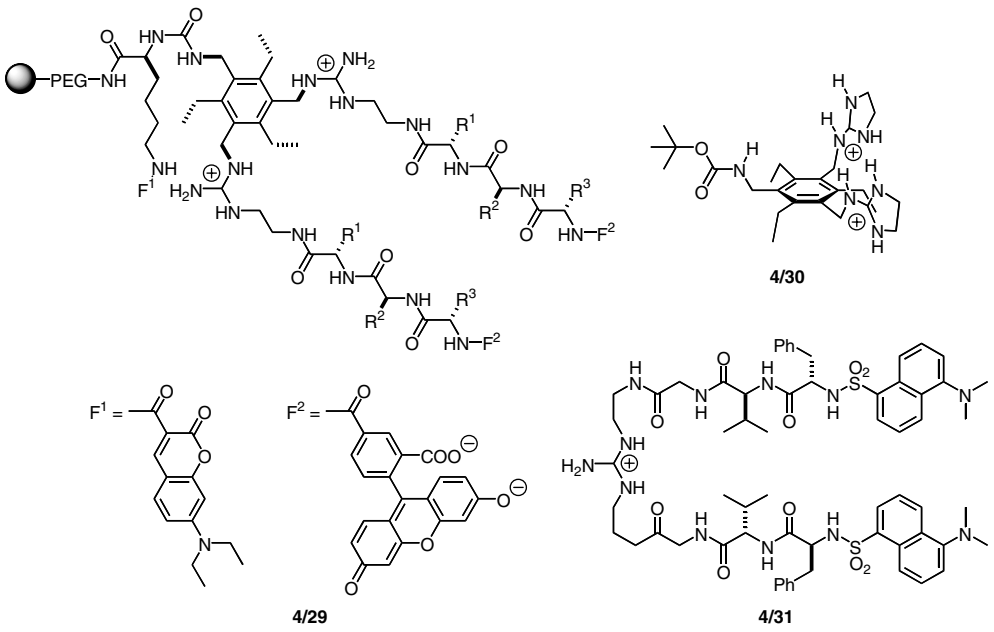
Figure 12. Schematic representation of the binding modes in the citrate complex of **4/24** (left) and in the tartrate complex of **4/26** (right).

imidazoline groups possess inferior receptor properties illustrating the importance of receptor preorganization and of hydrogen bonding interactions between the substrate and the guanidinium moieties. A schematic representation of the structure of the citrate complex of **4/24** that is based on a crystal structure is depicted in Figure 12. Host **4/24** was used in a dye displacement assay [182] to quantitatively determine the citrate content of various sports drinks [183]. A detailed microcalorimetric analysis of the interaction between **4/24** and citrate showed that formation of the 1:1 complex in water (5 mM phosphate buffer, pH 7.4) is an enthalpically ($\Delta H < 0$) as well as an entropically ($\Delta S > 0$) favorable process [170]. The observed stability constants agree with the ones obtained by NMR titration. Interestingly, complexes of higher stoichiometry, the most prominent having the composition [(**4/24**)$_2$·citrate], were observed in solution upon decreasing the citrate concentration while keeping the concentration of **4/24** constant. Formation of these complexes is endothermic and only favored by entropy which led to the conclusion that reduction of the concentration of one reactant can increase the apparent complexity of a system when aggregation is accompanied by solvation/desolvation processes [170].

Structural modifications of **4/24** were carried out with the aim to induce binding selectivity for other anions. Thus, **4/25** was designed as a receptor for inositol-1,4,5-triphosphate (IP_3) [184]. Binding affinity and selectivity was again characterized by a dye displacement assay that yielded a K_a of 4.7×10^5 M^{-1} for the IP_3 complex of **4/25** in water (10 mM HEPES, pH 7.4), slightly smaller than the K_a of the complex between the host's ammonium analog and IP_3 (5.0×10^5 M^{-1}). In the presence of 50 mM NaCl, IP_3 complex stability of **4/25** remains high ($K_a = 8.2 \times 10^4$ M^{-1}) while that of the ammonium analog decreased ($K_a < 1 \times 10^4$ M^{-1})clearly demonstrating the specific interactions in the complex between **4/25** and the guest. The stability of the complex between **4/25** and IP_3 in methanol is much higher ($K_a = 1.0 \times 10^8$ M^{-1}) and working in methanol in the presence of 5-carboxyfluorescein as indicator has the additional advantage that complex formation can easily be detected by a color change of the solution from fluorescent yellow to non-fluorescent colorless which can be ascribed to a displacement of the dye from its complex with **4/25** upon IP_3 binding and the simultaneous conversion of its dianionic form preferred in the complex to its cyclized form preferred in methanol [184]. The same color change is also observed at pH 4.0 in 40% methanol/water, or at the same pH in water in the presence of 2% of the surfactant Triton-X-100 [185]. Under these conditions, complex stability amounts to 5.0×10^6 M^{-1} (in 40% methanol/water) and 1.2×10^6 M^{-1} (in 2% Triton-X-100/water). Complex formation in the presence of Triton-X-100 is thus believed to occur to a significant extent in the lower dielectric environment of the micelles.

The boronic acid residues in 4126 and in 4127 were introduced to induce binding selectivity for anions containing vicinal hydroxy groups (Figure 12). The amine in the linker to the boronic acid serves two purposes. First, it promotes the transesterification reaction between the diol and the boronic acid, and second, it geometrically orients the boronic acid toward the cavity of the hosts. **4/26** indeed does bind tartrate with a high binding constant of 5.5×10^4 M^{-1} in 25% water/methanol (10 mM HEPES, pH 7.3) [186]. With the exception of malate, every other investigated anion is bound considerably less tightly thus allowing a quantitative determination of the tartrate/malate content of several beverages by dye displacement. Recently, a mathematical method was derived for the analysis of indicator displacement assay isotherms which was employed for the determination of malate in Pinot Noir must using host **4/26** and alizarine complexone [187]. Host **4/27** was shown to bind gallate and phenolic acids of similar structure in 25% water/methanol (10 mM HEPES, pH 7.0), a property that was used for the development of a colorimetric assay for the aging of scotch [188]. **4/27** also binds tartrate and malate but, in contrast to **4/26**, it has a higher affinity for tartrate. Thus, a multicomponent sensing ensemble could be devised consisting of **4/26**, **4/27**, and two indicators that, in combination with pattern-recognition analysis of UV–VIS spectra, allowed the simultaneous quantitative estimation of the malate and the tartrate concentration of an aqueous solution (25% water/methanol, 10 mM HEPES, pH 7.4) [189]. Even a simple colorimetric test strip that makes use of an indicator displacement assay was devised for tartrate on the basis of host **4/27** [190]. A systematic comparison of the interaction of receptors **4/24**, **4/26**, **4/27**, and a derivative containing three boronic acid subunits with carboxylates, α-hydroxycarboxylates, and diols has recently been published that includes a microcalorimetric analysis of the binding equilibria [191]. Receptor **4/24** was shown to be selective for citrate while receptors that incorporate boronic acids have higher affinities for guests containing α-hydroxycarboxylate or catechol moieties over guests containing only carboxylate or hydroxy groups. Isothermal titration calorimetry demonstrated that all binding events are exothermic with positive entropy. Moreover, enthalpy/entropy compensation was observed. Thus, boronic acid containing receptors form more tightly bound complexes (more negative ΔH) in comparison to the much looser complexes of receptors containing only guanidinium groups (less negative ΔH). The entropic component in the formation of the tighter complexes is, however, smaller than that in the formation of the looser ones most probably because it involves a less extensive solvent reorganization and leads to structurally better defined complexes [191].

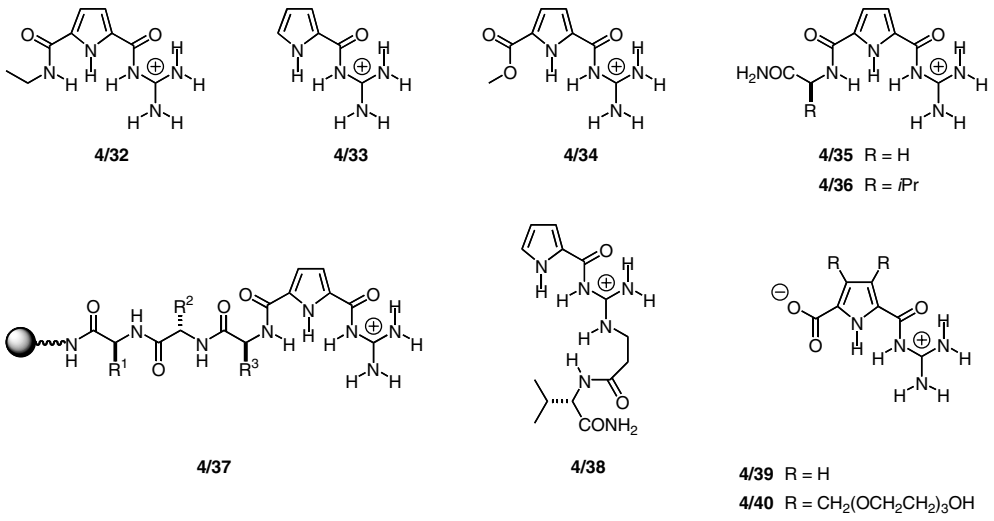
Receptor **4/28** is a metal containing anion receptor that is presented here and not in chapter 7 'Hosts containing metal centers' only because of its close structural relationship to hosts **4/24**–**4/27**. The metal-free form of **4/28** binds citrate with a stability constant of 3.9×10^6 M^{-1} in 15% water/methanol (0.1 mM HEPES,



Scheme 15.

0.1 mM NaCl, pH 7.4) whereas a stability constant of at least 8.3×10^6 M^{-1} was estimated for the complex between citrate and **4/28** · Cu^{2+} [192]. Moreover, the fluorescence of the phenanthroline moiety that is quenched in the copper complex of **4/28** returns upon citrate binding thus allowing an optical sensing of the anion. The cooperative effect of copper indicates a participation of the metal center in citrate complexation and detailed studies involving model compounds showed that the return in fluorescence is indeed due to metal anion interaction and not to a displacement of the metal from the host. Also in this case, the sensing properties of **4/28** allowed the quantification of the citrate content of various beverages [192].

A combinatorial approach to ATP sensing reported by the Anslyn group involved resin bound host **4/29** consisting of a hexasubstituted 1,3,5-triethylbenzene core, two guanidinium groups for anion binding, two identical peptide side chains for selectivity and two fluorophores F^1 and F^2 for optical sensing [193]. **4/29** is based on the structurally much simpler structure **4/30** which has been shown to interact with ATP with a binding constant of 3.5×10^2 M^{-1} in water. Screening of a library containing 4913 members differing in the peptide sequence along the side chains furnished a host that binds ATP ca. 10 times stronger than the core structure **4/30** alone ($K_a = 3.4 \times 10^3$ M^{-1} in 200 mM HEPES, pH 7.4). More important is, however, that the Ser–Tyr–Ser sequence in the peptide chains of this host induces selectivity for ATP over GTP and AMP. In a slightly different approach, a selection of 12 beads of the host library (before attachment of the fluorophores) was chosen to construct a chip-based array for the optical differentiation between structurally similar anions such



Scheme 16.

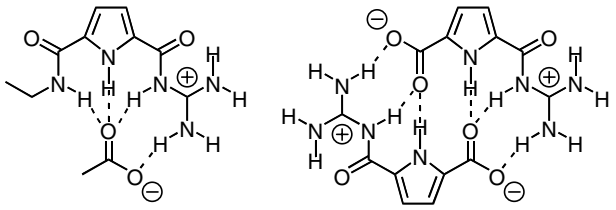


Figure 13. Schematic representation of the binding modes in the acetate complex of **4/32** (left) and in the dimer of **4/39** (right).

as AMP, GTP, and ATP using an indicator displacement assay [194].

Structurally somewhat related to **4/29** is the tweezer-type receptor **4/31** that was developed by Kilburn and co-workers for the sequence selective recognition of short peptides. Complex formation was anticipated to involve a salt bridge between the C-terminal carboxylate group of a peptidic guest and the guanidinium moiety of the host. To test this idea, **4/31** was screened with a 1000-member, biased library of tripeptides containing mainly hydrophobic amino acids that was attached to a TentaGel resin via the amino terminus. **4/31** was found to bind to ca. 3% of the library members and showed 95% selectivity for Val at the carboxy terminus of the tripeptides and 40% selectivity for Glu(O*t*Bu) at the amino terminus [195]. A stability constant of 4×10^5 M^{-1} was determined for the complex between **4/31** and Z-Glu(O*t*Bu)–Ser(O*t*Bu)-Val-OH in 16.7% DMSO/water (1 mM sodium borate buffer, pH 9.2) by means of microcalorimetry. No other thermodynamic parameters are reported. The reverse experiment involved a resin bound library of symmetrical tweezer-type receptors with identical peptide fragments appended to both sides of the guanidinium scaffold. Screening with a dye-labeled tripeptide as substrate allowed the identification of a host that was shown to bind this tripeptide in 15% DMSO/water with appreciable selectivity over the corresponding enantiomer [196].

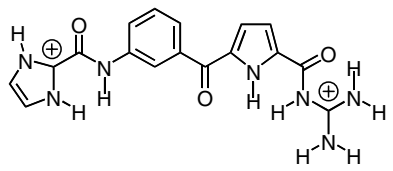
The 2-(guanidiniocarbonyl)pyrrole group was introduced by Schmuck as binding site for carboxylates to combine the anion binding properties of guanidinium cations with that of pyrrole rings [197]. The potential of this strategy was first demonstrated using the simple 2-(guanidiniocarbonyl)pyrrole derivative **4/32** [198]. This compound was shown to interact with various carboxylates in highly competitive solvent mixtures such as 40% water/d_6-DMSO. Strongest binding was observed for acetate with the stability constant K_a of the complex amounting to 2700 M^{-1}, much larger than the stability constant of the corresponding acetate complex of *N*-acetyl guanidinium (50 M^{-1}) in the same solvent. This result was ascribed to cooperative effects of the pyrrole and amide NH groups of the host in carboxylate recognition, an assumption that is consistent with the complexation induced shifts observed in the ^{1}H NMR spectrum of **4/32** upon complex formation and with detailed molecular modeling studies. Thus, complex structure in solution can be represented schematically as shown in Figure 13. In the crystal structure of 2-(guanidiniocarbonyl)pyrrole acetate, no discrete host/guest entities were observed but an extended two-dimensional network in which the acetate carboxylate group simultaneously interacts with the guanidinium moiety of one molecule of **4/32** and with the pyrrole NH of another [199]. The affinity of **4/32** toward *N*-acetylated amino acid carboxylates is somewhat reduced with stability constants ranging between 360 M^{-1} for the Ac-L-Lys-O$^-$ complex and 1700 M^{-1} for the one of Ac-L-Phe-O$^-$, which is most probably due to steric effects of the amino acid side chain that prevent the carboxylate group from adopting an optimal coplanar arrangement with the receptor [198]. The high binding constant of the phenylalanine complex was ascribed to stabilizing cation-π interactions between the guest's aromatic side chain and the receptor's pyrrole subunit.

Further work involved a systematic investigation of the influence of substituents in the 5-position of the pyrrole ring on anion affinity and selectivity [200]. To this end, hosts **4/33**–**4/36** were synthesized and their interaction with, for example, Ac-l-Ala-O$^-$ characterized. A selection of stability constants is summarized in Table 7 that demonstrate the cooperative contribution of the pyrrole substituents to binding. Individual energetic contributions of the acylguanidinium moiety and of the NH groups to complex stability were estimated. Moreover, the chiral host **4/36** also allowed enantioselective amino acid recognition.

In a combinatorial approach, a library of hosts of the general structure **4/37** were prepared on solid support and binding affinity was screened toward the *N*-protected tetrapeptide L-Val–L-Val–L-Ile–L-Ala-O$^-$, the C-terminal sequence of the amyloid-β-peptide responsible for the formation of protein plaques within the brains of patients suffering from Alzheimer's disease [201, 202]. A first investigation involved a library of 125 receptors of which ca. 7% showed selective binding to the target substrate [201]. After selection of the most efficient receptors, an on-bead binding assay was used to determine relative association constants of L-Val–L-Val–L-Ile–L-Ala-O$^-$ binding in methanol in the presence of formate as counterion. Anion affinity of the best receptors amounted to 9300 M^{-1} in L-Val–L-Val–L-Val–Gua recognition and to 9800 M^{-1} in that of L-Phe–L-Val–L-Val–Gua. Recently, a larger library containing 512 potential hosts was synthesized and screened in water (5 μM bis-Tris, pH 6) [202]. In this solvent,

Table 7. Stability constants in M^{-1} of the Ac-l–Ala-O$^-$ complexes of hosts **4/32**–**4/36** as picrate salts in 40% water/d_6-DMSO at T = 298 K

Host	K_a
N-Acetyl guanidinium picrate	50
4/33	130
4/32	770
4/34	940
4/36	1610



4/41

Scheme 17.

binding affinity of the most potent receptors toward the target is less than half of that observed in methanol. More important is, however, that the best receptors in water are structurally quite different from the ones in methanol, which underlines the important influence of the solvent on supramolecular complex formation both in terms of binding affinity and selectivity. Characterization of the binding properties of some receptors in solution essentially confirmed the results of the on-bead assay and molecular modeling studies revealed structural information on the complexes formed.

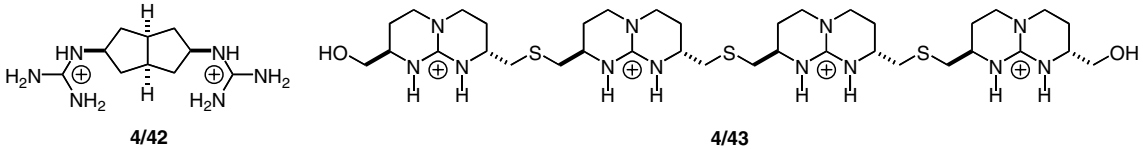
An alternative approach to strengthen interactions between 2-(guanidiniocarbonyl)pyrroles and carboxylates involved introduction of substituents into the guanidinium group. Thus, *N*′-alkylated guanidinium derivatives such as **4/38** were synthesized and shown to possess improved affinity toward carboxylates with a remarkable stability constant of, for example, the Ac-L-Val-O^- complex of 1750 M^{-1} in water (3 mM bis-Tris, pH 6.1) [203].

The zwitterionic compound **4/39** and its water soluble analog **4/40** form remarkable stable dimers in solution whose structure is schematically depicted in Figure 13 [204, 205]. In DMSO, the stability constants of the dimers of **4/39** and **4/40** range between an estimated 10^{10}–10^{12} M^{-1} while for the dimer of **4/40** in water, a surprisingly high association constant of 170 M^{-1} could still be detected demonstrating the efficient complementary interactions in the aggregate. An isomer of **4/40** with the carboxylate group in the 4-position of the pyrrole ring self-assembles in DMSO in the form of linear oligomers, a process that was shown to be endothermic and thus driven by the entropically favorable release of ordered solvent molecules into the bulk solution [206].

The most recent development in the Schmuck group involved host **4/41** that was designed to bind dipeptides in aqueous solution by a three-point interaction, namely two hydrogen bonds between hydrogen bond donors and acceptors in **4/41** and complementary ones in *N*-acetylated dipeptides in addition to the carboxylate guanidinium binding motif [207]. In accordance with this expectation, high binding constants ranging from 1.6×10^4 to 5.4×10^4 M^{-1} were determined for the complexes of dipeptides with **4/41** in 10% d_6-DMSO/D_2O (0.5 mM bis-Tris, pH 5.5). The dipeptide Val–Val is bound significantly more strongly than Ala–Ala or, even more pronounced, Gly–Gly which indicates that complex stability correlates with the flexibility and hydrophobicity of the substrate.

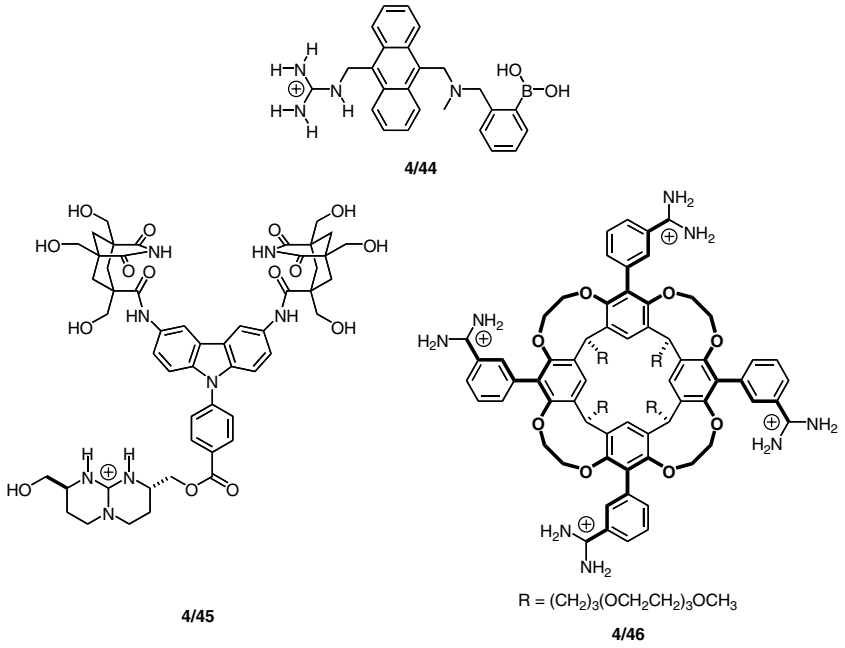
A central research theme in the Hamilton group is the development of synthetic receptors for the selective recognition of secondary structures of proteins or of protein surfaces [15]. An early attempt involved bis(guanidinium) receptor **4/42**, which binds glutarate with an association constant of 3900 M^{-1} in 10% water/methanol [208]. Investigation of the interaction between **4/42** and different 16-mer α-helical peptides containing two aspartate residues separated by a variable number of other amino acids revealed a preference for the binding of helices with aspartates in the i and the $i+3$ position [208, 209]. Circular dichroism spectroscopy indicated that the addition of **4/42** to such a peptide in 15% water/methanol at 25 °C causes a 23% enhancement of helicity while the increase of helicity in peptides with aspartate residues located farther apart is significantly lower. In buffered aqueous solution, similar but weaker binding and helix induction were observed. These results suggest that the two guanidinium moieties of **4/42** can simultaneously interact with appropriately spaced carboxylate groups on the surface of a helical peptide.

In a similar approach, the linear tetraguanidinium host **4/43** was synthesized and its interactions with aspartate containing oligopeptides tested [210, 211]. Molecular modeling studies indicated that **4/43** is able to wrap around an ideal right-handed α-helix of a peptide containing aspartate residues at positions $i + 3n$ ($n = 0$, 1, 2, 3) with an almost perfect match of each guanidinium moiety with the corresponding aspartate carboxylate groups. Consistent with these calculations, an increase of the helicity of such a peptide from 21 to 45% was observed in 10% water/methanol in the presence of 2 equivalents of **4/43** with the association constant of the aggregate amounting to 3.4×10^5 M^{-1}. Interestingly, spermine that has been shown to enhance the helical content of a peptide containing glutamate residues in an i, $i + 4$, $i + 7$, $i + 11$ arrangement from 19 to 38% helicity [25] had no effect on the helicity of the target peptide used in these studies which demonstrates the importance of the geometrical fit between the binding sites in the

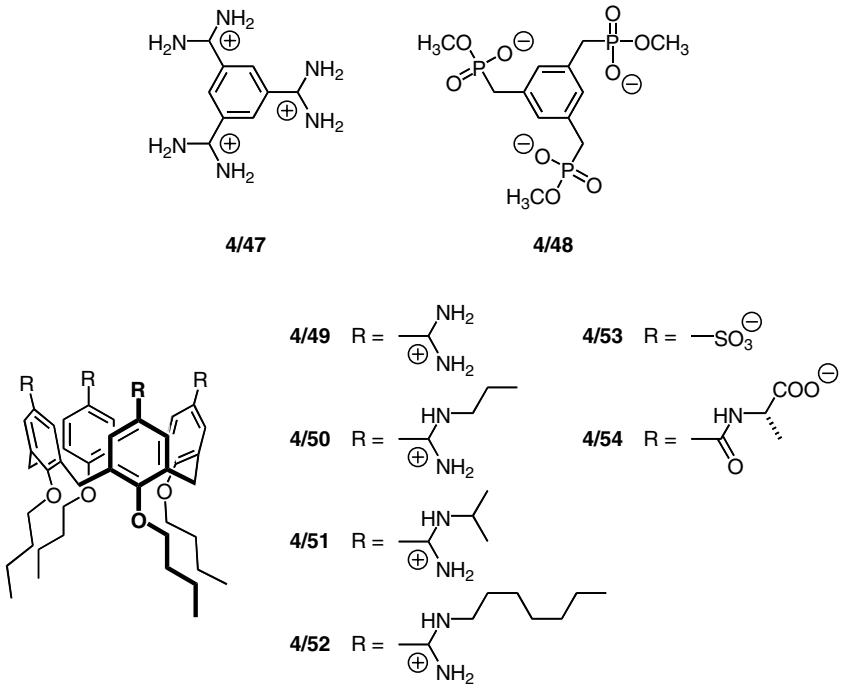


4/42 **4/43**

Scheme 18.



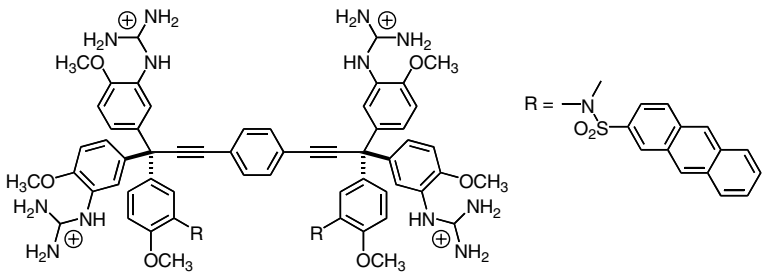
Scheme 19.



Scheme 20.

host and in the substrate. In water, the peptide is conformationally much more flexible and, although interactions seem to occur, no significant induction of a helical structure was found in the presence of **4/43** [210, 211].

Design of guanidinium and amidinium based synthetic receptors for anionic biomolecules also targeted monosaccharides and nucleotides. For monosaccharide sensing in aqueous solution, for example, receptor **4/44** was developed [212]. This compound takes advantage of the ability of the boronic acid functionality to interact reversibly with diols in aqueous media and uses the guanidinium moiety to introduce affinity for carboxylates. Consequently, sugar carboxylates such as D-gluconate ($K_a = 1.5 \times 10^3\ M^{-1}$) and, even more pronounced, D-glucarate ($K_a = 5.1 \times 10^3\ M^{-1}$) are bound by **4/44** in 50% methanol/water (0.1 M HEPES, pH 7.4) significantly more strongly than neutral monosaccharides, for example D-glucose ($K_a = 62\ M^{-1}$). The high binding affinity for glucarate is explained by simultaneous interaction of both carboxylate groups of the substrate with the guanid-



4/55

Scheme 21.

inium group. Host **4/44** has the additional advantage that complex formation can be detected optically by an increase in fluorescence intensity.

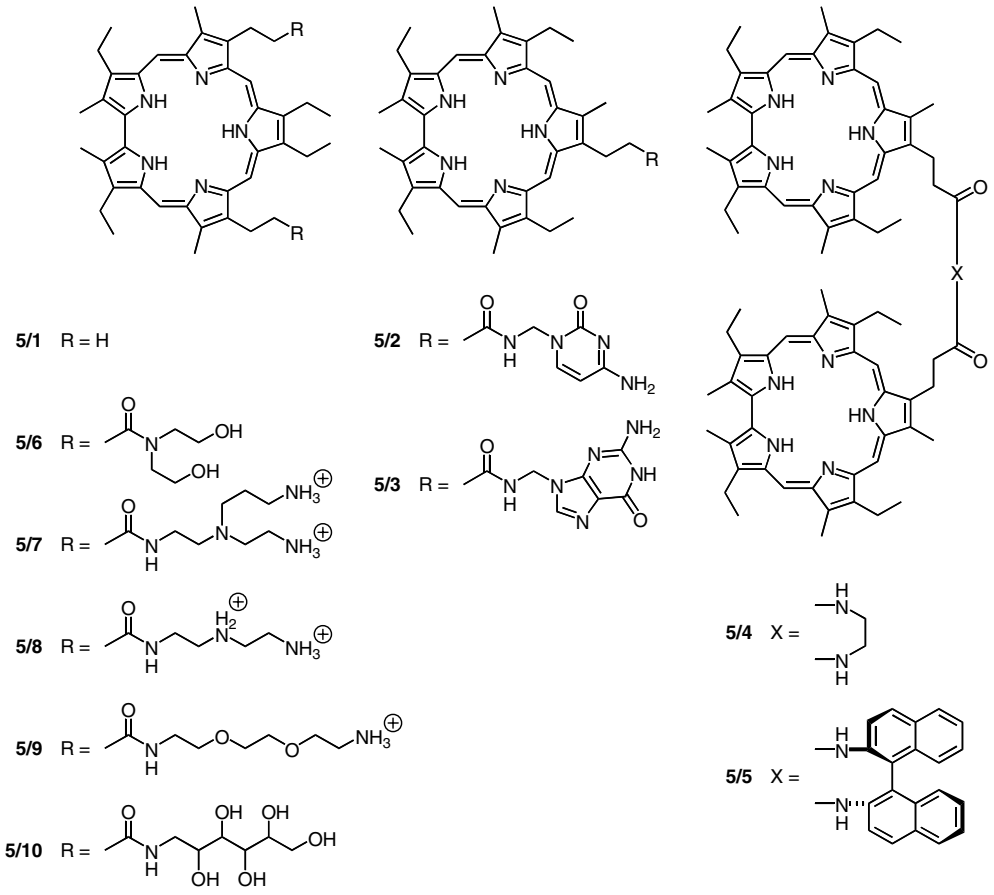
Receptors for nucleotides containing guanidinium or amidinium groups were developed in the groups of Rebek and Diederich. Rebek's host **4/45** arranges a guanidinium moiety and two Kemp's triacid imides around a 3,5-diaminocarbazole platform [213]. In water (10 °C, pH 6.0, 51 mM NaCl) **4/45** binds 2′3′-cAMP and 3′,5′-cAMP with association constants of, respectively, 660 and 600 M^{-1}. The slight preference for 2′3′-cAMP vanishes, however, upon increasing the ionic strength of the solution. Adenosine or 9-ethyladenine are bound significantly less strongly by the host clearly illustrating the cooperative effect of the guanidinium group in nucleotide recognition. Binding thus involves hydrophobic interactions, Watson–Crick and Hoogsteen hydrogen bonding, and electrostatic phosphate-guanidinium interactions with the latter contributing on average 2.5 kJ mol^{-1} (51 mM ionic strength) or 1.3 kJ mol^{-1} (501 mM ionic strength) to complex stability. These results are in good agreement with values reported by Schneider for the strength of exposed ionic interactions between ammonium ions and various oxoanions in water [83, 84]. Host **4/45** and a derivative containing two carbazole moieties linked by a bicyclic guanidinium were also used for the extraction of nucleotides and dinucleotides from water into dichloromethane and for nucleotide transport across liquid membranes [214–216].

As demonstrated by Diederich and co-workers, a completely different host architecture consisting of four amidinium groups arranged around the cavity of a resorcin[4]arene can also be used for nucleotide recognition [217]. The corresponding cavitand **4/46** forms 1:1 complexes with cAMP, AMP, ADP, and ATP in water (2.5 mM Tris, pH 8.3) with stability constants ranging from 1.4×10^3 M^{-1} (for cAMP) to 6.6×10^5 M^{-1} (for ATP). The increase in binding strength with increasing charge of the substrate indicates a large contribution of electrostatic interactions to binding. Additionally, a selectivity for AMP over nucleotide monophosphates containing other bases was also found, which was explained, on the basis of detailed ^{1}H NMR studies, by an inclusion of the nucleobase part of AMP into the bowl-shaped cavity of **4/46**. The major driving force of complex formation is believed to come from ion pairing and hydrogen bonding interaction between the substrate and the host. Apolar interactions and hydrophobic desolvation most probably do not make a large contribution to complex stability.

The group around Schrader used the amidinium phosphonate interaction for the assembly of ball-shaped molecular complexes in water. The trisamidinium derivative **4/47** and the trisphosphonate **4/48**, for example, form a defined 1:1 complex in protic solvents with an association constant of 1.1×10^6 M^{-1} in methanol and 1.0×10^3 M^{-1} in water [218–220]. The cavity between the two components of this aggregate is too small, however, to accommodate a guest molecule. Larger capsules that use a similar type of interaction for subunit assembly were developed by Reinhoudt and co-workers [221, 222]. Their approach involved tetraamidinium calix[4]arenes **4/49**–**4/52** and tetrasulfonato calix[4]arene **4/53** which were shown to form capsule-like assemblies in methanol and water/methanol mixtures with a cavity large enough for the inclusion of acetylcholine, tetramethylammonium or *N*-methylquinuclidinium cations [221]. Information on the interactions stabilizing these complexes came from a crystal structure of the complex between **4/50** and **4/53** [222]. Isothermal titration calorimetry revealed that complex formation in 40% water/methanol is an endothermic process and driven by the entropic favorable release of ordered solvent molecules into the bulk solvent (Table 8) [221]. Interestingly, assembly of **4/50** and the tetracarboxylate **4/54** leads to a water soluble capsule with a stability constant of 3.3×10^4 M^{-1}, a process that is not only favored by entropy but by enthalpy as well. This capsule was shown to include an *N*-methylquinuclidinium ion [222].

Table 8. Thermodynamic parameters in kJ mol^{-1} for the complexes of tetraamidinium calix[4]arenes **4/49**–**4/52** and tetrasulfonato calix[4]arene **4/53** in 40% water/methanol in the presence of 0.01 M Bu_4NClO_4 at $T = 298$ K [221]

	K_a [M^{-1}]	ΔH	$T\Delta S$
4/49	1.9×10^6	33.3	68.8
4/50	8.5×10^6	14.1	53.6
4/51	6.4×10^6	13.7	52.4
4/52	1.1×10^6	17.9	52.4



Scheme 22.

A chemosensor for dicarboxylates on the basis of tetraguanidinium pinwheel receptor **4/55** was developed in the group of Glass [223]. Binding of a suitable dicarboxylate to **4/55** was expected to restrict the rotation around the trityl groups which would preorganize the receptor for a stronger (cooperative) second binding event and simultaneously bring the fluorophores into close proximity thus causing a quenching of their fluorescence. A decrease in fluorescence emission of **4/55** was indeed observed in water (10 mM Tris, pH 7.5) in the presence of various dicarboxylates that allowed a quantitative estimation of binding affinity and cooperativity. These measurements showed that the stabilities of the dicarboxylate complexes of **4/45** decrease in the order oxalate > malonate > succinate > glutarate making **4/45** a more selective host for rigid dicarboxylates than for flexible ones, a property that was explained on the basis of entropic considerations. Interestingly, even high background concentrations of monocarboxylates do not significantly interfere in dicarboxylate binding of **4/45**.

A number of guanidinium or amidinium based receptors have been described that are able to extract anions from an aqueous into an organic phase [224–227]. These investigations are not summarized in the context of this review as binding takes place in a lipophilic environment.

5. Hosts containing pyrrole groups

In 1990 the Sessler group discovered the anion binding ability of sapphyrins, expanded porphyrins containing five pyrrole subunits [228–230]. First evidence came from an X-ray structural analysis of diprotonated sapphyrin **5/1** with what were expected to be two hexafluorophosphate counterions. Surprisingly, the crystal structure revealed only one PF_6^- per sapphyrin moiety, while the second anion turned out to be fluoride residing inside the centers of each sapphyrin ring within hydrogen bonding distance to the five pyrrole nitrogens. The almost perfect size complementarity of F^- and **5/1**, the radius of the sapphyrin cavity amounts to 2.5 Å, allowed for a coplanar arrangement of the anion and the macrocycle [231]. Anion coordination to NH groups was also detected in the crystal structures of, for example, chloride [232, 233], carboxylate [234, 235], and phosphate [236, 237] salts of monoprotonated or diprotonated sapphyrins, but in these complexes, the anions are located above the plane of the macrocycle because of their larger size with respect to fluoride. These findings immediately prompted the question of whether similar interactions would also occur in solution and much effort has therefore been directed toward investigations of the anion binding capabilities of sapphyrins in organic and aqueous solution.

Table 9. Stability constants in M^{-1} of sapphyrin complexes with various anions.

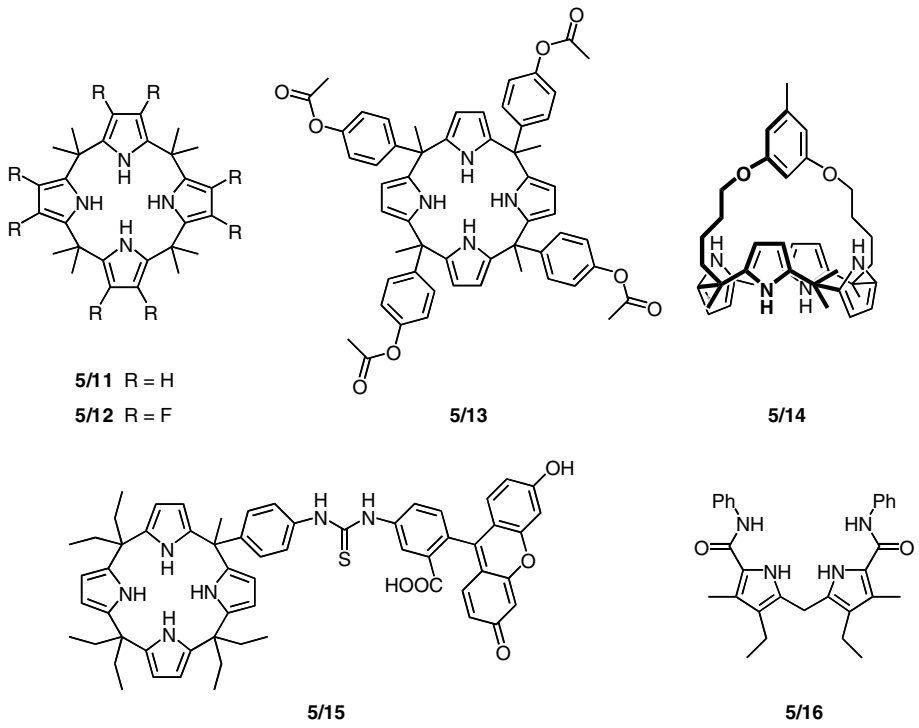
Sapphyrin	Solvent	Anion	K_a
5/1	CH_2Cl_2 [233]	F^-	$>10^8$
		Cl^-	1.8×10^7
		Br^-	1.5×10^6
5/1	CH_3OH [233]	F^-	9.6×10^4
		Cl^-	1×10^2
		Br^-	$<1 \times 10^2$
5/2	CH_3OH [239, 240]	2′-GMP$^-$	2.2×10^4
		5′-GMP$^-$	8.1×10^3
		5′-AMP$^-$	1.7×10^3
		5′-CMP$^-$	8.8×10^2
5/4	CH_3OH [234, 241]	Nitroterephthalate	9.1×10^3
		Malonate	4.5×10^2
		Oxalate	2.6×10^2
5/6	H_2O (10 mM aqueous Tris buffer, pH 6.1) [236]	Phenyl phosphonate	310
		Diphenyl phosphate	280
		Phenyl phosphate	300

One method employed extensively in this context involved transport experiments using a standard Pressman-type U-tube membrane model, in which the rate of anion transport is determined between two aqueous phases separated from each other by an organic solvent such as dichloromethane. It was found that the presence of **5/1** in the organic layer accelerates the transport of fluoride with respect to the background rate by two orders of magnitude at neutral pH [238]. At this pH, **5/1** is monoprotonated, and the interaction between the sapphyrin and the anion is expected to be similar to that found in the crystal structure. Chloride is transported by **5/1** even more efficiently than fluoride while significantly smaller transport rates were observed when porphyrins were used as anion carriers instead of **5/1** [238]. The latter result is consistent with the fact that the sapphyrin is positively charged under the experimental conditions allowing for electrostatic interactions with the anions whereas porphyrins are neutral. Nucleotide transport was tested by using cytosine and guanosine functionalized sapphyrins, for example **5/2** and **5/3** [239, 240]. These systems enhance the transport of substrates that form complementary Watson-Crick base pairs in comparison to mismatched nucleotides by a factor of ca. 10. Interestingly, transport rate is also sensitive to the position of the phosphate group on the ribose moiety as 2′, 3′, and 5′-cytosine monophosphate are transported by **5/3** with different rates. Anion transport of various dicarboxylates was studied with, for example, the bis(sapphyrin) **5/4** [234, 241].

We summarize these studies although they involve anion binding in non-polar media because they demonstrate that sapphyrins are not only structurally related to prodigiosins, sapphyrins can formally be regarded as conformationally restricted cyclic prodigiosins with an additional dipyrrolylmethene subunit, they also possess similar anion transport properties. First transport studies using sapphyrins were carried out before the biological role of prodigiosins became clear but in light of the close relationship between prodigiosins and sapphyrins in terms of anion transport properties that has only emerged recently, these studies have gained considerable significance prompting speculations about potential pharmaceutical applications of sapphyrins [230].

Several methods were used to quantitatively estimate anion affinity of sapphyrins in solution. Stability of the halide complexes of diprotonated **5/1** in CH_2Cl_2 was determined, for example, from effects of anion binding on the fluorescence lifetime of this host [233]. In this solvent, the stability of the fluoride complex turned out to be too high to be measured accurately. Visible and NMR spectroscopy (^{31}P for phosphate binding and ^1H for carboxylate binding) provided other means of monitoring complex formation [236, 239, 240]. These studies showed that even in competitive solvents such as methanol, highly stable complexes are formed (Table 9). Especially noteworthy is the observed selectivity of fluoride over chloride or bromide of three orders of magnitude that is consistent with the in-plane hydrogen bonding motif found in the crystal structure of the fluoride complex. It also turned out that protonated sapphyrins generally bind phosphates more efficiently than chloride, bromide, or carboxylates, but still an order of magnitude less efficiently than fluoride. Chiral sapphyrin derivatives such as **5/5** are able to bind dicarboxylates enantioselectively in 5% methanol/dichloromethane [234, 241].

Hydrophilic sapphyrins such as **5/6** were prepared for investigations on anion binding in water [236]. These compounds are monoprotonated at pH 7, pK_{a1} and pK_{a2} of the diprotonated form of **5/6**, for example, amount to, respectively, 4.8 and 8.8 [236], which should in principle enable them to interact with all the anions also bound in less polar solvents. Unfortunately, hydrophilic sapphyrins are also highly aggregated in water making quantitative investigations on anion binding difficult. Three distinct aggregation states were detected in polar protic media that could clearly be distinguished



Scheme 23.

by UV–vis spectroscopy [236]. In the absence of a surfactant such as sodium dodecyl sulfate (SDS) or of phosphate anions, a broad band with a maximum around 400 nm is visible in the spectrum of, for example, **5/6** that corresponds to the aggregated state. The absorbance maxima of these aggregates shift to red upon dilution, a characteristic feature of H-type aggregation. Adding SDS to the solution causes the appearance of first, a band at $\lambda_{max} \approx 420$ nm that was assigned to the absorbance of a sapphyrin dimer, and successively at higher concentrations of SDS, of a narrow band with a maximum at ca. 450 nm corresponding to the monomer, the only sapphyrin state in aqueous solution that is fluorescent. Addition of phosphate anions to the aggregated state of **5/6** caused similar effects on the UV–vis spectrum allowing the calculation of apparent association constants for the interactions of phosphate with the dimeric aggregate of the sapphyrin (Table 9). Only recently these investigations were extended to include the water soluble sapphyrin derivatives **5/7**–**5/10** and also involved a quantitative assignment of phosphate complex stability of the sapphyrin monomers in water [242]. Depending on the sapphyrin used, stability constants ranging between 6 and 19 M^{-1} were determined in buffered solution (25 mM PIPES, pH 7.0) in the presence of 150 mM NaCl. Although these constants may seem small, the strong increase of fluorescence emission that is observed upon complex formation allows even small changes in phosphate concentration to be detected visually. Moreover, chloride does not interfere in phosphate recognition even at high concentrations, which is advantageous for the use of hydrophilic sapphyrins as selective anion sensors in water [242]. In line with this idea, methods for the fluorimetric determination of fluoride in aqueous solution [243, 244] and for the optical detection of the environmentally important pertechnate anion employing protonated sapphyrins have recently been developed [245]. In the latter case, binding constants for the interaction of pertechnate and phosphate with the aggregated state of

Table 10. Stability constants in M^{-1} and thermodynamic parameters in kJ mol^{-1} of the complexes between calix[4]pyrrole **5/11** and various anions

Method	Solvent		F^-	Cl^-	Br^-	$H_2PO_4^-$
NMR titration in the presence of Bu_4N^+ counterions	CD_2Cl_2 [252]	K_a	17,170	350	10	97
	CD_3CN [253]	K_a	> 10,000	5000		1300
	d_6-DMSO [254]	K_a	1060	1025		
microcalorimetry in the presence of $\{K\cdot[2.2.2]\}^+$ counterions	CH_3CN (< 10 ppm H_2O) [255]	K_a	153,000	185,000		16,800
		ΔH	−34.5	−44.4		−45.8
		$T\Delta S$	−4.4	−13.8		−21.2

5/6 could be determined because under the conditions of the measurements and the concentration regime used (2.5% methanol/water, pH 7.0) deaggregation was not observed. These stability constants turned out to be quite high, $3900 \pm 300\ M^{-1}$ for pertechnate and $23{,}000 \pm 3000\ M^{-1}$ for phosphate.

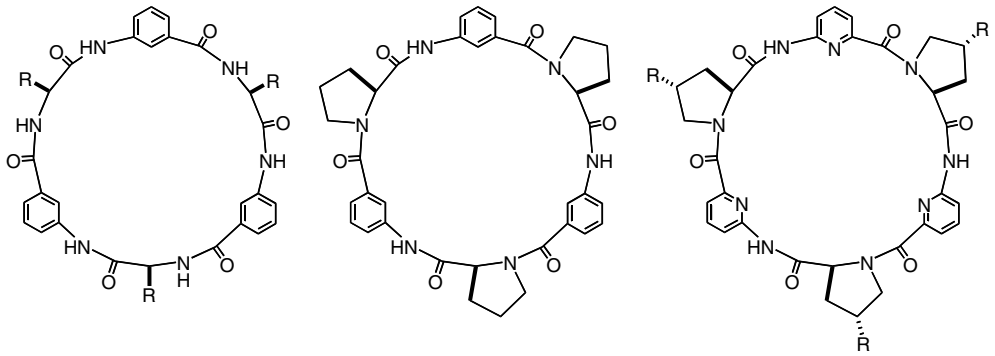
Interaction of Sapphyrins with phosphate in water is not restricted to inorganic phosphate or simple phosphate esters, also DNA can serve as a possible target. First evidence for strong interactions between sapphyrins and DNA came from the observation that addition of **5/6** to double stranded DNA at pH 7 caused precipitation of green fibers [237, 246, 247]. The formation of these fibers were ascribed to charge neutralization arising from the interaction between the DNA phosphodiesters and the protonated sapphyrin. These interactions, termed 'phosphate chelation', were proposed to involve specific contacts between phosphate oxygens and sapphyrin NH groups similar to those found in some crystal structures. They thus differ from other mechanisms of DNA recognition, namely groove binding, intercalation, or simple electrostatic interactions, an assumption that was confirmed by a number of independent experiments [237, 246, 247]. Interestingly, sapphyrins were also found to catalyze the photocleavage of DNA, a property that could be suppressed, for example, by addition of SDS, a reagent known to inhibit sapphyrin–phosphate interactions [248]. Appending nucleotide conjugates to the sapphyrin core even allowed site-specific photocleavage of complementary DNA strands, which again suggests a potential value of sapphyrins in medicinal application [249].

Larger sapphyrin derivatives and heterosapphyrins containing one or more furane, thiophene, or selenophene subunits have been prepared possess significantly reduced anion affinity with respect to sapphyrin itself [230]. Strong anion binding was observed, however, for another class of pyrrole containing macrocycles, so-called calixpyrroles of which calix[4]pyrrole **5/11** is the best studied derivative [250, 251]. This compound, whose name derives from its resemblance, not only structurally but also in terms of conformational flexibility, to calixarenes, contains four pyrrole subunits linked between the 2 and 5 positions via disubstituted methylene groups. In solution, **5/11** preferentially adopts a conformation with the pyrrole units alternately pointing upward and downward (1,3 alternate conformation) while in the complex with, for example, chloride a *cone* conformation is observed with all NH groups pointing into one direction thus allowing for four simultaneous hydrogen-bonding interactions with the anion [252].

Solution studies on the anion affinity of **5/11** mainly involved halide and phosphate complexation in organic solvents such as dichloromethane or acetonitrile. NMR titrations revealed, for example, a high selectivity for fluoride over chloride in CD_2Cl_2 and CD_3CN [252, 253]. This selectivity disappeared completely, however, upon changing the solvent to DMSO [254], and even the observed selectivity in acetonitrile was questioned by Schmidtchen who determined complex stability by means of microcalorimetry and found no significant difference between fluoride and chloride complex stability in the presence of cryptand[2.2.2] complexed potassium as counterion (Table 10) [255]. Complex formation is exothermic for both anions with an unfavorable contribution of entropy. Subsequent theoretical studies suggested that the discrepancy observed in the NMR spectroscopic and microcalorimetric investigations could be attributable to differences in the experimental conditions, namely in the amount of water in the solvent and the nature of the counterion [256]. Thus, while in dry acetonitrile binding of **5/11** to fluoride is preferred over chloride, this preference is reduced to less than 1 Kcal/mol when trihydrated tetrabutylammonium cations are considered as cosolute.

A number of calix[4]pyrrole derivatives have been synthesized some of which possess remarkable anion selectivity or affinity, for example **5/12** containing fluorinated pyrrole subunits [253], **5/13** in which four aromatic substituents line a deep cavity [257], **5/14** whose conformational flexibility is reduced by a flexible strap bridging two opposite pyrrole rings [258, 259], or **5/15** in which a fluorescent label signals complex formation [260]. The dihydrogenphosphate complex of fluorinated calix[4]pyrrole **5/12** is seven times more stable in DMSO than the one of **5/11** [253]. **5/13** exclusively binds fluoride in DMSO but does not interact with chloride, bromide, iodide, dihydrogenphosphate, or hydrogen sulfate [257], and the stability of the chloride complex of **5/14** amounts to impressive $4.3 \times 10^6\ M^{-1}$ in CD_3CN (as determined by microcalorimetry and in the presence of $\{K{\cdot}[2.2.2]\}^+$) [258, 259]. With the exception of **5/15**, however, none of these derivatives have been studied in aqueous solution probably because of too low water solubility. **5/15** binds dihydrogenphosphate and hydrogenpyrophosphate in 4% water/acetonitrile with high association constants of, respectively, $6.8 \times 10^5\ M^{-1}$ and $> 2 \times 10^6\ M^{-1}$ most probably due to two-point interactions of the anions with the calix[4]pyrrole and the thiourea moiety of the receptor [260].

Calix[*n*]pyrroles [261] with $n > 4$ as well as calix[*n*]bipyrroles [262] have been synthesized and shown to possess improved affinities for larger anions such as chloride or bromide, but again binding was only studied in acetonitrile and DMSO or by anion transport experiments. This is also true for most non-cyclic pyrrole containing anion hosts that, not surprisingly, in general possess a lower anion affinity than corresponding cyclic counterparts. Some receptors, however, do tolerate small amounts of water in the solvent mixture used to study complex formation, the most notable example probably being **5/16** whose stability constant of the fluoride complex amounts to $1.1 \times 10^2\ M^{-1}$ and of the dihydrogenphosphate complex to $2.3 \times 10^2\ M^{-1}$ even in 25% water/DMSO [263]. The



6/1 R = CH_3
6/2 R = $CH_2CH_2COOCH(CH_3)_2$

6/3

6/4 R = H
6/5 R = OH

Scheme 24.

non-cyclic receptors developed by Schmuck containing a pyrrole and a guanidinium moiety for anion recognition are described in Chapter 4 'Hosts containing guanidinium or amidinium groups'.

6. Hosts containing amide or urea groups

All of the anion hosts presented in the previous three sections contain at least one positive charge. Although hydrogen bond formation often constitutes a decisive element in the interaction of these hosts with anions even in water, binding is primarily due to strong Coulomb attraction. Electroneutral (non-zwitterionic) anion hosts containing hydrogen bond donors such as the NH groups of amide, urea, or thiourea moieties, on the other hand, have to rely solely on hydrogen-bonding interactions for anion recognition. These compounds do possess several advantages over charged anion hosts one being their electroneutrality that prevents a competition in solution of counterions for the binding sites of the host, and another one the directionality of hydrogen bonds that allows a well defined arrangement of binding sites in the molecular framework of a host to translate into predictable substrate selectivity. One major disadvantage of hydrogen-bonding interactions is, however, their weakness in polar and particularly in protic solvents, which is probably the reason why there are only few examples of neutral anion hosts active in water [7, 264, 265].

In general, the strength in association of an anion and a hydrogen bond donor decreases upon increasing the solvent polarity, often in the order CCl_4 > $CHCl_3$ CH_3CN > DMSO > CH_3OH > H_2O [80, 266]. Although deviations from this sequence are possible, anion affinity of a neutral host in chloroform is usually much reduced in DMSO and completely vanishes in water. Thus, strategies commonly used to prepare hosts with high anion affinity in competitive solvents such as DMSO or acetonitrile, for example increasing the number of binding sites around a macrocyclic cavity, ideally in combination with a well defined converging arrangement of these binding sites, usually failed to generate hosts active in water or at least in aqueous solvent mixtures. This problem has been ascribed to the better solvation of anions and the polar binding sites of a host in more competitive solvents that prevents the gain in enthalpy originating from the interaction of host and guest to compensate for the enthalpic cost required to desolvate the binding partners [80]. If this simple

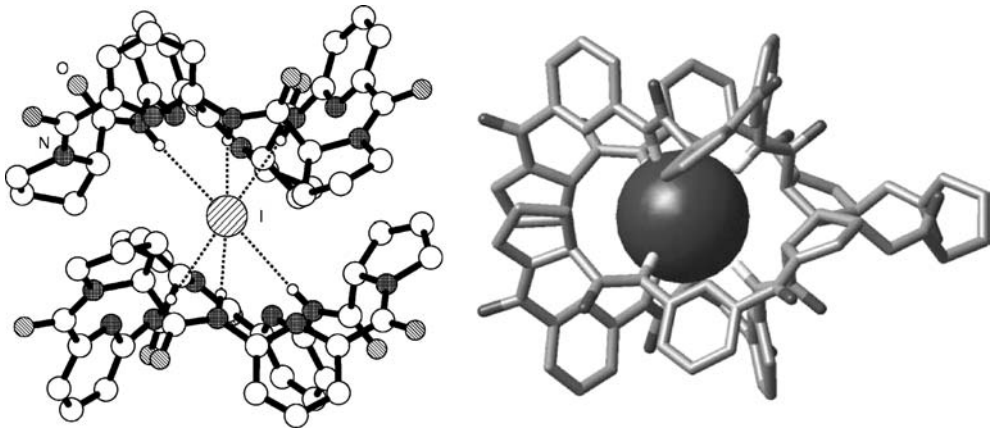


Figure 14. Crystal structure of the iodide complex of **6/4** (left) and calculated structure of the iodide complex of **6/6** (right).

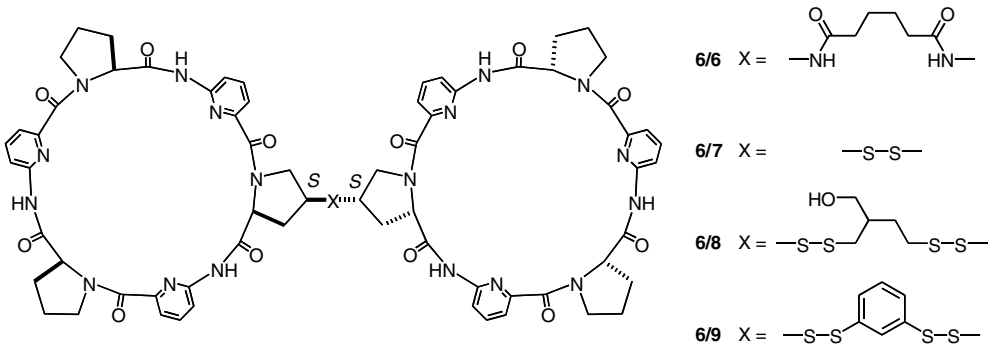
Table 11. Stoichiometries and stabilities of the iodide complexes of hosts **6/3**–**6/9** in various solvents at $T = 298$ K [278]

Host	Solvent[a]	Host/guest	K_1[b]	K_2[b]	K_T[b]
6/3	d_6-DMSO	1:1	< 10		
6/4	d_6-DMSO	1:1	150		
6/4	80% D_2O/CD_3OD	2:1	22	7.38×10^3	1.62×10^5
6/4	50% D_2O/CD_3OD	2:1	30	7.67×10^3	2.30×10^5
6/5	D_2O	1:1	14		
6/5	80% D_2O/CD_3OD	1:1	19		
6/5	50% D_2O/CD_3OD	1:1	26		
6/6	50% D_2O/CD_3OD	1:1	8.90×10^3		

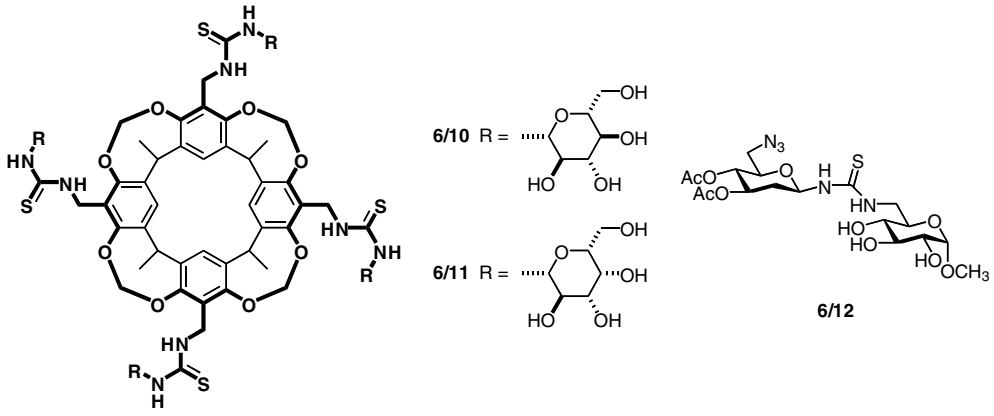
[a]Counterions in d_6-DMSO *n*-butyltrimethylammonium, in D_2O/CD_3OD Na^+, and in H_2O/CH_3CN K^+.
[b]Stability constants K_1 and K_2 in M^{-1}; K_T in M^{-2}.

enthalpic reasoning holds, however, it is unlikely that anion binding in water by neutral receptors can be achieved at all, yet the example of the sulfate-binding-protein (SBP) clearly demonstrates that this is not the case (*vide supra*). SBP very efficiently and selectively recognizes sulfate in water relying on the formation of multiple hydrogen bonds. Competing effects of water molecules in complex formation are minimized by localizing the binding event in a cavity well shielded from the surrounding solvent. In addition, the release of solvent molecules from the solvation sphere of the anion and probably also from the active site of the protein could provide favorably entropic contributions to binding. Design of synthetic receptors for anions in water must therefore not only focus on the optimization of binding enthalpy but should also consider entropic parameters such as desolvation of the binding partners and/or structural factors such as shielding of the substrate from the solvent upon complex formation. However, even with the sophisticated modeling software available today this task is very challenging. Thus, the fact that we discovered a system in our group that, despite being neutral and having to rely on hydrogen bonding for complex formation, binds to halides and sulfate with high affinity in aqueous solvent mixtures has to be considered serendipitous although our approach did contain an element of design.

For some time now, we are interested in the use of cyclic peptides as artificial receptors. These peptides, most of which are hexameric, contain natural amino acids and 3-aminobenzoic acid derivatives in an alternating sequence. The ability of such macrocyclic hosts to strongly interact with anions was first demonstrated by Ishida and co-workers who showed that a hexapeptide composed of alternating L-alanine and 3-aminobenzoic acid subunits **6/1** strongly binds to *p*-nitrophenyl phosphate ($K_a = 1.2 \times 10^6$ M^{-1}) in DMSO [267]. **6/1** turned out to be the most efficient receptor in comparison to larger peptides. Moreover, binding affinity is not significantly affected by the nature of the amino acid side chain. Our investigations revealed how anion (and cation) affinity depends on the conformational behavior of these peptides. Not surprisingly, the optimal conformation for anion complexation is one with the maximum number of NH groups converging toward the center of the peptide ring where anion binding takes place. Cyclic hexapeptides containing non-cyclic α-amino acid subunits such as **6/1** or a derivative with glutamic acid side chains **6/2** are, however, rather flexible in aprotic polar solvents such as DMSO and are therefore not well preorganized for anion binding [268]. The conformational freedom of the peptide ring can be reduced by using proline as the natural amino acid, but although the proline containing hexapeptide **6/3** binds, for instance, iodide in $CDCl_3$ quite strongly [269], there is practically no interaction with this anion in DMSO. Only when preorganization is further improved by replacement of the 3-aminobenzoic acid subunits in **6/3** with 6-aminopicolinic acid to give **6/4** can structures with converging NH groups be stabilized



Scheme 25.



Scheme 26.

[270]. The preference of **6/4** to adopt such conformations is due to the influence of the lone pairs of the aromatic ring nitrogens on the neighboring NH bonds, an orienting effect that is in part also responsible for the stable helical conformations of certain 2,6-diaminopyridine containing linear oligoamides [271–274]. Table 11 clearly shows the effect of the better preorganization of **6/4** on complex formation. In absolute terms, the anion affinity in DMSO resulting from the structural optimization of the peptide is only moderate, however, and it was therefore rather surprising when we detected strong interactions of **6/4** with anions such as sulfate and halides in polar protic solvent mixtures such as, for example, 80% D_2O/CD_3OD [270]. The reason for this unusual behavior turned out to be the special structure of the complexes formed. While 614 binds to anions with an 1:1 stoichiometry in DMSO 2:1 complexes are formed in water in which two cyclopeptide subunits interact with one anion. In these complexes, a completely desolvated anion is bound by six hydrogen bonds in a cavity formed by two almost perfectly shape-complementary peptide rings. To illustrate this binding motif, the crystal structure of the iodide complex of **6/4** is depicted in Figure 14. Other sandwich-type anion complexes are known [126, 275–277], but only **6/4** combines a sufficient water solubility with the ability to completely enclose anions in a cavity between two associating receptor moieties, a feature that somewhat resembles the binding mechanism of SBP. That complexes of different stoichiometries are formed in water and in DMSO can be ascribed to hydrophobic interactions since the release of solvent molecules, not only of those from the solvation sphere of the anion but in particular also of those surrounding the non-polar proline rings of **6/4** that approach van- der- Waals contact in the sandwich complex should be especially favorable in water. In DMSO, proline rings are better solvated and the energetic gain associated with the aggregation of two molecules of **6/4** is presumably significantly reduced. This assumption is supported by the fact that cyclopeptide **6/5** containing hydroxyproline subunits instead of prolines only forms 1:1 complexes with anions in aqueous solution most probably because the desolvation required for an aggregation of 2 molecules of **6/5** is energetically more difficult than that of **6/4** [278].

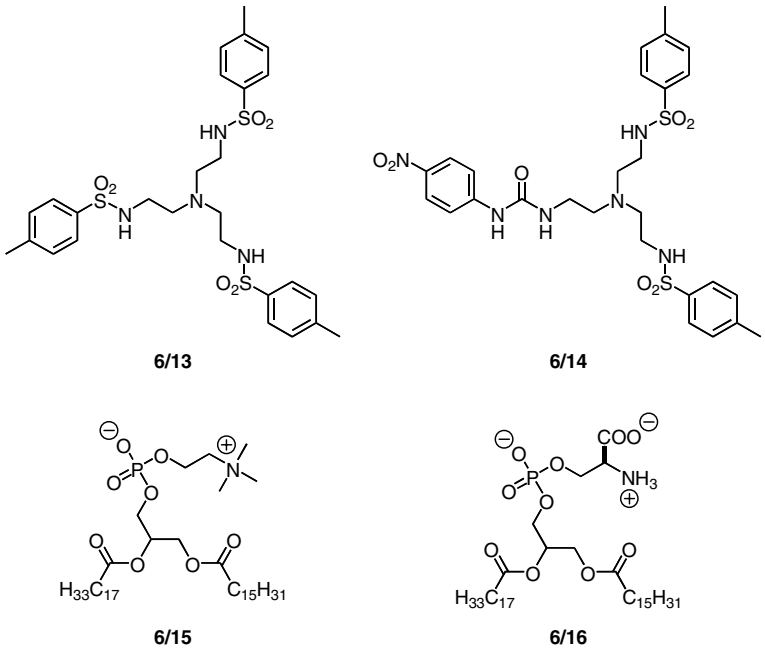
The stability constants of the iodide complexes of cyclopeptide **6/4** and **6/5** are compared in Table 11. **6/5** obviously possesses a weak affinity for iodide in 50% CD_3OD/D_2O. As expected, complex stability decreases upon increasing the water content of the solvent mixture, but even in D_2O iodide complexation is clearly detectable [278]. K_1, the stability constant of the first binding step in the interaction between **6/4** and iodide is similar to the K_a of the iodide complex of **6/5**. The second binding step representing the formation of the 2:1 complex from the 1:1 complex is, however, characterized by a significantly larger stability constant which means that once formed, the 1:1 complexes of **6/4** have a strong tendency to bind a second cyclopeptide molecule making complex formation a cooperative process [278]. Microcalorimetric investigations showed that sulfate binding by **6/4** in 50% water/methanol is an exothermic process accompanied by a favorable entropic term (log $K_T = 6.48 \pm 0.08$; $\Delta H = -19.3 \pm 1.6$ kJ mol^{-1}; $T\Delta S =$ 17.7 kJ mol^{-1}), a result that agrees well the assumption that hydrophobic interactions are important for complex formation [279].

In subsequent work, we converted the 2:1 complexes formed by peptide **6/4** into 1:1 complexes by covalently linking two peptide units together via adipic acid. This linker was chosen because molecular modeling indicated that it should have the appropriate length and flexibility to bridge two cyclopeptide subunits in the iodide

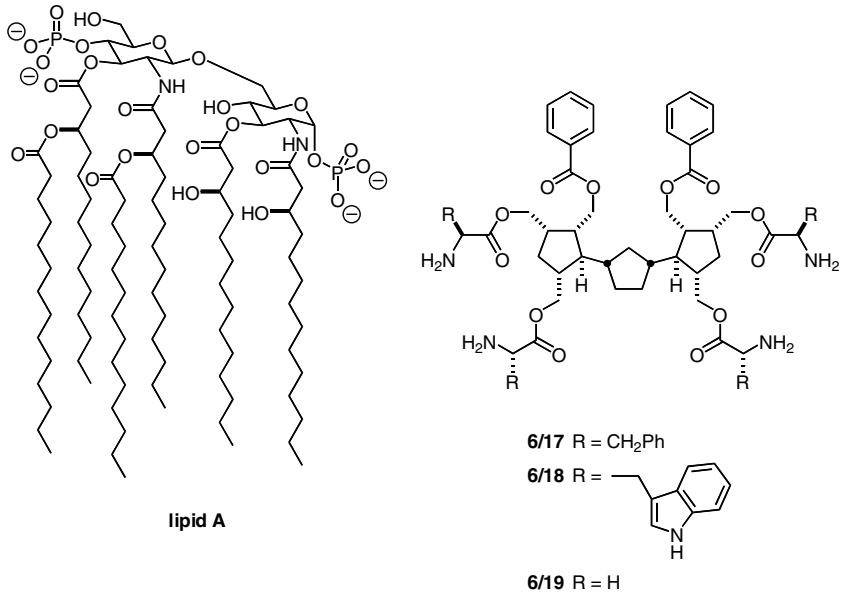
Table 12. Thermodynamic parameters for the iodide and sulfate complexes of **6/6**, **6/8**, and **6/9** in 33% H_2O/CH_3CN at T = 298 K [280][a].

	Iodide			Sulfate		
Host	K_a	ΔH	$T\Delta S$	K_a	ΔH	$T\Delta S$
6/6	3.3×10^3	−4.3	15.7	2.0×10^5	10.7	41.0
6/8	2.9×10^4	−20.7	4.8	5.4×10^6	1.8	40.1
6/9	5.6×10^4	−13.4	13.7	6.7×10^6	3.7	42.7

[a] K in M^{-1}; ΔG, ΔH, and $T\Delta S$ in kJ mol^{-1}; counterions K^+.



Scheme 27.



Scheme 28.

complex of **6/4** without preventing a cooperative action in anion binding. The calculated structure of the iodide complex of the corresponding host **6/6** is depicted in Figure 14. Because of the structural resemblance of this complex to a clam shell containing a pearl we termed **6/6** a 'molecular oyster' [279].

Job plots and ESI mass spectrometry demonstrated that **6/6** indeed forms 1:1 complexes with halides and sulfate, and a ROESY NMR spectrum of the sulfate complex of **6/6** showed that the ditopic host adopts folded conformations in the complex most probably resembling the calculated structure depicted in Figure 14. More important is, however, that **6/6** exhibits a high affinity for sulfate with the K_a of the complex approaching 10^5 M^{-1} in 50% water/methanol. Halides are bound somewhat weaker with the association constants of the complexes increasing in the order $Cl^- < Br^- < I^-$ thus correlating with the size of the anion (Table 11). A microcalorimetric characterization of the complex equilibria revealed that, similar to **6/4**, anion binding of **6/6** is enthalpically as well as entropically driven in 50% H_2O/CH_3OH (Na_2SO_4: log K_a = 4.55 ± 0.23; $\Delta H = -15.0 \pm 0.9$ kJ mol^{-1}; $T\Delta S$ = 11.0 ± 2.2 kJ mol^{-1}; NaI: log $K_a = 3.79 \pm 0.26$;

$\Delta H = -21.6 \pm 1.5$ kJ mol^{-1}; $T\Delta S = 8.5 \pm 2.5$ kJ mol^{-1}) [279].

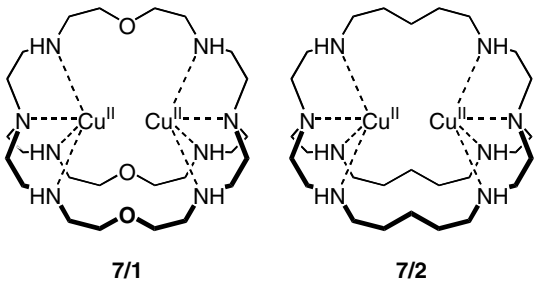
Peptides **6/4** and **6/6** can be regarded as clear proof that anion binding by neutral host molecules in aqueous solution is possible and that by the right choice of host structure, remarkably high anion affinity can be achieved. An advantage of our ditopic hosts is the possibility to control receptor properties by varying the nature of the linker. This feature recently allowed us to improve the binding properties of **6/6** by using a dynamic combinatorial approach [280]. Formation of a dynamic library (DCL) [281–286] containing potential anion receptors involved equilibrating of cyclopeptide disulfide **6/7** with six dithiols in 33% H_2O/CH_3CN. Upon addition of K_2SO_4 as an anionic template to this mixture, an amplification of the two bis(cyclopeptides) **6/8** and **6/9** was observed whose concentration also increased in the DCL when iodide or bromide were used as templates. The effect of bromide was much smaller, however, than that of iodide or sulfate, while chloride and fluoride salts had no effect on DCL composition. Subsequently, hosts **6/8** and **6/9** were synthesized in larger amounts and their binding properties compared with those of the designed receptor **6/6**. These investigations showed that **6/8** and **6/9** bind iodide ca. one order of magnitude stronger than **6/6**, and that this effect mainly stems from a more favorable enthalpic contribution to complex stability (Table 12).

Dynamic combinatorial optimization of the receptor properties of **6/6** has thus furnished anion receptors with, for neutral species, unprecedented binding affinities that most probably would not have been found by design. We therefore expect that this method will prove very valuable to develop anion receptors on the basis of our cyclopeptides whose binding properties even more closely resemble those of natural systems.

Two neutral receptor both containing thiourea and monosaccharide moieties have only recently been described that also bind anions in aqueous solution. The fully acetylated derivatives of the saccharide-thiourea functionalized cavitands **6/10** and **6/11** developed in the Reinhoudt and Verboom groups possess high affinity for anions in acetonitrile [287]. The most stable complexes are formed with Cl^- and affinity decreases in the order $Cl^- > HSO_4^- > NO_3^- > Br^- > I^- > ClO_4^-$, which is consistent with the assumption that hydrogen bond formation between the anions and the thiourea moieties of the hosts are mainly responsible for complex stability. In line with this interpretation is the observation that the nature of the sugar substituent has no large effect on anion selectivity or affinity. Anion affinity is not lost but much reduced in acetonitrile/water 1:1, a solvent mixture that required the use of the better water soluble deacetylated hosts **6/10** and **6/11** for the binding studies. The stability constants K_a of, for example, the chloride complexes amount to 1.5×10^4 M^{-1} for the fully acetylated derivative of **6/10** in acetonitrile and to 250 M^{-1} for **6/10** itself in acetonitrile/water 1:1. Interestingly, anion selectivity is essentially retained upon changing to the more polar solvent mixture. It should also be pointed out the characterization of the anion affinity of these cavitands also involved a successful demonstration of the use of electrospray mass spectrometry for not only a qualitative detection of complex formation between a neutral host and a charged guest but, more importantly, also for a quantitative estimation of complex stability [287].

The simple pseudodisaccharide **6/12** interacts with phosphates in water, albeit only weakly with stability constants of $K_a = 2.5$ M^{-1} and $K_a = 39$ M^{-1} for the complexes of, respectively, dimethyl phosphate and phenyl phosphate [288]. Regarding the structural relationship of **6/12** and the independently developed cavitands **6/10** and **6/11** it seems as if the combination of sugar and thiourea moieties could be a promising basis for the development of new anion hosts active in aqueous solution.

The group around Smith is interested in the development of small molecular mimics of translocases, enzymes that flip phospholipids in biological membranes from one side to the other [289–291]. It was demonstrated, for example, that tren sulfonamide **6/13** facilitates phosphatidylcholine (**6/15**) translocation across synthetic vesicle and erythrocyte membranes [292, 293]. Activity of **6/14** proved to be even higher and includes the translocation of phosphatidylserine (**6/16**) [294]. Mechanistic studies indicate that hosts **6/13** and **6/14** can form a complex with the phosphatidyl head group of a lipid by hydrogen bond formation between the sulfonamide and urea NH groups and the phosphate oxygens thus reducing head group hydrophobicity and promoting diffusion through the non-polar interior of the bilayer membrane. These



Scheme 29.

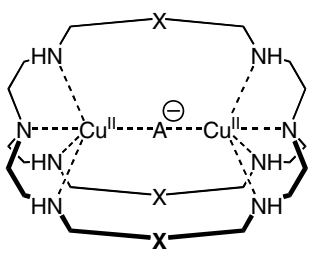
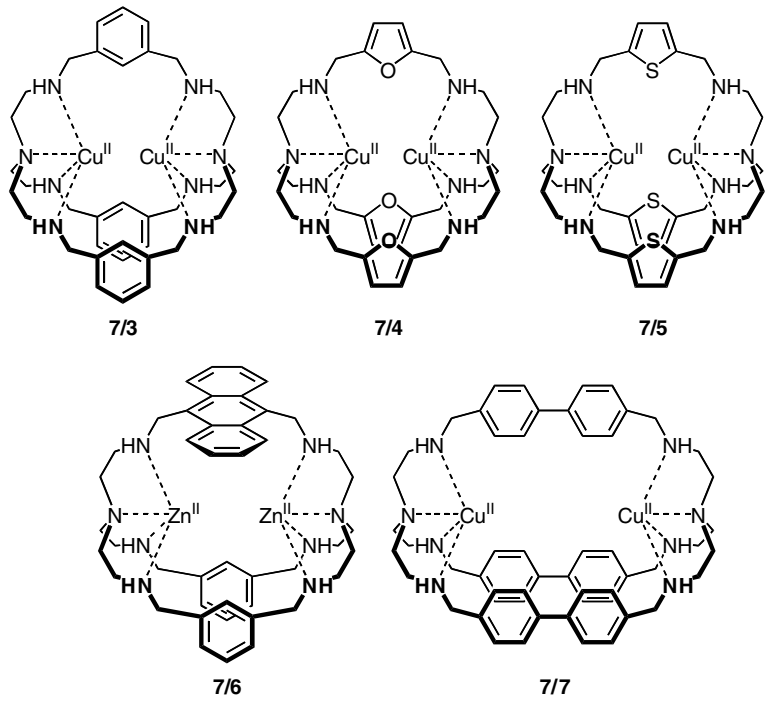


Figure 15. Schematic representation of a cascade complex between an anion and a dimetallic bis-tren based cryptate.



Scheme 30.

complexes are most stable in a non-polar environment which explains why the majority of binding studies between anions and **6/13**, **6/14**, or other structurally related tren derivatives were carried out in organic solvents such as chloroform [295]. If one assumes that translocation is initiated at the surface of the membrane and not in its interior, the primary binding event must occur at the aqueous interface, however. Moreover, since pK_a measurements showed that the tren derivatives **6/13** and **6/14** are not protonated at physiological pH [294], these systems can also be regarded as neutral hosts binding to anions in an aqueous medium.

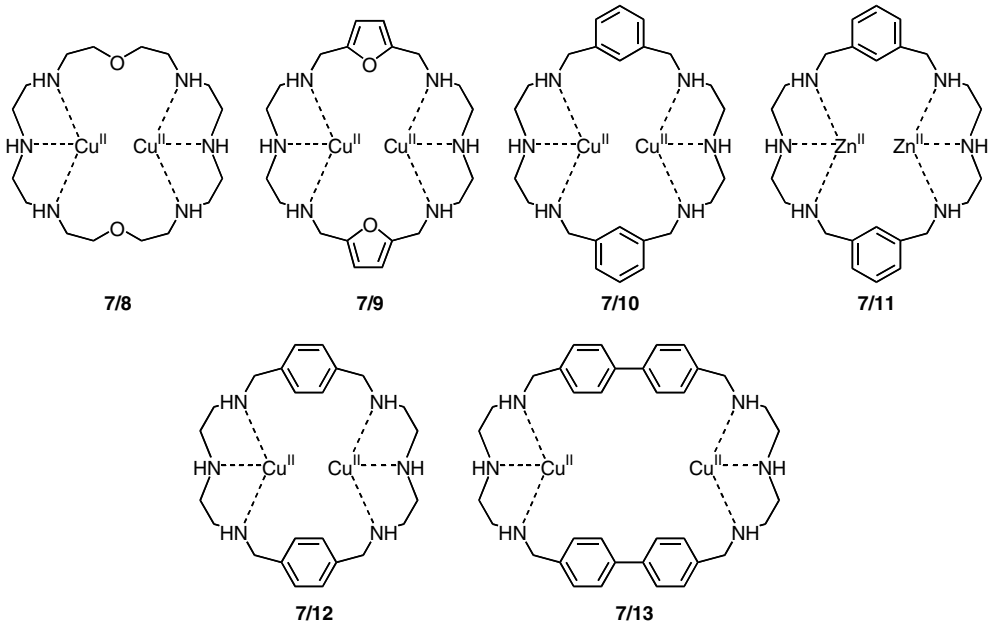
Finally, interactions between the neutral *ter*-cyclopentanes **6/17** or **6/18** and the anionic bisphosphate lipid A in phosphate-buffered saline at pH 7.4 were demonstrated by Miller and co-workers.[296] Job plots indicate that defined 1:1 complexes are formed with dissociation constants of 587 and 592 nM for the complexes of, respectively, **6/17** and **6/18**. Because only weak binding was detected between **6/19** and lipid A, complexation is obviously promoted by the hydrophobic substituents of **6/17** or **6/18**.

7. Hosts containing metal centers

Incorporation of metal ions in anion hosts can serve several purposes. According to a systematic classification proposed by Gale [8, 10, 297], metals can be co-bound guests in an ion pair receptor, can act as organizing elements in the structural stabilization of a receptor, can withdraw electron density from a π-system to increase the anion affinity of a hydrophobic host, can act as reporter groups signaling complex formation without being directly involved in anion binding, and finally, can serve as Lewis acidic coordination sites for the substrate. The latter strategy provides a means to employ strong coordinative interaction in anion recognition often in combination with Coulomb attraction and a well defined structural preorganization of the receptor cavity. In addition, binding selectivity benefits from the predictable directionality of coordinative interactions. As a consequence, anion recognition in water of anion hosts containing Lewis acidic binding sites, on which we will mainly focus in this chapter, is often very efficient [298].

To allow for introduction of metal ions, a host molecule must contain a suitable arrangement of several basic centers that can form a chelate-type complex with the metal leaving at least one coordination site unsaturated or saturated by only a weakly bound solvent molecule or counterion. This site will be the one involved in anion binding. Metals frequently encountered in this type of anion hosts are copper(II) or zinc(II) although also other metals have been used. Copper has the advantage that anion binding most often causes a profound change in the absorption spectrum of the host thus allowing complex formation to be followed by spectroscopic methods. Zinc, on the other hand, is diamagnetic making NMR spectroscopic binding studies possible.

Early examples of metal containing anion receptors are the dicopper(II) cryptates **7/1** and **7/2** developed in the groups of Martell and Lehn of which **7/1** is based on the azacryptand **3/30** [299–301]. In these cryptates, each tren subunit coordinates to a Cu(II) ion in a trigonal bipyramidal binding mode leaving one axial position

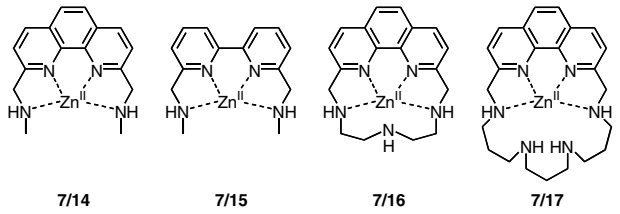


Scheme 31.

available for anion binding. If an anion can fill the vacant space between the two metal centers of the cryptate, it will bridge these binding sites leading to the formation of a so-called cascade complex (Figure 15). Stability of such complexes can be expected to depend on the complementarity between shape and size of the anion and the host cavity.

Copper binding to the ligands used for the construction of **7/1** and **7/2** and binding of hydroxide, fluoride, and chloride to the corresponding mononuclear and binuclear copper(II) cryptates was investigated by potentiometric measurements at 25 °C in 0.1 M $NaClO_4$[301]. It was shown that the tetracationic dicopper(II) species **7/1** and **7/2** form the most stable complexes while, despite the higher charge, protonated derivatives exhibit a reduced anion affinity as do monocopper cryptates even if protonation of amino groups not involved in metal coordination causes their charge to be the same as that of **7/1** or **7/2**. The anion affinity of the protonated metal free ligands is still lower. Thus, the high stability of the anion complexes of **7/1** and **7/2** results from the simultaneous coordination of the anionic guests to both metal centers of the hosts. Comparison of the anion affinity of **7/1** and **7/2** showed that, in general, **7/1** forms more stable complexes, which was ascribed to the better fit of the anions into the cavity of this cryptate. The exceptionally large stability constant of the hydroxide complex of **7/1** ($\log K_a = 10.0$) that is ca. four orders of magnitude larger than the stability constant of the hydroxide complex of **7/2** ($\log K_a = 6.2$) was rationalized by a stabilizing hydrogen bond between the anionic guest and an oxygen atom in the linker of **7/1** [301].

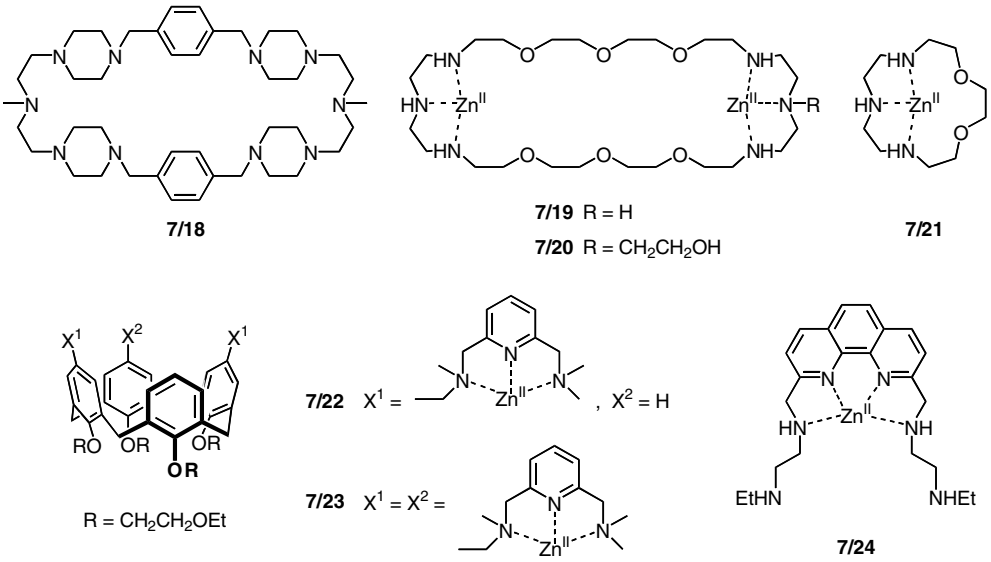
On the basis of this pioneering work, substantial contributions to the concept of the use of cascade complexes for anion recognition [302] and sensing [303, 304] in aqueous solution came from the Fabbrizzi group. The relatively rigid dicopper(II) cryptate **7/3** was shown, for example, to interact with various anions such as N_3^-, OCN^-, SCN^-, SO_4^{2-}, $HCOO^-$, CH_3COO^-, HCO_3^-, and NO_3^- [305]. Complex formation can easily be detected by the color change of an aqueous solution of this host from blue in the absence of these anions to green in their presence. **7/3** forms the most stable complex with N_3^- ($\log K_a = 4.78$) followed by OCN^- ($\log K_a = 4.60$) and HCO_3^- ($\log K_a = 4.56$), a result that was rationalized by the almost perfect fit of azide and, to a lesser extent, hydrogencarbonate and cyanate between the two copper centers. All other anions studied, even the twofold charged sulfate or the strongly coordinating thiocyanate, are bound considerably less tightly leading to the conclusion that the host does not recognize the donor tendencies or the shape, but the bite length of the anionic guest [305]. X-ray diffraction studies confirmed that in the azide complex of **7/3**, the anion collinearly bridges the two metal centers by coordination with the terminal nitrogen atoms [306]. In contrast to **7/3**, the somewhat more flexible cryptate **7/4** interacts also with smaller anions such as halides as evidenced by the color change of a solution of this host from pale blue to bright yellow upon addition of fluoride, chloride, bromide or iodide [307]. Also hydroxide [308], azide, and thiocyanate form stable complexes with **7/4** the latter two of which cause a solution of **7/4** to turn green. Thus, this cryptate represents a rather versatile anion receptor being able to expand and contract its cavity to include anions of various size and shape. Interestingly, the anion selectivity of the thiophene containing dicopper(II) cryptate **7/5** is significantly higher than that of **7/4** in spite of the close structural relationship between these two compounds [309]. Again, large association constants were determined for the OH^-, N_3^-, OCN^-, and



7/14 7/15 7/16 7/17

Scheme 32.

SCN^- complexes of **7/5**. The affinity of this host for SO_4^{2-}, NO_3^-, HCO_3^-, $HCOO^-$, CH_3COO^-, Cl^-, Br^-, and I^- is significantly smaller, however, an observation ascribed to the atomic radius of the sulfur atom in the thiophene moieties that increases the distance between the two copper centers in **7/5** with respect to **7/4** thus reducing the affinity for certain anions [309]. The anthracene moiety in host **7/6** was introduced to allow the optical sensing of azide ions in aqueous solution [310]. Because copper(II) would quench the emission of the proximate fluorophores either through an electron transfer (eT) or an energy transfer (ET) process, zinc(II) ions had to be used for anion coordination because they cannot be engaged in an eT mechanism and, due to the filled 3d level, cannot participate to any ET process. A solution of **7/6** is strongly fluorescent at pH 8.5 but fluorescence is completely quenched upon addition of one equivalent of N_3^- most probably due to electron transfer. The stability constant log K_a of the azide complex of **7/6** amounts to 5.8. No quenching was observed in the presence of SO_4^{2-}, NO_3^-, HCO_3^-, Cl^-, Br^-. Moreover, azide complexation is not affected when the receptor solution contains 10 equivalents of each one of the above anions which indicates that they do not compete with N_3^- complexation. Interestingly, OCN^- does not quench the fluorescence of the anthracene moiety in **7/6**, it does, however, strongly alter the binding isotherm observed for azide complexation indicating a competition of cyanate for inclusion within the cage [310]. An alternative approach for the optical sensing of anions also realized by Fabbrizzi and co-workers made use of dye displacement [182]. Upon binding of the carboxylate group of coumarine 343 to **7/3**, complete quenching of the coumarine emission occurs which was ascribed to an intramolecular ET process involving the photoexcited coumarine fragment and the copper ions [311]. A 1:1 complex is formed between **7/3** and coumarine 343 in degassed aqueous solution (50 mM HEPES, pH 7) with an association constant of log $K_a = 4.8$. Anions that form more stable complexes with **7/3** such as N_3^-, OCN^-, and HCO_3^- are able to displace the dye from the cryptate causing a regeneration of fluorescence. Only a slight enhancement of fluorescence is observed with anions, however, whose complexes with **7/3** are less stable than the coumarine 343 complex (SCN^-, NO_3^-, SO_4^{2-}, $HCOO^-$, CH_3COO^-, HPO_4^{2-}) thus allowing the combination of **7/3** and coumarine 343 to be used, for example, for a quantitative determination of carbonate in mineral waters [311]. In a similar way, host **7/7** with an enlarged cavity was used for the optical detection of dicarboxylic acids [312]. Binding of **7/7** to carboxyrhodamine, a fluorescent dye containing a terephthalate subunit, leads to the formation of a non-fluorescent complex in aqueous HEPES buffered solution at pH 7 with a stability constant log K_a of 7.0. Of the isomeric benzene dicarboxylic acids, only terephthalic acid is able to compete with carboxyrhodamine in binding to the host thus causing a fluorescence enhancement while almost no effect on fluorescence was observed in the presence of 1,2- or 1,3-benzene dicarboxylic acid. In the family of aliphatic dicarboxylates $^-OOC\text{-}(CH_2)_n\text{–}COO^-$ with $n = 0$-5, the receptor-dye ensemble can be used for glutarate ($n = 3$) and adipate ($n = 4$) sensing. No displacement of the dye from the host occurs in the presence of the longer or the shorter dicarboxylic acids with the exception of oxalate ($n = 0$),



7/18 7/19 R = H 7/20 R = CH_2CH_2OH 7/21

7/22 X^1 = , X^2 = H

7/23 $X^1 = X^2$ =

R = CH_2CH_2OEt

7/24

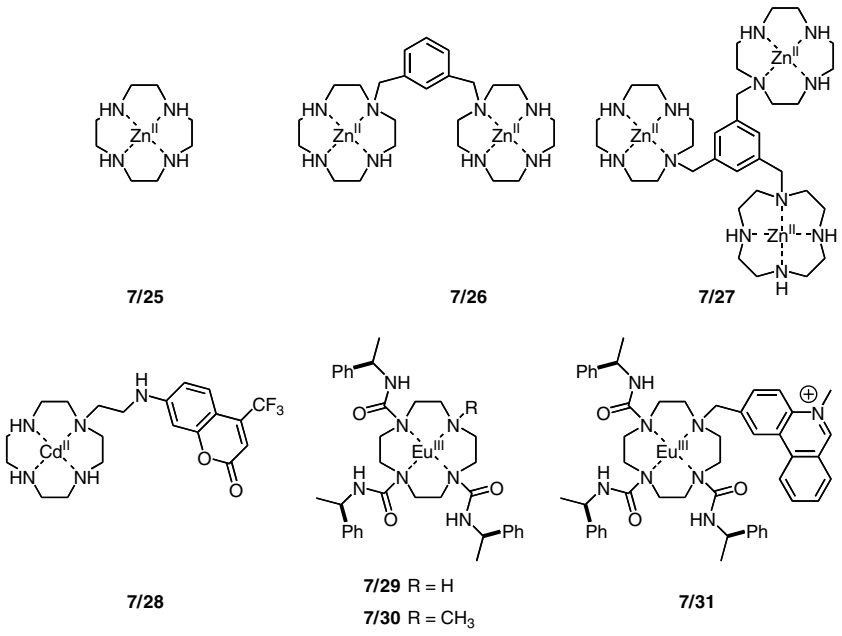
Scheme 33.

which was ascribed to the formation of a 2:1 complex between **7/7** and two molecules of this dicarboxylic acid [312].

Dimetallic anion hosts are not restricted to bicyclic polyaza frameworks. The examples of hosts **7/8**–**7/13** show that also monocyclic host topologies have been realized. These hosts are conformationally significantly more flexible than bicyclic systems. As a consequence, the distance between the two metal centers varies in a wider range allowing the complexation of anions that cannot be included into the cavity of hosts **7/3**–**7/7**. Host **7/8**, for example, was shown to bind pyrophosphate $P_2O_7^{4-}$ in water (0.1 M KCl) with an association constant of log $K_a = 8.5$ [313]. In comparison with an equally charged mononuclear derivative in which two amino groups are protonated, **7/8** shows stronger binding for pyrophosphate by 2.25 log units. A mononuclear species, in which three amino groups are protonated binds the anion 1.43 log units more strongly than **7/8**, however, which was attributed to the organizing effect of the three ammonium groups on the ligand that causes a better fit of the pyrophosphate anion inside the host cavity [313]. Metal binding, pH dependent protonation equilibria, as well as anion complexation of **7/9**–**7/11** were investigated in a similar fashion [314–317]. These hosts interact with, for example, orthophosphate, pyrophosphate, oxalate, malonate, maleate, and fumarate. In addition, **7/10** and **7/11** also form complexes with various amino acids in which the carboxylate group of the guest coordinates to one metal center and the amino group to the other [316, 317]. Another example for a host that strongly interacts with pyrophosphate in water is **7/12** (log $K_a = 7.2$) [318]. Complex formation involves bridging of the two copper ions of the host by oxygens of both phosphate moieties. To allow for binding of the smaller orthophosphate anion, the macrocycle has to contract, which causes a strained situation and, as a consequence, a reduced stability of the corresponding complex. Expansion of the cavity of **7/12** leads to host **7/13** that tightly binds triphosphate (log $K_a = 8.0$) and ATP (log $K_a = 7.8$) [319]. By the careful choice of an anionic fluorescent indicator whose complex with the host is almost as stable as that of a target substrate but more stable than complexes of potential other substrates, fluorescent sensors employing the dye displacement strategy were devised on the basis of **7/12** and **7/13** for the detection of, respectively, pyrophosphate and ATP [318, 319].

Also the zinc containing complexes **7/14**–**7/17** form complexes with ATP [320]. Association constants increase in the order **7/14** (log $K_a = 3.25$) < **7/15** (log $K_a = 3.74$) < **7/16** (log $K_a = 4.35$) < **7/17** (log $K_a = 5.18$) which was rationalized in terms of a more 'open' coordination sphere at the zinc ion in hosts **7/15** and **7/16**. ^{31}P NMR spectroscopy showed that the terminal phosphate group of ATP and not the ring nitrogens in the adenine moiety interacts with the metal in the complexes. Contributions to complex stability from the adenine system were detected in the form of π-stacking interactions. Protonation of amino groups of the host not involved in metal coordination results in a further increase in complex stability due to the formation of salt bridges. Because protonated metal free ligands form much less stable complexes, the high ATP affinity of protonated species of **7/14**–**7/17** was ascribed to synergetic effects of the metal ion and of the ammonium groups [320].

Another systematic binding study revealed a pronounced pH dependence in the interaction between **7/18** and pimelic acid ($HOOC(CH_2)_5COOH$, H_2pim), hydrogenpimelate ($Hpim^-$) and pimelate (pim^{2-}) in the presence of copper(II) [321]. While H_2pim is recognized

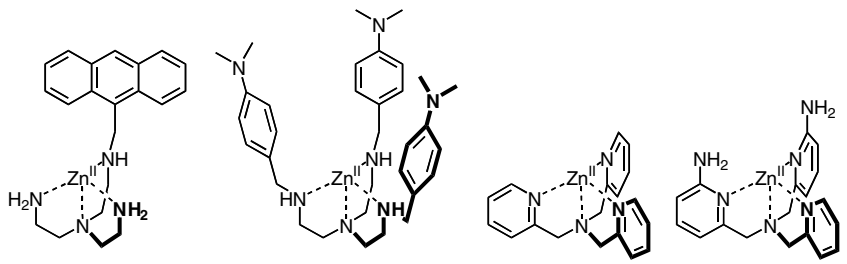


Scheme 34.

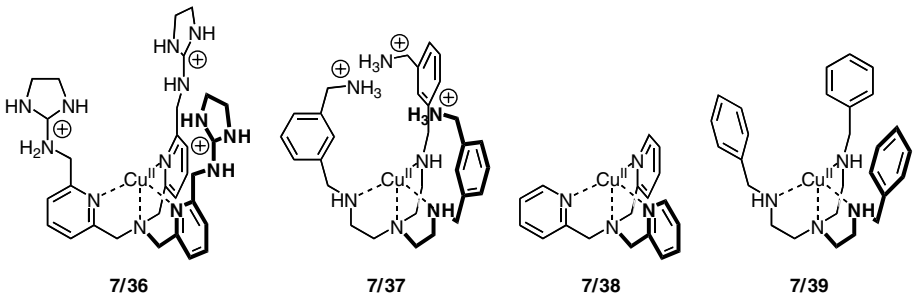
by the octaprotonated form of **7/18** at pH < 4, the most prominent complex in the pH range 4–6 is one formed between the pentaprotonated copper complex of **7/18** and $Hpim^-$. Above pH 6, dicopper species with a degree of protonation between 0 and 2 interact with pim^{2-}, and at pH > 8 the dicarboxylate is released due to the formation of a dihydroxylated dicopper complex.

Interaction of the binuclear dizinc(II) complex **7/19** with OH^- leads to the formation of hydroxo species in which, depending on the pH, one hydroxide ion bridges the two metal centers or two hydroxide ions are coordinated to each metal center [322]. The dihydroxo complex increases the rate of bis(*p*-nitrophenyl)phosphate hydrolysis in water by almost one order of magnitude with respect to the monohydroxo complex of **7/21** [323]. This indicates that both Zn(II) ions act cooperatively in the hydrolytic mechanism of **7/19**, most probably through a bridging interaction of the phosphate with the two metals and a simultaneous nucleophilic attack of a Zn-OH function on the substrate. This interpretation is consistent with the fact that there is no large difference in the effect of **7/19** and **7/21** on the rate of *p*-nitrophenyl acetate hydrolysis, a substrate that cannot bridge the two binding sites in **7/19**. Introduction into the macrocyclic ligand of an ethanolic side arm whose hydroxy group forms a strong nucleophile after deprotonation aimed at improving the catalytic activity of **7/19** [324]. Formation of the dizinc(II) complex **7/20** at pH > 6 is indeed accompanied by the almost simultaneous deprotonation of the pendant alcoholic OH group and the resulting species does possess catalytic activity in *p*-nitrophenyl acetate hydrolysis. A mixed complex present at slightly alkaline pH containing both a Zn-alkoxide and a Zn-hydroxide nucleophilic function is, however, by far more active. The rate of bis(*p*-nitrophenyl)phosphate hydrolysis in the presence of this complex is ca. 7 times higher than that in the presence of the dihydroxo complex of **7/19**. As in the alkaline phosphatase, both the alkoxide and hydroxide function of the mixed complex of **7/20** are obviously involved in the hydrolytic mechanism. In the first step of carboxylate or phosphate cleavage, the alkoxide acts as a nucleophile giving an acetyl or a phosphoryl intermediate, which is subsequently hydrolyzed via an intramolecular attack of the zinc-bound hydroxide group [324]. Examples of other zinc containing enzyme mimics are the calix[4]arene derivatives **7/22** and **7/23** that were developed in the groups of Reinhoudt and Engbersen and the zinc-phenanthroline complex **7/24** described by Guo *et al*. **7/22** and **7/33** were shown to promote the hydrolytic cleavage of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate as well as that of RNA dinucleotides [325–329]. Interestingly, large differences were found in the rate of hydrolysis of different dinucleotides. **7/22**, for example, cleaves GpG at least 8.5 times faster than any other RNA dinucleotide investigated [328]. Compound **7/24** and structurally related phenanthroline derivatives accelerate the hydrolytic cleavage of the terminal phosphate group from the triphosphate moiety of ATP [330]. A number of other metal containing systems have been described that catalyze phosphoester or phosphodiester hydrolysis. For an overview, we refer to a recently published review [331].

Work in the Kimura group centers around the development of synthetic hosts and enzyme mimics on the basis of metal complexes of macrocyclic polyamines, for example the zinc(II) complex of 1,4,7,10-tetraazadodecane (cyclen) **7/25** [332–334]. **7/25** was shown to reversibly form a 1:1 complex with various anions such as thiocyanate, acetate, chloride, HPO_4^{2-}, phenyl phosphate, and *p*-nitrophenyl phosphate in aqueous solution whereas the corresponding diprotonated metal free macrocycle only weakly interacts with these substrates under the same conditions [335, 336]. The stability constant log K_a of, for example, the *p*-nitrophenyl phosphate complex of **7/25** amounts to 3.3 [335, 336]. Linking two subunits of **7/25** together via a *m*-xylylene spacer increases the *p*-nitrophenyl phosphate affinity of the resulting host **7/26** with respect to **7/25** by ca. one order of magnitude [337]. A much more pronounced boost in phosphate affinity could be achieved, however, by arranging three zinc–cyclen complexes around an aromatic benzene scaffold [338]. The corresponding tripodal host **7/27** that was inspired by the crystal structures of the PO_4^{3-} complex of a derivative of **7/25** with an ethanolic residue on one cyclen nitrogen [336] and by the crystal structure of the *p*-nitrophenyl phosphate complex of **7/25** [338], in both of which three oxygens of the anion coordinate to the metal center of a zinc(II)-cyclen unit, indeed forms 1:1 complexes with phosphates in slightly acidic solution



Scheme 35.



Scheme 36.

(pH < 6) of which the *p*-nitrophenyl phosphate has a stability constant log K_a of 5.8. Thus, phosphate affinity of **7/25**, **7/26**, and **7/27** increases with the number of binding sites available for anion binding and the cooperative action of all three binding sites in **7/27** is obviously the reason for the highest phosphate affinity of this host. Phosphate affinity seems to parallel the basicity of the substrate as *p*-nitrophenyl phosphate ($pK_a = 5.2$; log $K_a = 5.8$), phenyl phosphate ($pK_a = 5.8$; log $K_a = 6.6$), α-D-glucose-1-phosphate ($pK_a = 6.1$; log $K_a = 7.0$), and phenyl phosphonate ($pK_a = 7.0$; log $K_a = 7.9$) are bound increasingly more strongly [338]. In basic solution, phosphate is displaced by hydroxide ions from the metal centers of **7/27**.

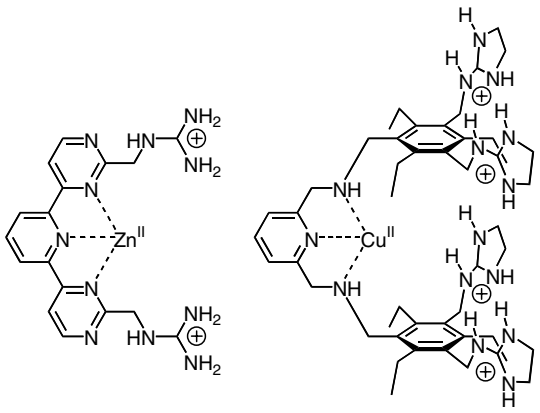
Host **7/28** that also contains a metal–cyclen complex was designed as a fluorescent sensor for anions in neutral aqueous solution [339]. In the absence of suitable anions, the 7-amino-4-trifluoromethylcoumarine subunit coordinates via the amino group to the cadmium center which causes a blue shift in the emission spectrum of **7/28** with respect to the spectrum of the metal free host. Upon addition of pyrophosphate or citrate to an aqueous solution of **7/28** (100 mM HEPES, pH 7.4), recovery of the emission spectrum of the free chromophor was observed showing that binding of these anions causes a displacement of the chromophor from the metal center. Among the various anions studied, pyrophosphate (log $K_a = 4.12$) and citrate (log $K_a = 4.05$) are bound best. The phosphate complex of **7/28** is significantly less stable (log $K_a = 1.82$) most probably because of the reduced charge of this anion with respect to pyrophosphate. The stability of the halide complexes of **7/28** decreases in the order I^- (log $K_a = 2.04$) > Br^- (log $K_a = 1.49$) > Cl^- (log $K_a = 1.05$) demonstrating the higher affinity of **7/28** for soft bases. Fluoride and perchlorate are not bound at all. Also sensing of ATP (log $K_a = 4.85$), ADP (log $K_a = 4.59$), and AMP (log $K_a = 3.36$) in aqueous solution is possible whereas no change in the emission spectrum was observed in the presence of *c*AMP, which allowed the use of **7/28** to monitor in real-time the phosphodiesterase catalyzed cleavage of this cyclic nucleotide [339].

Anion binding in aqueous solution using the chiral Eu(III)–cyclen complexes **7/29**–**7/31** and analogous Tb(III) complexes was characterized by NMR spectroscopy and by changes in the emission intensity and circular polarization following direct excitation or, in the case of **7/31**, sensitized excitation via the alkylphenanthridinium chromophor [340–342]. The affinity for carbonate, phosphate, lactate, citrate, acetate and malonate at pH 7.4 (0.1 M collidine/HCl) was found to decrease as a function of the overall negative charge of the complex. Thus, citrate and malonate are bound most strongly with association constants log K_a > 4.6. The association constant log K_a of the acetate complex of, for example, **7/30** amounts to 2.40 whereas that of the HCO_3^- complex to 3.75 demonstrating that the latter anion is bound ca. one order of magnitude more strongly than the former one. Analysis of the rate constants for the radiative decay of the Eu $^5D_0$ or Tb $^5D_4$ excited states in H_2O and D_2O showed that citrate, malonate, and lactate form chelate complexes at the metal center of the hosts by displacing both metal-

Table 13. Thermodynamic parameters in kJ mol^{-1} for various complexes of hosts **7/36**, **7/37** and their analogs **7/38**, **7/39** in 2% methanol/water (5 mM HEPES, pH 7.4) at T = 298 K

Host	Guest	ΔG	ΔH	$T\Delta S$
7/36[348][a]	HPO_4^{2-}	−22.2	−15.9	6.3
7/37[348][a]	HPO_4^{2-}	−27.2	2.5	29.7
7/38[348][a]	HPO_4^{2-}	−17.2	−3.3	13.9
7/39[348][a]	HPO_4^{2-}	−15.9	−3.8	12.1
7/37[350][a]	Acetate	−14.3	2.9	17.2
7/37[350][a]	Glutarate	−15.1	13.8	28.9
7/37[350][a]	Tricarballate	−24.3	−2.0	22.3
7/37[350][a]	1,2,3,4-Butanetetracarboxylate	−24.3	−1.2	23.1

[a]Determined by van't Hoff analysis.
[b]Determined by isothermal titration calorimetry.



7/40 7/41

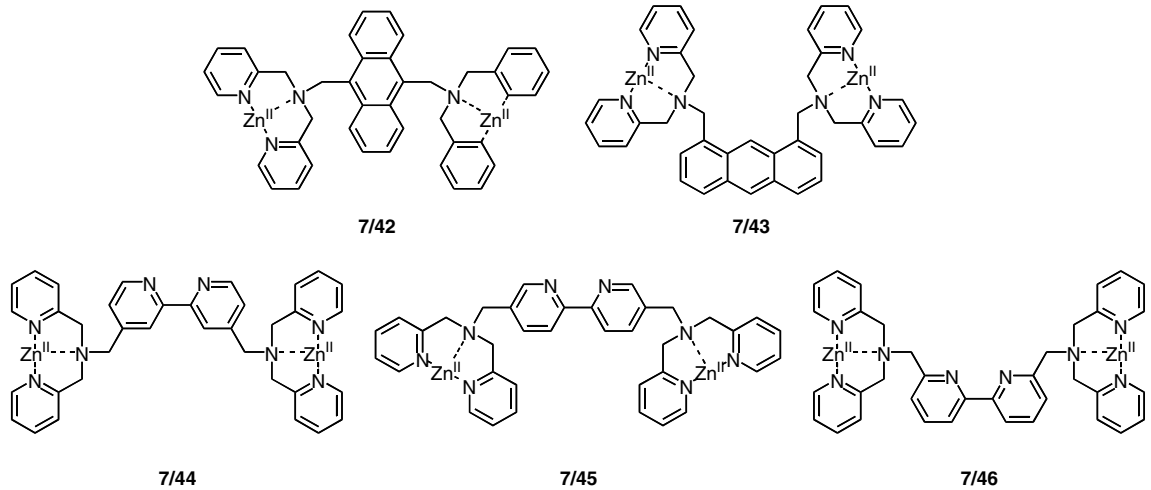
Scheme 37.

bound water molecules. Also acetate and carbonate chelate leading to the conclusion that the higher affinity of **7/30** and **7/31** toward HCO_3^- has to be ascribed to a preferential binding of the symmetrical anion. HPO_4^{2-} is bound in a monodentate fashion, giving a mono-aqua adduct [342]. These structural assignments were confirmed by the X-ray crystallographic determination of several structures of anion complexes of **7/29**. The structure of the citrate complex, for example, clearly revealed the coordination of the metal to the four nitrogens in the cyclen ring, to the three oxygens of the cyclen side arms, and to the oxygens of a carboxylate group of the substrate [343]. Also the anion affinity of Yb(III) and Gd(III) complexes of **7/29** was recently studied in detail [343].

Non-cyclic metal containing anion hosts are often based on copper(II) or zinc(II) complexes of tripodal tetraamines such as tren or ligands of similar topology. In these complexes, four coordination sites of the metal are occupied by the amino groups of the ligand, while the axial position, opposite to the tertiary amine, remains vacant and is thus available for anion binding. Hosts **7/32** and **7/33** were developed in the Fabbrizzi group. **7/32** interacts with benzoates in methanol but to allow complex formation to be followed via the anthracene quenching, the aromatic subunit of the substrates must contain either a donor such as a dimethylamino substituent or an acceptor such as a nitro group in the 4-position [344]. Quenching was ascribed to an electron transfer process involving the aromatic guest and the photoexcited anthracene moiety of the host. Similarly, host **7/33** also forms complexes with various aliphatic and aromatic carboxylates but only the aromatic guests cause a quenching of the dimethylaminobenzene chromophors arranged around the cavity [345].

That such simple systems are not only able to interact with anions in methanol but also in water was, among others, demonstrated by Mareque-Rivas and co-workers. Host **7/34**, for example, forms a complex with phenyl phosphate in water (50 mM HEPES, pH 7.0) whose stability constant log K_a amounts to 3.6 [346]. Interestingly, the phenyl phosphate complex of a derivative of **7/34** containing two additional amino groups on the ligand is ca. one order of magnitude more stable (log $K_a = 4.4$) despite the fact that steric and electronic effects of the amino groups should reduce phosphate affinity of **7/35** with respect to **7/34**. The increase in complex stability has therefore been attributed to NH···OP hydrogen bonds between the amino groups and the guest, an assumption that was supported by the crystal structure of the nitrate complex of **7/35** [346].

The C_{3v} symmetrical hosts **7/36**–**7/39** that structurally resemble **7/32**–**7/35** were developed in the Anslyn group. Anion complexation of these hosts involves a combination of coordinative interactions to a copper(II) center with Coulomb attraction and hydrogen-bonding to protonated amino imidazoline moieties or amino groups arranged around the cavity. In addition, the shape of the cavity of these hosts provides an optimal environment for the inclusion of tetrahedral anions. As a consequence, **7/36** and **7/37** strongly bind to, for example, HPO_4^{2-} in 2% methanol/water (5 mM



7/42 7/43

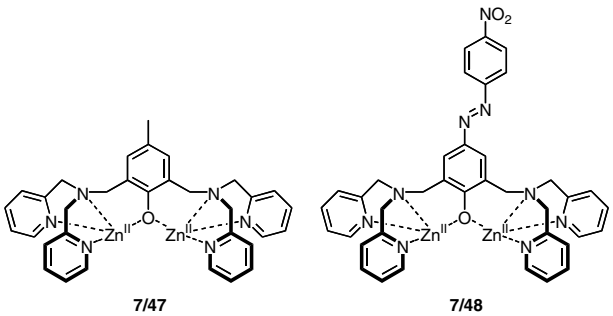
7/44 7/45 7/46

Scheme 38.

HEPES, pH 7.4) with stability constants K_a of, respectively, 1.5×10^4 M^{-1} and 2.5×10^4 M^{-1} [347, 348]. Very weak or no interactions of both receptors with anions such as acetate, sulfate, nitrate, hydrogencarbonate, and chloride were detected. Arsenate is bound by both receptors comparably well as is phosphate, whereas the perrhenate complex of **7/37** has a ca. one order of magnitude smaller stability constant than the phosphate complex most probably because of the larger size and the reduced charge of this anion. Interestingly, binding of **7/36** to perrhenate is almost negligible ($K_a < 100$ M^{-1}) demonstrating that this host has a lower affinity but a higher selectivity for phosphate in comparison to **7/37**. The lower selectivity of **7/37** was initially attributed to the higher flexibility of this receptor [347]. Subsequently, a thermodynamic characterization of the complexation equilibria revealed another reason for the different properties of both hosts, however. Table 8 shows that phosphate binding to **7/36** is accompanied by favorable enthalpic and entropic changes whereas binding to **7/37** is endothermic and driven by entropy only [348]. Because enthalpy and entropy changes during phosphate binding of derivatives of **7/36** and **7/37** lacking the positively charged binding sites in the periphery of the cavity are comparable, both being slightly exothermic with a favorable entropy contribution, the differences in the thermodynamic parameters observed during complex formation of **7/36** and **7/37** were ascribed to principal differences in the solvation of ammonium and guanidinium groups. Because water molecules around ammonium groups are more tightly bound and better organized than those around guanidinium groups, desolvation of guanidinium groups is easier but the entropic gain is smaller than in the case of ammonium groups explaining why phosphate binding to **7/37** is accompanied by a larger entropic contribution, but binding to **7/36** is enthalpically more favorable [348]. The high phosphate affinity and selectivity of **7/36** has recently been used in a dye displacement assay in combination with the indicator carboxyfluorescein for the quantitative determination of phosphate in horse serum and saliva [349].

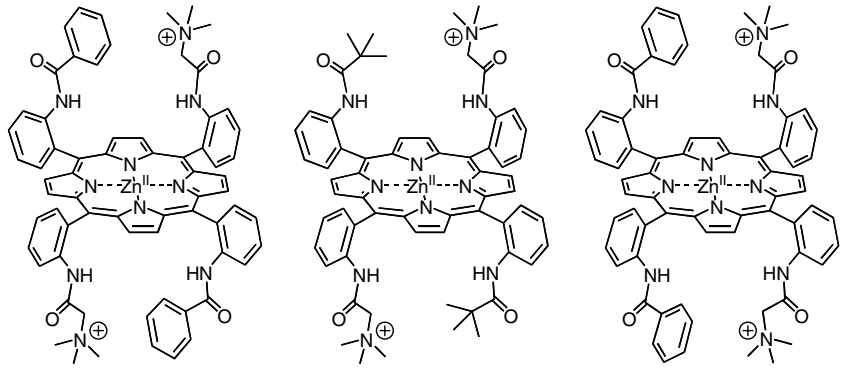
Host **7/37** was also used to investigate the thermodynamic origin of cooperativity in ion-pairing host–guest interactions in water [350]. To this end, complexation of different carboxylic acids bearing one to four carboxylate groups was studied by means of isothermal titration calorimetry. It was expected that the coordination of one carboxylate group of the substrate to the metal center of **7/37** is the primary binding event in these interactions, and that ion-pairing of the ammonium groups on the host with additional carboxylate groups on the substrate would further stabilize the aggregate formed. Table 8 shows that this is indeed the case. A systematic analysis of the data revealed, however, that the overall binding strength observed for, for example, the tricarballate or the 1,2,3,4-butanetetracarboxylate complex is weaker than the combined binding energies of individual parts of these substrates. Thus, negative cooperativity is observed in this system whose thermodynamic origin is, according to the microcalorimetric measurements, primarily entropy. An explanation for this result could be the smaller number of solvent molecules released upon binding of a substrate A–B, in which different binding sites are linked covalently, to a given host with respect to binding of the individual parts A and B. Not only will A–B occupy a smaller volume of the host cavity than A and B, individual molecules A and B will also have larger solvation spheres than a covalently linked analog. The authors thus conclude that if this 'volume analysis' is correct, it may be generally difficult to achieve positive cooperativity in ion-pairing recognition in water [350].

Determination of the stability of the zinc complex **7/40** showed that the formation constant in aqueous solution (10 mM HEPES, pH 6.8) increases from < 100 M^{-1} in the absence of phosphate to 4300 M^{-1} in the presence of one equivalent of Na_2HPO_4 which was ascribed to the combined stabilizing effects of coordination of one phosphate oxygen to the zinc, ion-pairing, and hydrogen-bonding interactions between the phosphate and the guanidinium moieties of **7/40** [351]. In combination with pyrocatechol violet, a dye displacement assay was established on the basis of **7/40** that allowed the detection of α-amino acids in 50% methanol/water (10 mM HEPES, pH 7.4) by a color change from blue to yellow [352]. The association constants of most amino acid complexes studied range between 0.8×10^4 and 2.3×10^4 M^{-1}. A comparing with association constants determined for complexes of model substrates containing only an amino or a carboxylate group shows that amino acid binding involves simultaneous coordination of the carboxylate and the amino group to the zinc center of the host. The large stability constant observed for the aspartate complex of **7/40** ($K_a = 1.5 \times 10^5$ M^{-1}) was attributed to cooperative ion-pairing interactions between a guanidinium group of the host and the side chain carboxylate group of the guest. Moreover, the fact that the glutamic acid complex is much less stable ($K_a = 2.2 \times 10^4$ M^{-1}) indicates that an optimal complementarity between the interacting groups also contributes to the high stability of the complex with aspartate [352].



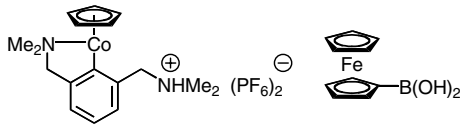
7/47 7/48

Scheme 39.



7/49 7/50 *rac* - 7/51

Scheme 40.



7/52 7/53

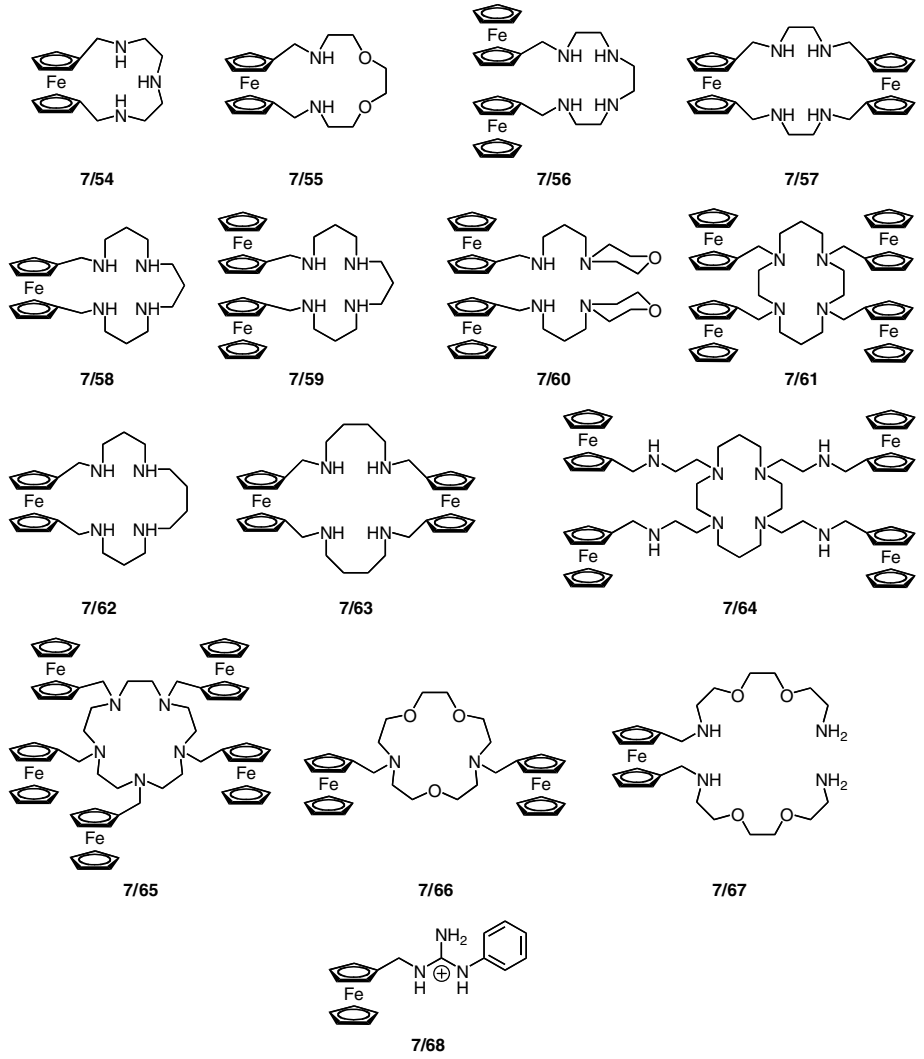
Scheme 41.

Compound **7/41** was designed to selectively recognize 2,3-bisphosphoglycerate (2,3-BPG), an allosteric effector that modulates the oxygenation level of hemoglobin, in aqueous solution [353]. The K_a of 8×10^8 M^{-1} determined for the complex between 2,3-BPG and **7/41** in 50% methanol/water (10 mM HEPES, pH 7.4) is indeed remarkable and it only slightly decreases to 4×10^7 M^{-1} upon changing the solvent to pure water (pH 6.8). Moreover, monophosphate esters or carboxylates are bound much less tightly to the host under the same conditions. This allowed the use of **7/41** to modulate the oxygenation affinity of hemoglobin in horse red-cell hemolyzate in 20 mM phosphate buffer solution at pH 7.2. Thus, decrease of oxygen affinity of hemoglobin resulting from interactions with 2,3-BPG could be reversed upon addition of **7/41** to the solution which indicates that the synthetic host is able to strip the natural effector from the protein. One reason for this ability is the significantly higher affinity of **7/41** to 2,3-BPG in comparison to hemoglobin [353].

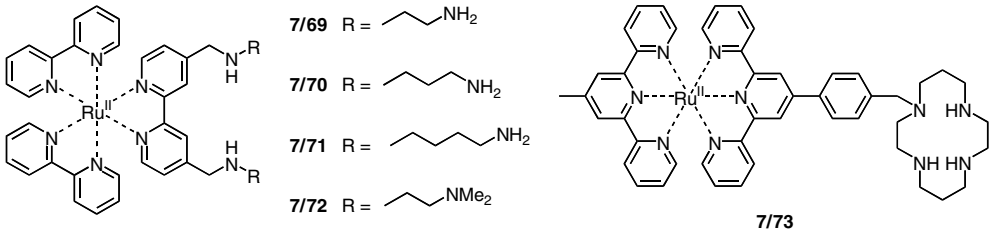
Hamachi and co-workers developed a series of Zn(II) dipicolylamine-based receptors **7/42**–**7/46** that interact with the imidazole moiety of histidine [354] and, more important in the context of anion recognition, with phosphate in aqueous solution [355]. Addition of inorganic phosphate or mono phosphate esters such as phenyl phosphate, *o*-phospho-L-tyrosine, or methyl phosphate to a solution of the binuclear hosts **7/42** or **7/43** (10 mM HEPES, pH 7.2) causes an increase of fluorescence intensity thus allowing a determination of complex stoichiometry and stability by fluorescence spectroscopy. Both hosts form 1:1 complexes with the different phosphate esters whose stability constant range between 10^4–10^5 M^{-1} [356, 357]. Binding affinity toward pyrophosphate, ADP, or ATP is higher [357, 358], most probably because of the higher charge of these anions. No interaction could be detected with diphosphate esters, cAMP, or other anions such as carbonate, sulfate, nitrate, acetate demonstrating the selectivity of the hosts for phosphate.

Binding of **7/42** and **7/43** to several phosphorylated peptides containing optimal consensus sequences that are phosphorylated by certain kinases was also studied [356, 357]. These investigations showed that binding strength becomes stronger with increasing negative charge on the substrate. Thus, both receptors scarcely sense peptides bearing net charges of 0 or +2 even at 10^{-4} M concentrations whereas they form highly stable complexes with an eight fold negatively charged peptide whose stability constants amount to 9.5×10^5 M^{-1} for **7/42** and 8.9×10^6 M^{-1} for **7/43**. Moreover, binding of phosphorylated peptides is much stronger than of non-phosphorylated analogs showing that the hosts can distinguish phosphorylated peptides from non-phosphorylated ones. This ability and the fact that the interactions with a negatively charged phosphorylated peptide are much stronger than with inorganic phosphate allowed the phosphatase-catalyzed dephosphorylation of a model peptide to be monitored in real time in the presence of **7/42** and **7/43** [357]. Isothermal titration calorimetry indicated that binding of phosphorylated peptides to the hosts is endothermic and accompanied by a large positive entropy change, consistent with the assumption that the interactions are driven primarily by a release of solvent molecules from the solvation spheres of host and guest [356, 357].

Also the binuclear complexes **7/44** and **7/45** containing two Zn(II) dipicolylamine subunits in different positions on a 2,2′-bipyridine scaffold interact with phosphorylated peptides [359]. Circular dichroism spectroscopy demonstrated that **7/44** and **7/45** but not **7/46** induce α-helical conformations in 17-mer peptides containing two phosphorylated serine residues in the 5,16, the 9,16, or the 12,15 position. The strongest effect, namely a change of an almost random coil conformation to one with a helical content of 30%, was observed upon addition of **7/45** to a peptide in which the phosphorylated serine residues are located in positions



Scheme 42.



Scheme 43.

6 and 19. **7/44** and **7/45** do not alter the conformation of monophosphorylated peptides while mononuclear analogs of the hosts less effectively induce helical conformations in diphosphorylated peptides than **7/44** and **7/45** suggesting that two-point interactions are optimal for helix stabilization [359].

The interaction of **7/45** in water (10 mM borate, pH 8.0) with phosphorylated peptides causes a decrease in the emission intensity at 389 nm of this host that was used to quantify binding affinity and selectivity. These investigations showed that selectivity in binding to 17-mer model peptides differing in the positions of the phosphate groups along the peptide chain is only moderate. Affinity, on the other hand, is 10 times higher for the diphosphorylated model peptides with respect to monophosphorylated analogs and an even larger difference was detected in the interactions between **7/45** and monophosphorylated and diphosphorylated derivatives of a naturally occurring peptide fragment of the insulin receptor kinase [359]. In this case, the complex of the monophosphorylated peptide has a stability constant K_a of 0.07×10^6 M^{-1}, whereas the diphosphorylated deriv-

ative is bound ca. 20 times more strongly with a stability constant K_a of 1.7×10^6 M^{-1} [359]. Thus, the Zn(II) dipicolylamine-based receptors developed in the Hamachi group are promising systems for the recognition and the sensing in aqueous solution of phosphorylated peptide surfaces of biological importance.

Two anion sensors that also make use of Zn(II) dipicolylamine binding sites were described recently by other groups. Based on **7/47**, a sensing ensemble for phosphate was devised using pyrocatechol violet as indicator [360]. In the absence of phosphate, an equimolar mixture of the host and the indicator in water (10 mM HEPES, pH 7.0) gives rise to a blue solution. Addition of phosphate to this solution causes the color to change to yellow whereas no color change is observed in the presence of other anions such as sulfate, halides, carbonate, acetate, azide, or nitrate. A thermodynamic characterization of the underlying equilibria showed that this system represents one of the few examples in which binding of the host to the substrate (either pyrocatechol violet or phosphate) is exothermic in water and accompanied by a negative entropy change. The HPO_4^{2-} complex of **7/47** is slightly more stable ($K_a = 11.2 \times 10^4$ M^{-1}) than the one of pyrocatechol violet ($K_a = 5.3 \times 10^4$ M^{-1}) which is the prerequisite for an operating indicator displacement assay [360]. The azophenol based chromogenic sensor **7/48** is selective for pyrophosphate [361]. Binding can be detected by the color change of an aqueous solution (10 mM HEPES, pH 7.4) of this host from yellow to orange, whereas in the presence of other anions such as phosphate, citrate, acetate, or fluoride, the color of the solution remains unchanged. The stability constant K_a of the pyrophosphate complex of **7/48** amounts to 6.6×10^8 M^{-1} and is thus three orders of magnitude larger than the one of the HPO_4^{2-} complex [361].

Although in natural systems anion coordination to metal centers often involves porphyrin ligands, there are only few examples of synthetic porphyrin-based anion hosts that are active in aqueous solution. Examples are hosts **7/49**–**7/51** described by Imai and co-workers [362]. These compounds interact with α-amino carboxylates (aqueous $NaHCO_3/Na_2CO_3$ buffer, pH 10.4) most probably by coordination of the metal ion to the carboxylate group. Coulomb interactions and/or hydrophobic interactions between the hosts and additional functional groups on the substrates cause the complexes with, for example, aspartate or tryptophane to be significantly more stable than those with glycine [362].

An unusual organocobalt compound was introduced by Pfeffer and co-workers for anion recognition [363]. Complex formation of **7/52** with various anions such as Cl^-, Br^-, I^-, CH_3COO^-, $H_2PO_4^-$, and NO_3^- is due to electrostatic interactions in combination with coordination of the anionic guests to the cobalt atom. Among the different anions tested, acetate and dihydrogenphosphate are bound best with association constants log K_a of, respectively 3.0 and 3.1. The host is most efficient in slightly acidic solution. If the pH is too low, **7/52** decomposes while at too high pH, a water molecule coordinates to the deprotonated form of the host that cannot be displaced by anions in aqueous solution [363].

The simple ferroceneboronic acid **7/53** strongly interacts with fluoride in water or water/methanol mixtures via coordination of the anion to the Lewis acidic boron center [364]. This binding event can easily be followed electrochemically by the perturbation of the ferrocenium–ferrocene redox couple in the presence of fluoride. Binding is stronger to the oxidized form of **7/53** because of the electron-withdrawing nature of the ferrocenium unit that increases the electron-deficiency at the boron center. The association constant of the fluoride complex of the oxidized form of **7/53** amounts to 700 M^{-1} in water. Practically no binding of other anions was detected in this solvent with the exception of hydroxide that only interacts with the host at high pH, however [364].

Work in the Beer group centers around the development of sensors that allow an electrochemical detection of anions in solution [298, 365, 366]. This research has significantly advanced the field of anion coordination chemistry toward practical applications and some of the many elegant systems described in recent years are compounds **7/54**–**7/67** of which all are active in aqueous solvent mixtures [367–371]. These hosts do not, however, belong into the same category of metal containing anion hosts described so far because they use metal ions as electrochemically active reporter groups and not as Lewis acidic binding sites. In the oxidized state, ferrocene subunits do contribute to anion binding by electrostatic interactions, the primary recognition event between anions and **7/54**–**7/67** involves the linear or cyclic polyammonium moieties, however. As a consequence, binding properties of these hosts are essentially comparable to those of other polyammonium based anion receptors (*vide supra*). Anion affinity, for example, also increases with increasing degree of protonation of the hosts and with increasing negative charge of the guests. The major advantage of **7/54**–**7/67** is, of course, that anion binding causes a shift in the oxidation potential of the appended ferrocene subunits thus allowing complex formation to be detected by electrochemical methods. Moreover, if only one substrate is able to shift the redox wave at a certain pH, selective detection can be achieved. In this context it was shown, for example, that hosts **7/57**, **7/59**, **7/61**, and **7/63** can, at certain pH values, selectively detect sulfate and phosphate through an electrochemical response in the presence of competing anions such as nitrate, chloride, or acetate [369]. Besides sulfate and phosphate, most ferrocene containing polyamines also interact with ATP [367–371] and **7/63** even binds ADP and AMP [369], demonstrating the usefulness of these compounds for the electrochemical sensing of biologically active anions. That electrochemical anion sensing is not restricted to the combination of ferrocene units with polyamine frameworks demonstrates host **7/68**,

in which a guanidinium moiety mediates the detection of pyrophosphate in methanol/water mixtures [372].

Other examples for polyammonium based anion receptors that contain a metal complex as a reporter group are compounds **7/69–7/73**. Hosts **7/69–7/72** were shown to bind an sense phosphate and ATP in water via MLCT (metal to ligand charge transfer) luminescent emission quenching [373]. Also **7/73** binds ATP in 70% acetonitrile/water. Complex formation at pH 4 causes a remarkable enhancement of the emission properties of this host, an effect not observed for other anions such as chloride, sulfate, or phosphate. Thus, **7/73** allows a selective sensing of ATP in aqueous environment [374]. No direct participation of the metal centers in anion binding was observed in hosts 7169-7173, however.

8. Conclusion

Although anion coordination chemistry may have had a slow start this review clearly shows that today, it is a thriving field in supramolecular chemistry. Based on the pioneering efforts of a few groups, many structurally diverse synthetic anion receptors have been developed in the last two decades, some of which possess impressive anion affinity and selectivity. Moreover, the activity of these receptors is not restricted to organic solvents. Even in water, the medium in which biochemical anion binding processes occur, and in some cases in the presence of a large excess of competing salts, selective anion binding and sensing can be achieved with some systems. The types of interactions used in the synthetic systems for anion coordination are essentially the same as those found in Nature. Thus, receptors have been described that bind anions by electrostatic interactions, by metal coordination and by hydrogen-bonding in aqueous solution.

Microcalorimetric binding studies are becoming increasingly popular for the characterizations of interactions between a synthetic receptor and an anionic substrate since they provide a deeper insight into the thermodynamics of complex formation. The various examples presented in this review clearly demonstrate the important role entropy often plays in complex formation. Only in a few cases, for example in the interaction between vancomycin and its tripeptidic substrate in water, has a decrease in entropy been observed. Much more often, entropic factors favorably contribute to complex stability, and in systems where binding is athermic or endothermic, complex formation is entirely entropy driven. Entropic contributions are unfortunately much more difficult to predict than enthalpic ones in the deliberate design of a new receptor by, for example, molecular modeling. One can therefore only hope that the accumulating information regarding the interplay of enthalpy and entropy to binding will eventually facilitate the design of anion receptors with properties even more closely resembling those of natural systems. One promising approach could involve a more extensive use of synthetic receptors that employ a combination of different binding mechanisms for anion recognition.

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