Allosteric Supramolecular Receptors and Catalysts

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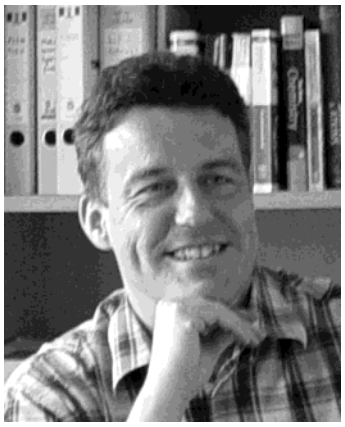
Larisa Kovbasyuk was born in Bila Tserkva (Ukraine) in 1973. She studied chemistry at the National Taras Shevchenko Kyiv University, where she undertook her doctoral study in the field of coordination chemistry. Following completing of her doctorate in 1999, Larisa Kovbasyuk joined Professor. Dr. R. Kaemer's research group as a postdoctoral fellow. She is currently working on discovery of novel allosteric catalysts based on metal complexes with polydentate organic ligands.



R. Krämer holds a chair of inorganic chemistry at the University of Heidelberg. After completing his undergraduate studies at the Universität Karlsruhe and Universität München (Germany), he received a Ph.D. in chemistry with W. Beck at Universität München. In 1991, he joined J.-M. Lehn at Universite Louis Pasteur, Strasbourg, as a postdoctoral fellow. After returning to Germany, he joined the Chemistry Department at the University of Münster, were he finished his habilitation in 1997. He moved to his present position in Heidelberg in 1999. His research is focused on coordination and bioinorganic chemistry, in particular synthetic mimics of metalloenzymes, bioinspired catalysis, and metal complex conjugates of oligonucleotides and peptide nucleic acids.

1. Introduction

The concept of allosteric proteins was developed in the early 1960s by Monod[1] and Koshland.[2] Allosteric is derived from the Greek root *allo*, meaning "the other". The binding of a regulatory molecule or ion to a specific allosteric site of the protein, structurally distinct from the active site, brings about an alteration of the conformation of the protein that indirectly modifies the properties of the biologically active site. This indirect mode of action via a distinct regulatory site is today the most commonly accepted meaning of the word "allosteric".[3]

Allosteric modulation of activity is fundamental for cellular function and is a common feature of biological receptors and enzymes, in particular those involved in metabolic pathways. The effector can either enhance (*positive* allosterism) or decrease (*negative* allosterism) the binding or catalytic efficiency of the protein. Ultimately, activity is switched ON (see Scheme 1) or OFF. The simplest mode of allosteric regulation, represented by a monomeric protein having distinct subunits for the binding of substrate and effector, is illustrated in Scheme 1.

In the case of an enzyme, the effector can influence either the affinity (the binding constant) for a substrate or the catalytic efficiency (rate of conversion of active site bound substrate), or both. In practice, monomeric allosteric proteins are very rare. Most allosteric proteins are oligomeric: they consist of several subunits, often two or four. The regulatory process is usually more complicated since conformational changes induced by an allosteric effector bind-

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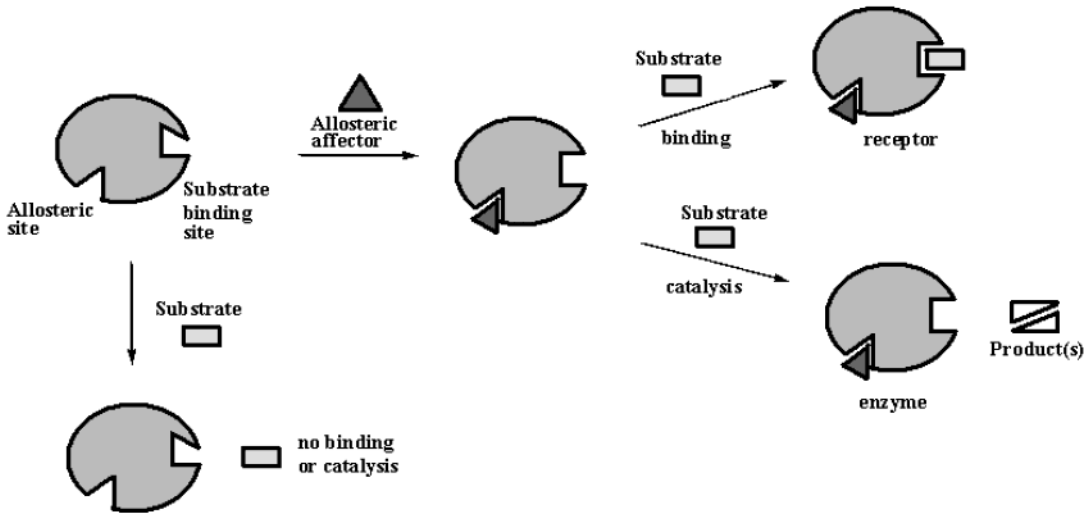
Scheme 1. Monomeric Allosteric Protein: Activation of Substrate Binding (If Protein Is a Receptor) or Catalytic Conversion (If Protein Is an Rnzyme) by an Allosteric Effector



ing to one subunit can be transmitted to the other subunit(s).

Depending on whether the binding of a molecule to the protein influences the interaction with a different or the same molecule, interactions are referred to as *heterotropic* or *homotropic*. Transmission of homotropic effects between protein subunits is an important aspect of cooperativity. The classic example is the binding of four dioxygen molecules by four subunits of hemoglobin in a cooperative fashion.[4]

Along with the development of supramolecular host−guest chemistry, fully synthetic allosteric receptors have been designed. Pioneering contributions by Rebek in the early 1980s[5−7] describe a 2,2′-bipyridine crown ether in which the alkali metal affinity of the crown unit (= receptor site) is significantly influenced by coordination of a transition metal to the 2,2′-bipyridine unit (= allosteric site).

The use of allosteric interactions enables chemists to control molecular function by external stimuli, to transduce chemical signals, and to achieve chemical feedback regulation. These are important aspects in the development of functional supramolecular devices of increasing complexity.

The aim of this review is to give a comprehensive, up-to-date overview of synthetic allosteric systems in which the interaction of a receptor or catalyst with a substrate is influenced by an effector not identical to the substrate (i.e., heterotropic allostery). Some of the compounds included here have been mentioned in Nabeshima's 1996 review on "regulation of ion recognition",[8] which focused on metal ions as guests or effectors. A recent short review by Shinkai[9] included only a brief section on heterotropic allosteric systems, with particular focus on the authors' own recent research achievements.

The present review does not cover the following topics related to allosteric behavior (or which may be considered so):

- homotropic allosteric systems, in which binding of one substrate molecule influences the binding of a second, identical substrate (such systems have been discussed in the context of cooperativity in an early review by Tabushi[10] and two recent short reviews by Shinkai,[9,11] with particular emphasis on the amplification of chemical or physical signals and on information transduction);
- indirect regulation of molecular function by light or redox processes (i.e., electron as an effector);[12]
- self-assembled systems in which one species, e.g. a metal ion, directs the self-assembly process of a receptor, which then binds a second species;[13]
- allosteric ribozymes and desoxyribozymes, which have emerged as an interesting new class of nucleic acid-based catalysts but have been covered by several recent reviews.[14−16]

A particular aim of this review is to identify the molecular design criteria and structural requirements for supramolecular allosteric systems, and to indicate how strong allosteric effects can be achieved, corresponding to an ON or OFF switching, for example, of a receptor property. A most recent development, the design of supramolecular allosteric catalysts which are synthetic analogues of allosteric enzymes, is discussed in detail.

2. Allosteric Receptors

2.1. Receptors for Metal Cations and Simple Anions

Many of the reported synthetic allosteric receptors bind alkali metal ions as substrates or effectors. This work has been inspired in part by the role of Na^+ and K^+ in the transduction of nerve signals. Some ion channels directly respond to concentration of these ions; additionally, membrane transport of Na^+ and K^+ is allosterically controlled, and changes in their concentration effect a biological response, such as the opening of ion channels.

2.1.1. Metal Ions as Allosteric Effectors

In nearly all supramolecular allosteric receptors, metal ions are either the effector or the substrate, or both. This is not surprising in view of the predicatble coordination behavior of metal ions with directed coordinative bonding, which facilitates the design of specific binding sites for metal ions. In

contrast, recognition of organic guests generally involves weaker and often nondirected interactions. Which of the two molecules or ions that interact with the receptor is the effector and which is the substrate is, in principle, arbitrary and is a question of interpretation. Often, the stronger binder, e.g. a transition metal ion, is considered the effector, and its influence on the second, weaker binder, e.g. an alkali metal ion, is significant and readily detectable, while this would be difficult the other way around.

The first example of an allosteric supramolecular receptor was described by Rebek and co-workers in 1979.[5−7] It is a 2,2′-bipyridine (bpy) to which a crown ether moiety was attached in 3,3′-positions (**1**) (Table 1). A $W(CO)_4$ fragment has the role of an allosteric effector when coordinated to the bidentate bpy site and affects interaction of Na^+ with the crown ether site. Relative binding constants were obtained by determination of partition coefficients for the extraction of Na^+ from the aqueous into the organic phase. The affinity of the crown ether for Na^+ is reduced about 5-fold by coordination of a $W(CO)_4$ fragment to the 2,2′-bipyridine unit.[5] Also, the K^+/Na^+ transport preference across a $CHCl_3$ liquid membrane is inverted by the allosteric effector.[6] Experiments were performed with the isolated, kinetically stable $W(CO)_4$ complex of **1**.

Not only the binding constant of $Hg(CF_3)_2$ to the crown unit of **1** is influenced by coordination of $PdCl_2$ to the bpy unit, but also the rate of its release from the host−guest complex in acetone/benzene, determined by ^1H and ^{19}F NMR spectroscopy.[7] Remarkably, allosteric Pd^{II} slows Hg release by 7 orders of magnitude and acts as an ON−OFF switch for the uptake and release of the linear $Hg(CF_3)_2$ complex by the crown ether. This is interpreted in terms of a rotaxane-type structure of the $Hg(CF_3)_2$ crown complex, in which the passage of a $-CF_3$ group through the crown ring is hindered by conformational restrictions in the Pd complex.

Two 2,2′-bipyridine derivatives are attached to a linear polyether chain in compound **2**, reported by Nabeshima and co-workers.[17] On addition of CuCl, formation of a cyclic complex is assumed in which the heavy metal ion is coordinated by both 6,6′-substituted bpy's of **2** in a tetrahedral fashion. Formation of such a pseudocrown structure increases the preorganization of the polyether chain and should provide a better selectivity for alkali metal ion binding. The selectivity of alkali metal ion transport rate across a CH_2Cl_2 liquid membrane is significantly influenced. With $n = 4$, K^+−picrate is transported 2.5 times faster than Na^+−picrate anion in the absence but 10 times faster in the presence of the allosteric effector Cu(I). Besides formation of the cyclic structure, electrostatic repulsion between Cu(I) and alkali metal ion is suggested to affect the selectivity of transport rates.[18]

A related idea was followed with system **3**, in which two polyether arms with appended 2, 2′-bipyridine derivatives were attached to a diazacrown ether. [19] Here, a pseudocryptand structure is expected to form on addition of a heavy metal ion which is complexed intramolecularly by the two bpy units. However, in contrast to the pseudocrown system **2**, practically no effect on the K^+/Na^+ transport preference across the CH_2Cl_2 membrane is caused by addition of Cu(I).

A Schiff base bis(crown ether) ligand described by Beer and co-workers forms, in an acetone (75%)/chloroform (25%) solution, a 1:1 complex with KNO_3 in which the alkali metal is coordinated sandwich-like by both crown ether units. [20] The potassium-binding properties of **4** were followed by ^{13}C NMR titration in the presence of two different metal ions, Cu(II) and Ag(I), which coordinate to the tetradentate S_2N_2 site of the ligand. While with Cu(II) a 1:1 **4**−K^+ complex is preferred as in the case of **4** alone, the Ag(I) complex binds 2 equiv of K^+. This behavior is interpreted in terms of the preferences for different coordination polyhedra of Cu(II) and Ag(I): the square-planar geometry of Cu(II) favors the sandwich-type structure, while tetrahedral Ag(I) moves the two crown ether moieties apart.

Bis-crown ether receptor **5** was designed similarly.[21,22] $(bpy)_2Ru^{2+}$ and $(CO)_4Cr$ moieties were used as allosteric effectors, forming kinetically inert complexes with the 2,2′-bipyridine unit. Improved preorganization of the crown ethers in $[(bpy)_2Ru(\mathbf{5})]^{2+}$ favors formation of a 1:1 Na^+ complex, while a 2:1 complex is preferred between Na^+ and free **5** in methanol. In contrast, the organic "diquat" dication PF_6^- salt intercalates intramolecularly between the benzo crown ether units of free **5**, while binding to the Ru and Cr complexes is much weaker. Apparently, the rigid conformation of the metal complexes is not favorable for an intercalation of the organic guest.

Positive allosteric effects in alkali metal binding were observed by Kobuke with ionophores **6** in which two catechol groups were attached to a polyether chain.[23] Free **6** practically does not interact with Na^+ and K^+ in ethanolic solution. On addition of boric acid, boron forms an intramolecular tetrahedral complex with both catecholates, and this compound yields isolable Na^+ and K^+ complexes which were characterized by NMR and mass spectrometry. K^+ and Na^+ binding by **7** is also induced by boric acid.

Effects on alkali metal binding were also large with ionophore **8**, in which two β-diketone units were attached to the polyether.[24] Free **8** practically does not extract Na^+−picrate and K^+−picrate from the aqueous to the $CHCl_3$ phase. When Cu(II) ions are added, an intramolecular, planar bis(β-diketonate) complex is formed, similar to cupric bis(acetylacetonate). This complex, and in particular the larger ring form ($n = 2$), efficiently extracts Na^+ and K^+ picrates into the organic phase, with a preference for Na^+ of the smaller ($n = 1$) and for K^+ of the larger ($n = 2$) pseudocrown. The higher affinity for alkali metal ions than of **2** is explained by reduced electrostatic repulsion as a consequence of anionic diketonate ligands and contributions from diketonate oxygens to alkali ion complexation. The latter was confirmed by a crystallographic structure determination of a mixed copper(II)−potassium(I) complex of **8**.[25] Complexes with allosteric Ni(II) and Zn(II) ions also show selectivities, but these metals are much less effective than Cu(II). Hydrophobic anions such as anilino-

Table 1. Allosteric Receptors 1−47 Together with Their Substrates and Effectors [K_{Rel} Is the Substrate Binding Constant of the Receptor in the Presence of the Effector, Divided by the Substrate Binding Constant in the Absence of Effector (*, Relative Transport Rate through Liquid Membrane in the Presence/Absence of Effector; **, Relative Extractability from the Aqueous into the Organic Phase in the Presence/Absence of Effector)]

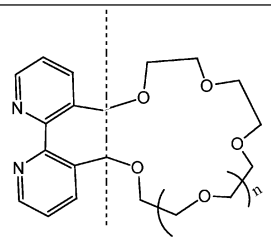
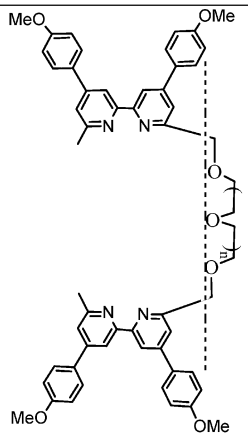
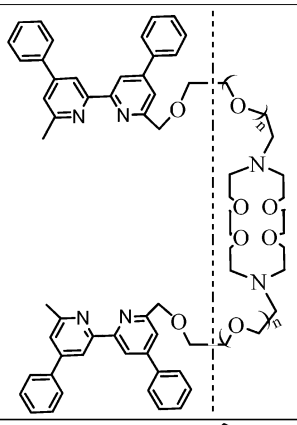
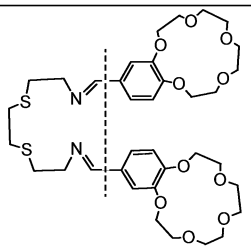
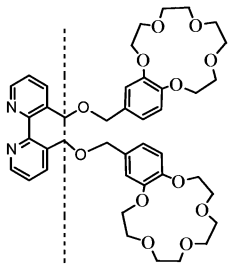
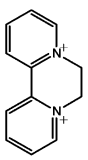
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
1	$W(CO)_4$		Na^+ (n=1) Li^+ (n=1) (n=2) K^+ (n=1) (n=2)	0.2** 0.8* 2.8* 0.2* 0.8*	5 6
	Pd^{2+}		$Hg(CF_3)_2$		7
2	Cu^+		Li^+ (n = 4) Na^+ (n = 4) K^+ (n = 4)	0.6* 0.04* 0.2*	17, 18
3	Cu^+		Na^+ K^+	≈ 1* ≈ 1*	19
4	Cu^+ Ag^+		Na^+ K^+		20
5	$[Ru(bipy)_2]^{2+}$ $Cr(CO_4)$		Na^+ 	<<1	21, 22

Table 1. (Continued)

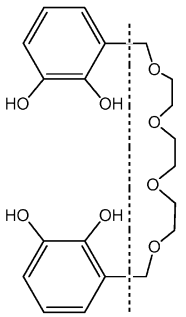
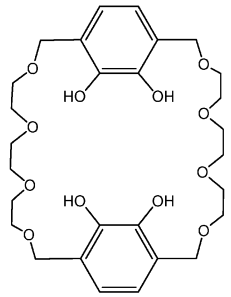
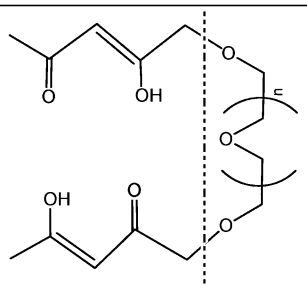
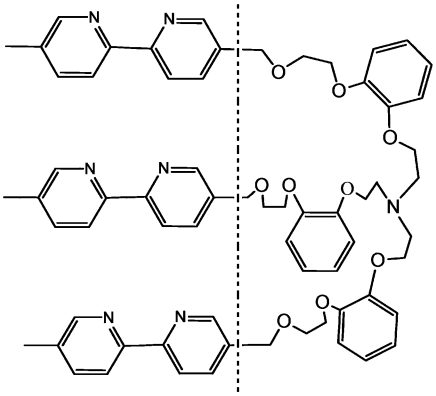
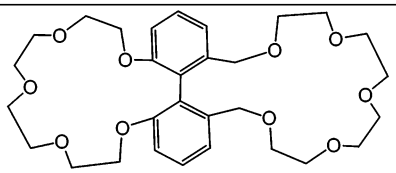
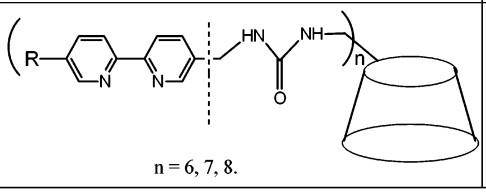
Nr.	Allost. effector	allosteric site : receptor site	substrate	K_{rel}	Ref
6	B^{3+}		K^+ Na^+	> 1	23
7	B^{3+}		K^+ Na^+	> 1	23
8	Cu^{2+} Zn^{2+} Ni^{2+}	 n = 1, 2.	Li^+ Na^+ K^+ Rb^+	>> 1**	24, 25
9	Fe^{2+}		K^+ Rb^+ Cs^+	0.2 2.3 39.0	26
10	$Hg(SCN)_2$		Na^+	< 1	27
11	Co^{2+}	 n = 6, 7, 8.	Eu^{3+} (n = 6, 7, 8)	<< 1	28

Table 1. (Continued)

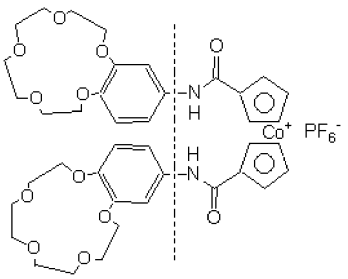
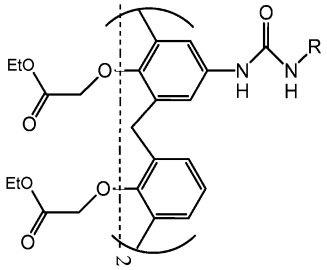
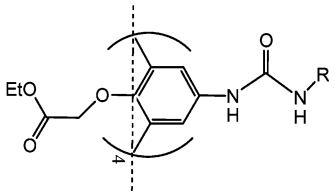
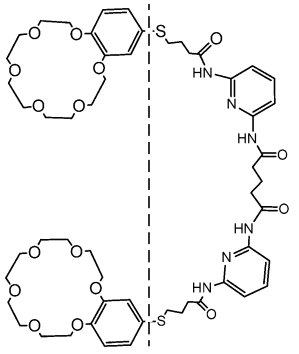
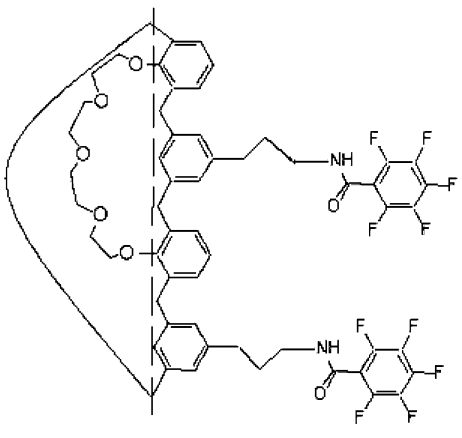
Nr.	Allost. effector	allosteric site | receptor site	substrate	K_{rel}	Ref
12	K^+		Br^- Cl^-	<< 1	29
13	Na^+, K^+		Br^- Cl^-	>>1	30
14	Na^+, K^+		Br^- Cl^-	>>1	30
15	Cs^+		Br^- Cl^-	10.0 45.0	31
16	K^+		$CH_3CO_2^-$	4.0	32

Table 1. (Continued)

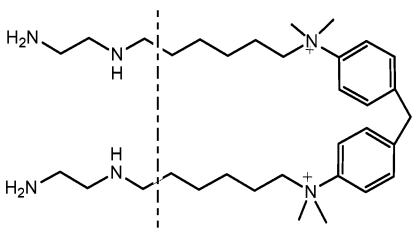
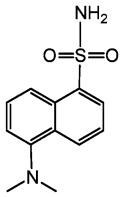
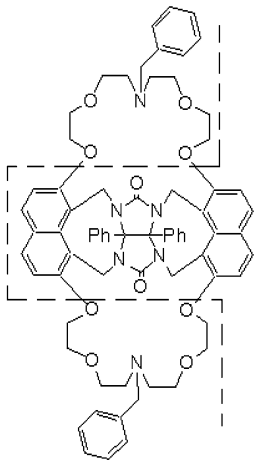
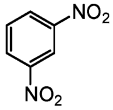
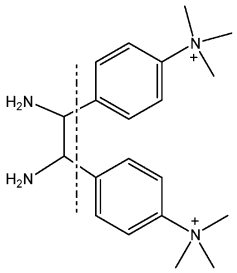
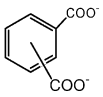
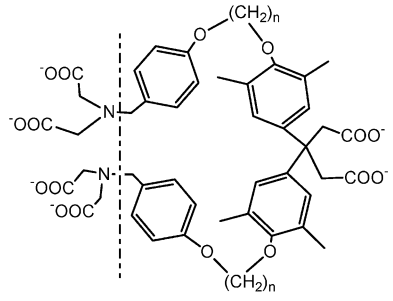
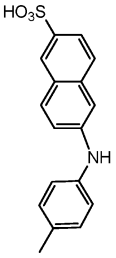
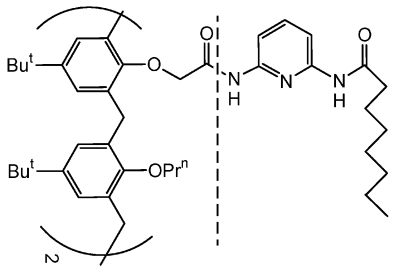
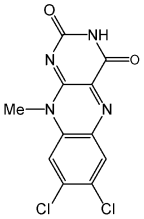
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
17	Cu^{2+} Zn^{2+}			10-100	33
18	K^+			6.0	34
19	Cd^{2+}			~ 0.5	35
20	Ca^{2+}			>>1	36
21	Na^+			>>1	37

Table 1. (Continued)

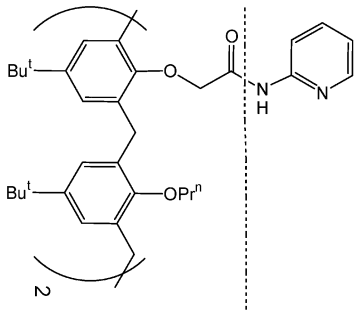
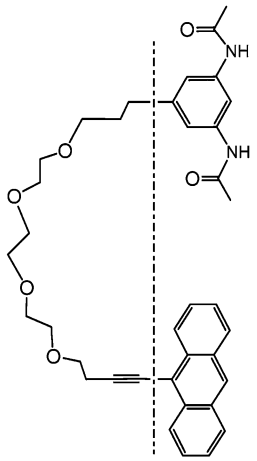
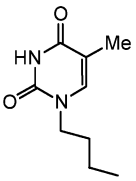
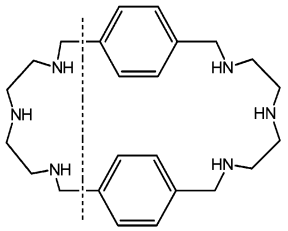
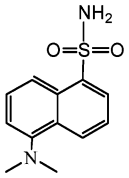
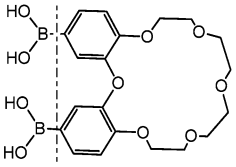
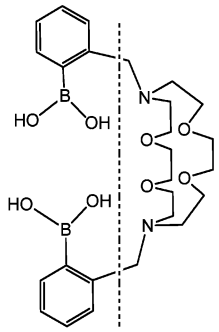
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
22	Na^+			>>1	38
23	Na^+			6.0	39
24	Zn^{2+}			100.0	40
25	Rb^+		D-allose	0.3	42
26	Ca^{2+} Sr^{2+} Ba^{2+}		D-fructose, D-glucose	<1	43, 44

Table 1. (Continued)

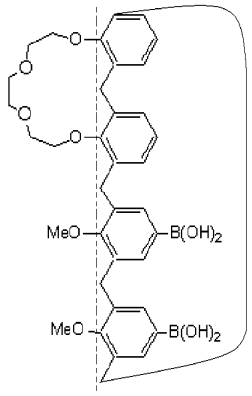
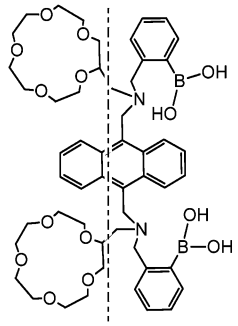
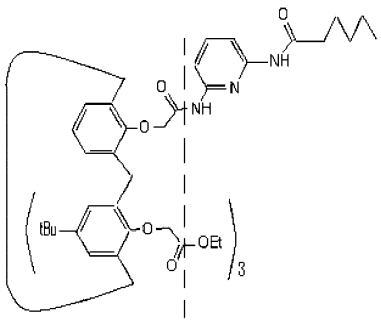
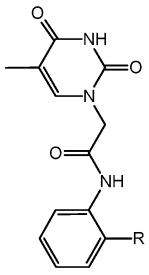
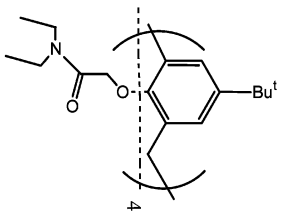
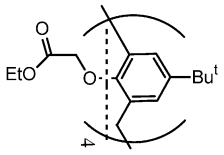
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
27	Mg^{2+} Ca^{2+} Li^{+} Na^{+} K^{+} Rb^{+} Cs^{+}		D-glucose	<1 >1	45
28	Na^{+} K^{+} Sr^{2+} Ba^{2+}		D-glucose	<1	46
29	Na^{+}		 R = Zn-porphyrin	>1	47
30	Na^{+} Na^{+} K^{+}		CH_3NO_2 CH_3CN	>1 	48 49
31	Na^{+}		CH_3CN 	>1 >>1	50 51

Table 1. (Continued)

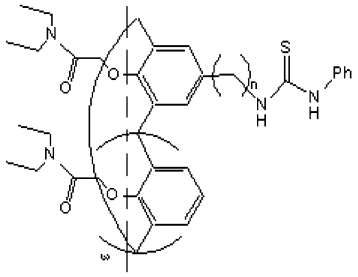
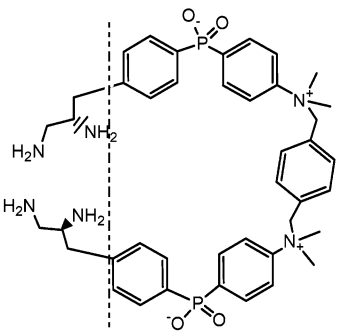
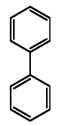
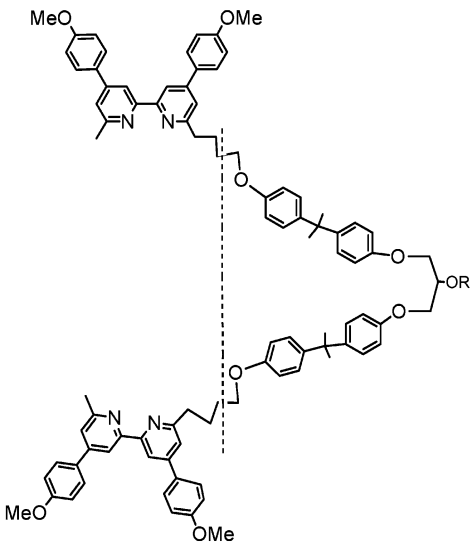
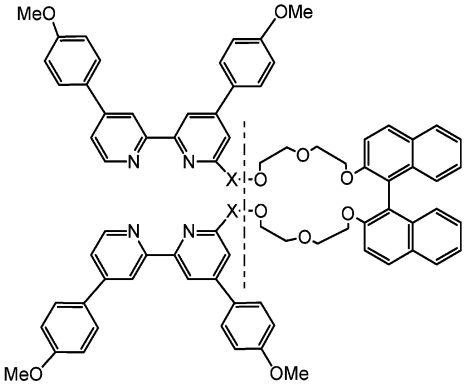
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
32	Na^+		Benzoate AcO^- Propionate	4.4 (n=0) 1.1 (n=1) 1.1 (n=0) 0.7 (n=1) 4.0 (n=0) 0.8 (n=1)	52
33	Cu^{2+}, Zn^{2+}		Dansylated aminoacids  	>1 >1 >1	53
34	Cu^+	 R_1 = CH_2-m-C_6H_4-$CH_2N^+Me_3Br^-$, R_2 = *t*-$BuMe_2Si$	Flavine mononucleotide	4.4* (R_1) 1.8*(R_2)	54
35	Cu^+	 X_1 = $CH_2CH_2CH_2$, X_2 = CH_2	L-tryptophane, D-tryptophane	4.7* (X_1) 4.3* (X_2) 5.8* (X_1) 4.2* (X_2)	55

Table 1. (Continued)

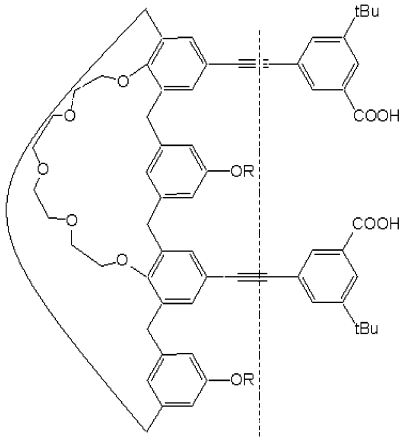
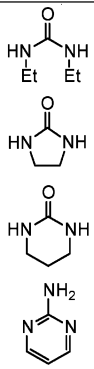
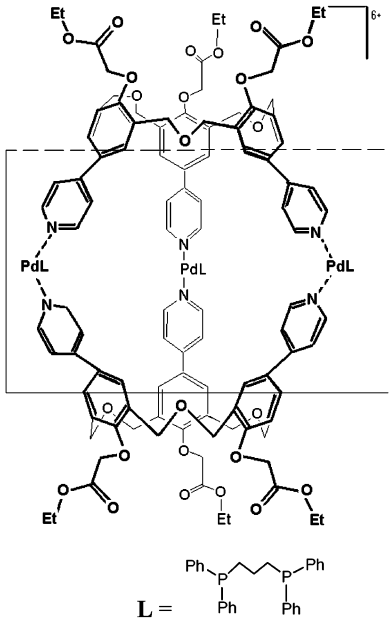
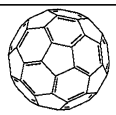
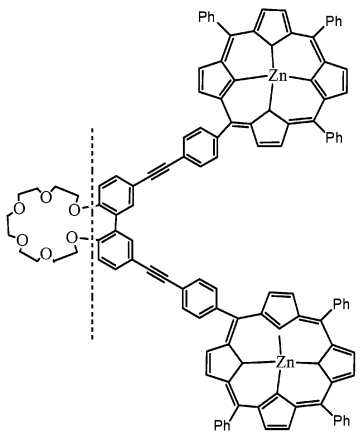
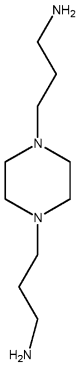
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
36	Na^+; K^+	 R = CH_2CH_2OEt		29.5; 3.9 10.0; 4.1 9.5; 2.1 1.5; 3.0	56
37	Li^+ Na^+	 L =		54.0 <<1	57
38	Ba^{2+}			< 1	58

Table 1. (Continued)

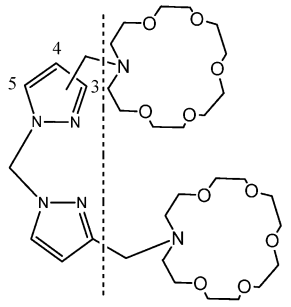
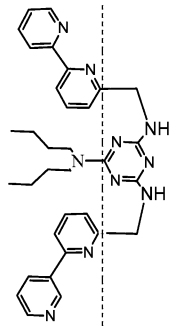
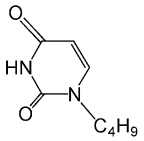
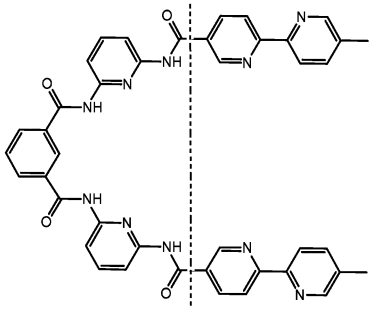
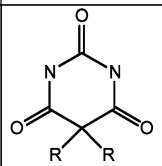
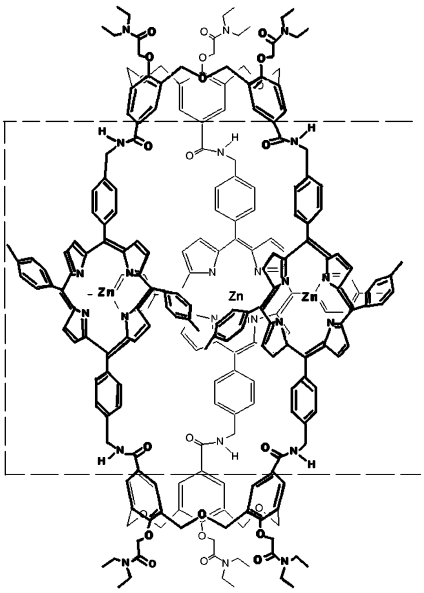
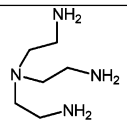
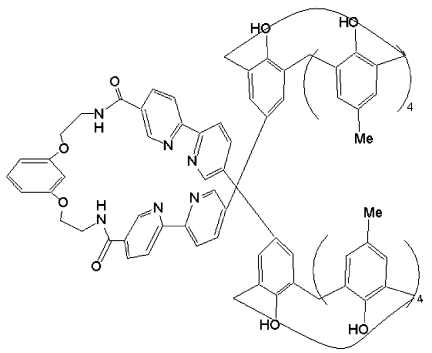
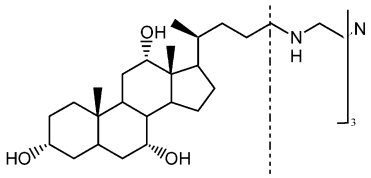
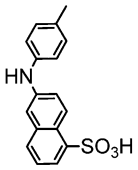
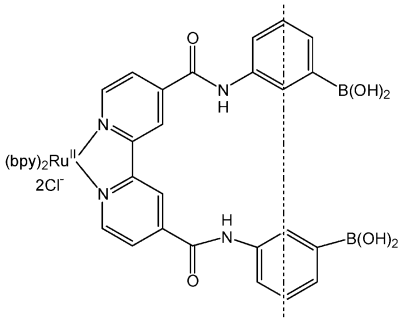
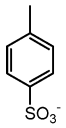
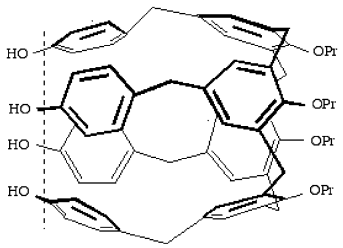
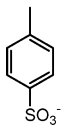
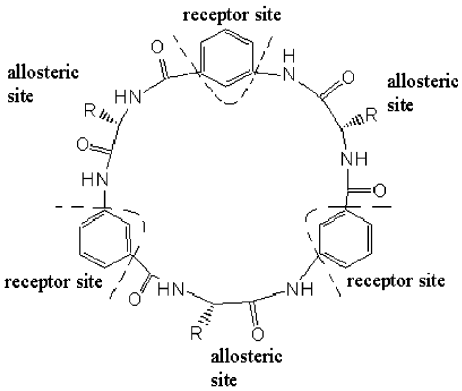
Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
39	Zn^{2+}		$NH_2(CH_2)_nNH_2$ n = 5, 6, 7, 8, 9	3-subst. 1.1;(n=5) 1.2,(n=9) 4 - subst. 0.9;(n=5) 0.9,(n=9) 5 – subst. 0.9;(n=5) 0.8,(n=9)	59
40	Cu^{+}			<< 1	60
41	Zn^{2+}			< 1	61
42	Na^{+}			900.0	62

Table 1. (Continued)

Nr.	Allost. effector	allosteric site ⋮ receptor site	substrate	K_{rel}	Ref
43	Cu^{+}		C_{60} C_{70}	39.0 4.0	63
44	Zn^{2+}			>>1	64
45	NaH_2PO_4 / Na_2HPO_4		Saccharides fructose	 2.1	65
46			$[NMe_4]^{+}$	11.0	66
47			$[NMe_3n\text{-}Bu]^{+}$	10^4	67

R = $CH_2CH_2COOCH(CH_3)_2$

naphthalene sulfonate improve the extractability and metal ion selectivity of (**8**)Cu(II).

Recently, Nabeshima[26] described allosteric effects in a pseudocryptand approach using the tripodal polyether−bpy receptor **9**. Fe(II) forms a 1:1 complex with **9** by coordination to the three bpy units. The polyether chains are highly preorganized in this complex. Binding constants for alkali metal perchlorate were determined by ^{1}H NMR titration. (**9**)Fe^{2+} binds Cs^+ 39 times better than free **9**, but K^+ 5 times worse. Similar trends were observed for ion transport through liquid membranes.

Costero's receptor **10** contains two crown ether units which are conformationally related by a biphenyl unit.[27] **10**, with $Hg(SCN)_2$ selectively bound to the allosteric crown site, transports NaPic about 5 times less effectively through a $CHCl_3$ liquid membrane than the corresponding monocrown receptor which lacks the allosteric crown unit. Complexation of the $Hg(SCN)_2$ unit by weak Hg−O interactions with all five O-donors of the allosteric crown unit is confirmed by the X-ray crystal structure of (**10**)$Hg(SCN)_2$.

In **11**, as recently reported by Marsura and co-workers,[28] six to eight 2,2′-bipyridine units have been tethered to the upper rim of a cyclodextrin by urea-based linkers. **11** has a "hard" binding site, formed by the urea groups, for the complexation of hard lanthanide ions such as Eu^{3+} and a soft 2,2′-bipyridine binding site suitable for, e.g., Co^{2+}. While a 1:1 **11**−Eu^{3+} complex in methanolic solution is identified by photometry, Eu^{3+} does not bind to **11** in the presence of 1 equiv of Co^{2+}, which is suggested to induce a conformational change by coordination to the bipyridine groups, resulting in negative allostery.

Few examples of the allosteric modulation of receptors for simple anions have been reported. A cobalticinium bis(benzo crown ether) **12** was introduced by Beer.[29] 1:1 complexes of **12** with chloride or bromide were detected by ^{1}H NMR spectroscopy in CD_3CN. Presumably, the anions interact with the two amide NH groups by H-bonding. In contrast, the potassium complex [(**12**)K][BPh_4]$_2$ does not interact with these anions. K^+ is a negative allosteric effector and, by coordination to the crown moieties, apparently disturbs the spatial arrangement of the anion-binding site. In contrast, no effect on the anion affninity of **12** is observed for Na^+, which forms a 1:2 complex, [(**12**)Na_2][BPh_4]$_3$.

In calixarene-based receptors reported by Reinhoudt's group,[30] the upper rim urea groups are not available for anion complexation due to intramolecular H-bonding in the case of **13** and formation of H-bonded dimers in the case of **14**. Therefore, **13** and **14** do not bind Cl^- or Br^- as their NBu_4^+ salts. However, complexation of the Na^+ and K^+ salts of these anions in $CDCl_3$ is observed by ^{1}H NMR. Binding of the alkali metal cation by the lower rim ester and ether oxygen donors stabilizes an open conformation of the calixarene and triggers simultaneous binding of the anion by the urea groups.

Nabeshima's bis(crown)-substituted hydrogen-bonding receptor **15** has a moderate affinity for Cl^- and Br^- ions in $CDCl_3/CD_3CN$ 4:1, as detected by NMR spectroscopy.[31] Formation of a 1:1 Cs^+ complex with both crown units organizes the two H-bond-donating 2,6-bis(acylamido)pyridine moieties. This leads to enhanced binding contansts, 10-fold in the case of Br^- and 45-fold in the case of Cl^-.

Casnati and Ugozzoli's calix[4]arene host **16**, with a lower rim crown moiety and upper rim amide functionalities, binds acetate in $CDCl_3$ 4 times better ($K = 140$ M^{-1}) in the presence of $KBPh_4$ than in its absence.[32] An X-ray crystal structure of the calixarene with complexed K^+ and acetate shows that the K^+ complex favors an open conformation but also a self-assembled 2:2:2 structure in the solid state. The acetate ions act as bridges and link two amide NH groups of different calixarenes.

2.2. Receptors for Organic Molecules

Many allosteric receptors which recognize organic molecules have been described, and in the majority of them a metal ion is the allosteric effector. Receptor−substrate interactions include aromatic π-stacking, solvophobic interactions, hydrogen bonding, and electrostatic interactions.

2.2.1. Metal Ions as Allosteric Effectors

Schneider and co-workers described in 1990 a receptor **17**, having a lipophilic subunit for binding of aromatic guests and an allosteric bis(ethylenediamine) subunit.[33] On addition of Cu^{2+} or Zn^{2+} ions, a pseudo-macrocycle with a hydrophobic pocket is formed by intramolecular bis(bidentate) coordination of the metal ion. The binding constants of aromatic guest molecules such as dansyl amide in water are increased 10−100-fold in the presence of the metal ions. Besides hydrophobic interactions, the quaternary ammonium groups of **17** also contribute to the binding of aromatic guests. Binding of dansyl amide is monitored by an optical signal: the fluorescence of dansyl amide (emission at 510 nm) bound to the metalated receptor is increased about 2-fold.

A "molecular clip" with allosteric binding properties was communicated by Nolte.[34] Receptor **18** displays two azacrown sites for binding of alkali metal ions and two naphthalene moieties which can potentially enclose an aromatic guest molecule, depending on the conformation of the molecule. A ^{1}H NMR conformational analysis of **18** in a $CDCl_3$-d_6−DMSO mixture indicates a predominant conformation which disfavors binding of aromatic guests, while the active conformation is less populated. The latter is induced by addition of 2 equiv of KPic or KSCN, with binding of K^+ to the azacrown sites. Consequently, **18** is turned into a receptor that has a 6-fold higher binding constant for 1,3-dinitrobenzene.

A scissors-shaped receptor **19** was presented by the group of Schneider.[35] The two quaternary ammonium groups interact in D_2O solution electrostatically with *o*-, *m*-, and *p*-phthalic acid dianionic guests. Coordination of Cd(II) ions to the chelating diamino site of **19** reduces the conformational flexibility and results in a weak negative allostery, with binding constants reduced by a factor of about 2.

A cyclophane host that is organized by Ca^{2+} binding was communicated by Deshayes and co-workers.[36] While **20** alone (60 μM) does not appear to bind a

6-toluidino-2-naphthalenesulfonic acid guest in aqueous solution, intramolecular coordination of Ca^{2+} to both iminodicarboxylate groups of **20** increases significantly the affinity. Binding is monitored by a strong increase of fluorescence of the substrate (which has a very low fluorescence intensity in water). Also, there is a substantial blue shift of emission from 500 to 420 nm. Guest binding to the hydrophobic part of the cavity is more efficient for a somewhat larger cavity with $n = 4$ versus $n = 3$.

An allosteric Na^+ ion converts the calix[4]arene receptor **21** studied by Shinkai et al.[37] from a closed into an open form. In the closed conformation, there is an intramolecular H-bonding interaction of the two 2,6-bis(acylamino)pyridine moieties which prevents binding of a pteridine-type host. Addition of $NaClO_4$ with coordination of Na^+ to the ether and amide oxygens of **21** is suggested to disrupt intramolecular H-bonding and affect conformation. The Na^+-coordinated "open" form interacts with the pteridine guest by hydrogen bonding. The association constant $K = 1200\ M^{-1}$ was determined in chloroform solution by fluorimetry since pteridine fluorescence at 513 nm is efficiently quenched on interaction with the 2,6-bis(acylamino)pyridine unit. No binding of pteridin guest is detected in the absence of Na^+. A related receptor **22**[38] is switched on by Na^+ for binding of γ-butyrolactam, as detected by NMR spectroscopy.

Another H-bonding receptor **23**[39] based on 2,6-bis-(acylamino)pyridine binds the nucleobase thymine in $CDCl_3$. On addition of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, **23** becomes a 6 times more effective thymine receptor. Inouye et al. suggested that the Na^+ ion, by coordination to the polyether chain, organizes **23** in a scorpion-like conformation. This would place the anthracene ring directly above the bound thymine substrate to add an additional binding force from aromatic π-stacking.

A second Zn^{2+}-activated receptor for dansyl amide is Schneider's polyaza-macrocycle **24**.[40] The allosteric effect is much stronger than in the case of **17**: while the affinity of dansylamide to **24** alone in neutral water is very small, the binding constant is increased about 100-fold by addition of 1 equiv of $ZnCl_2$, again monitored by fluorescence. The metal ion is expected to complex one tridentate subunit of **24**, and on the basis of force-field calculations it is suggested to contract the macrocycle and organize the two benzene groups for intercalation of the guest.

Shinkai and co-workers have developed diboronic acid-based receptors for saccharides[41] and have successfully combined these with allosteric binding sites for metal ions which allosterically modulate the receptor properties. **25** displays negative allostery on interaction of metal ions with a crown ether incorporated in the same molecule.[42] The receptor operates by reversible covalent interaction of the boronic acid pair with the sugar by formation of two cyclic diesters with the sugar hydroxo groups. Binding of saccharides D-glucose, D-talose, D-allose, and D-mannose in alkaline methanol/water 9:1 solution is monitored by circular dichroism. Addition of metal salts KSCN, $Ca(ClO_4)_2$, and $Mg(ClO_4)_2$ with complexation of the metal ion by the crown ether in **25** eventually leads to a decrease of substrate affinity of the receptor; e.g., with Ca^{2+} the binding constant for D-allose is reduced 4-fold from 1180 to 300 M^{-1}. This negative allostery is explained by a disposition of the boronic acids which is unsuitable for 1:1 binding of saccharides. A related receptor **26** containing a diazacrown unit displays negative allostery on interaction with Ca(II) and—with similar efficiency—Sr^{2+} and Ba^{2+}.[43, 44]

Calix[4]arene **27**, in which two boronic acids are attached to the upper rim and a crown loop to the lower rim, is a receptor for D-glucose and other monosaccharides in methanol/water 9:1.[45] Small alkaline and alkaline earth metal ions which bind to the lower rim of **27** stabilize a conformation that disfavors simultaneous glucose binding by both boronic acids, as monitored by circular dichroism. In contrast, positive allostery is observed with larger alkali ions K^+, Rb^+, and Cs^+. It is suggested that these metal ions are too large for lower rim chelation by ether oxygens and that rather a metal—π interaction is involved and stabilizes a conformation favorable for sugar binding

The glucose receptor **28** incorporates two crown units and an anthracene fluorescent reporter groups.[46] On binding of saccharides, fluorescence increases as a consequence of an increased acidity of boronic acid and a therefore strengthened Lewis-acid base interaction with the tertiary amine, which becomes a less efficient quencher of the fluorophore. Alkaline and alkaline earth metal ions, for example Na^+ and in particular Ba^{2+}, disfavor binding of D-glucose to the ditopic cleft of receptor, as monitored by fluorescence decrease and circular dichroism.

Allosteric behavior of higher complexity was observed by Reinhoudt and Rudkevich's groups with receptor **29**.[47] A 2,6-bis(acylamino)pyridine unit in this calix[4]arene host does not interact by H-bonding with complementary thymine derivatives, possibly as a consequence of intramolecular H-bonding with the calixarene ester groups. The ability to form H-bonds is switched on by addition of NaSCN, since Na^+ complexes the ester groups. The binding constant of (**29**)Na^+ for butylthymine in toluene, detected by ^1H NMR, is in the order of $10^3\ M^{-1}$. When the thymine guest is attached to a Zn—porphyrin and NaSCN is used, a stable quaternary complex (**29**)Na^+(Zn—porphyrin)SCN is formed, with SCN^- complexed axially to the Zn^{2+} ion. The affinity of the Zn—porphyrin for SCN^- is improved by more than 3 orders of magnitude as followed by photometry. This is interpreted as an additional electrostatic interaction of the SCN^- anion with the Na^+ cation in the quaterny complex. The complexation of Na^+ turns **29** into a ditopic receptor with a H-bonding site for thymine binding and a second site, generated by the allosteric effector itself, for interaction with the SCN^- anion, which in turn is associated with the first guest molecule.

Another example of a calix[4]arene host, the binding properties of which are switched on by complexation of Na^+ to lower rim ether and amide oxygen atoms, is compound **30**, studied by the group of Pochini.[48] While **30** alone in $CDCl_3$ does not interact significantly with nitromethane, a CH_3NO_2 binding

constant $K = 34\ M^{-1}$ is determined for its complex with NaPic by ^1H NMR spectroscopy.

Later, Pochini and co-workers reported crystal structures of both Na^+ and K^+ complexes of **30** with an included CH_3CN guest.[49] Coordination number 8 of the alkali ions, interacting with four ether and four amide oxygens, was confirmed. The calixarene adopts a cone conformation, and the CH_3 group of the CH_3-CN guest is located in the cavity formed by the arene groups. Subtle confomational differences between the Na^+ and K^+ complexes were identified: the cavity is somewhat wider with the smaller Na^+ ion as a consequence of contraction of the lower rim ligand sphere which results in an opening of the cavity.

Stibor's group has described a closely related calixarene receptor in which the amide groups of **30** are replaced by ester groups.[50] Host **31** is only a receptor for acetonitrile (^1H NMR study) on addition of NaSCN, with Na^+ stabilizing an open structure by coordination to the upper rim ester substituents.

In a study reported by S. Shinkai,[51] **31** did not interact with a potential fullerene (C_{60}) guest, possibly because the cavity prefers a "closed" conformation and is too small for C_{60} binding. On addition of $CsBPh_4$, Cs^+ coordinates the ester groups and appears to freeze in an open conformation. Binding of C_{60} to the 1:1 complex (**15**)Cs^+ in toluene solution was qualitatively detected by photometry (fullerene absorbance) and ^1H NMR. This effect is specific for Cs^+ and is not observed for other alkali metal ions. For smaller rim calixarene esters, Li^+ was shown to have a strong allosteric effect.

A calix[4]arene receptor **32**, similar to **30** but functionalized at the upper rim by a thiourea H-bond donor group, interacts with carboxylates.[52] Again, addition of $NaBPh_4$ with complexation of Na^+ to the lower rim rigidifies the calixarene apolar cavity. Coordination to the receptor with $n = 1$ induced negative allostery for most carboxylates, whereas with the $n = 0$ compound a positive allostery was determied by a NMR titration experiment in DMSO-d_6. This is explained by a combination of steric and electronic effects; the latter is suggested to originate from a small electron-withdrawing effect of the Na^+ ion which transfers to the NH group when thiourea is directly bound to the arene but not when a methylene group is inserted.

In an approach related to system **17**, Schwabacher and co-workers have prepared compound **33** which forms a hydrophobic cavity on intramolecular metal ion complexation by the two diaminoethane moieties.[53] On addition of Zn^{2+} and Cu^{2+} sulfates, the allosteric effect is strong, and interestingly, different coordination geometry preferences of Zn and Cu appear to affect the shape of the nonpolar cavity and influence the affinity and even selectivity for aromatic guests. A substituted biphenyl is bound 600 times more strongly by the Cu(II) complex than by the Zn(II) complex. The Zn(II) complex binds a substituted naphthalene about 3 times better than the biphenyl, while the Cu(II) complex has an inverse selectivity with an about 8-fold preference for the biphenyl (binding constants determined by ^1H NMR titration in D_2O). Metalated **33** is also a receptor for dansylated amino acids, the affinity depending strongly on amino acid structure. A mixture of **33** and a dansylated amino acid is a fluorescent sensor for Zn^{2+} ions, since fluorescence strongly increases on addition of Zn. The affinity and sensitivity of the sensor can be tuned by variation of the dansylated amino acid; i.e., an amino acid with high affinity to (**33**)Zn provides a sensor system with high Zn sensitivity.

Biomolecule receptors **34** and **35** were reported by Nabeshima.[54,55] The recognition site is either a hydrophobic pseudocyclophane cavity (**34**) or a crown ether (**35**), both organized by intramolecular coordination of Cu(I) to the allosteric bis(6,6′-disubstituted 2,2′-bipyridine) site, presumably in a tetrahedral fashion. **34** extracts flavin mononucleotide, which is an important cofactor for various redox enzymes, from the aqueous into the organic phase more effectively in the presence of CuCl. Release of flavin from the receptor is achieved by addition of bathocuprein, which forms a stable Cu(I) complex and removes copper from the allosteric site. **35** is a transporter for the amino acid tryptophane through a liquid CH_2ClCH_2Cl membrane. In the presence of Cu(I), transport is up to 6 times more efficient. This is attributed to the organization of the polyether chain into a pseudocrown structure which interacts well with the ammonium group of the amino acid.

Fukazawa's group has presented a study on the binding of various guests by allosteric monodeoxycalix-[4]arene crown ether **36**.[56] Two "long arms" with terminal carboxylic acid functions represent the guest-binding portion of the molecule. Binding constants for urea- and guanidine-type guest in $CDCl_3$ were determined by ^1H NMR spectroscopy. Simultaneous interaction of both $-CO_2H$ functions of **36** with the guest molecules by H-bonding is indicated by NH-proton downfield shifts. Addition of NaPic and KPic enhances the guest-binding constants. On the basis of molecular mechanics calculations, this is interpreted in terms of improved preorganization of the carboxylic groups for guest binding when Na^+ or K^+ is complexed by the crown moiety, while in metal-free **36** intramolecular H-bonding of the two $-CO_2H$ functions is relevant.

Shinkai and co-workers have investigated the C_{60}-binding properties of capsule **37** formed by Pd(II)-induced self-assembly of two homooxacalix[3]arene molecules.[57] An association constant $K = 40\ M^{-1}$ for C_{60} binding in $Cl_2CDCDCl_2$ was detected by NMR spectroscopy. In the presence of CF_3SO_3Li, the binding constant is $2100\ M^{-1}$, which corresponds to a 54-fold increase. Two Li^+ ions are suggested on the basis of NMR data to bind to the $-OCH_2CO_2Et$ groups of the lower rims and to induce a conformation of the cavity which is more suitable for the guest inclusion. On the other hand, addition of Na^+ ions to the C_{60} complex of **37** leads to a release of the guest molecule, indicating strong negative allostery.

Two Zn−porphyrin units in **38**, prepared by Kubo and co-workers, act as a tweezer when interacting with ω,ω-diamine substrates by amino coordination to Zn.[58] Incorporation of the diamine is easily followed by changes of the strong porphyrin optical absor-

bance. On addition of Ba^{2+}, the affinity of **38** for the diamine in CH_2Cl_2/CH_3CN (9:1) is diminished (negative allostery). Force-field calculations suggest that complexation of Ba^{2+} to the crown ether reduces the flexibility of the molecule and could block a diamine-induced conformation change.

Brunet and Roriguez-Ubis presented a bis(azacrown) receptor **39** which transports alkyl diammonium picrates through a liquid $CHCl_3$ membrane.[59] When Zn^{2+} ions are complexed by the bidentate pyrazolyl allosteric site, they improve the transport rate by 25% when both azacrowns are attached to the 3-positions of pyrazoles (3-substituted). In contrast, transport is slightly inhibited in the case of 4- and 5-substitution. The results are interpreted in terms of preorganization of the crown units which interact with the two ammonium groups of the guest by H-bonding in a 1:1 complex.

A case of strong metal-induced negative allostery is reported by Branda and co-workers.[60] Receptor **40** is based on the triaminotriazine scaffold, which presents a hydrogen bond surface well suited to act as host for imide guests such as uracil, as detected by ^{1}H NMR titration in $CD_3CN/CDCl_3$ 1:1. When **40** is exposed to $[Cu(CH_3CN)_4]PF_6$, the two bpy arms coordinate the metal ion and form a tetrahedral 1:1 complex. The conformational change relevant to allosteric behavior is evidenced by a crystal structure determination. Orientation of the exocyclic NH groups of the triazine moiety in (**40**)Cu(I) disfavors triple H-bonding with uracil, and affinity to uracil in solution is indeed very much reduced.

The studies have very recently been expanded to a barbiturate receptor **41** which binds a dibutyl barbiturate substrate by six-point recognition with an association constant of 2.8×10^3 M^{-1} in CD_2Cl_2/CD_3CN 9:1, as revealed by ^{1}H NMR titration experiments.[61] Zn^{2+}, when coordinated to the two 2,2′-bipyridine units of **41** in a tetrahedral fashion, is a strong negative allosteric effector: the complex (**41**)Zn^{2+} does not interact with the barbiturate.

Strong positive allostery has been quantified by Shinkai's group in the case of **42**, a huge covalent C_3-symmetric capsule containing Zn−porphyrin and calix[3]arene amide building blocks.[62] **42** in chloroform is a receptor for tris(2-aminoethyl)amine, which is incorporated in the central cavity with a binding constant $K = 1.7 \times 10^5$ M^{-1}, detected by UV−vis spectroscopy. Addition of $NaClO_4$ increases K to 1.5×10^8 M^{-1}, i.e., by almost 3 orders of magnitude. It is suggested that Na^+, when binding to the $-OCH_2-CONR_2$ moieties, disrupts intramolecular hydrogen bonds in **42** and rearranges the molecule into a conformation more suitable for inclusion of the amine guest. A positive but less pronounced effect on guest binding is also observed on addition of 10% MeOH, a H-bonding solvent, to the $CHCl_3$ solutions.

In Fukazawa's fullerene receptor **43**,[63] the strategy of allosteric regulation relies on the preorganization of two calix[5]arene units by the allosteric effector Cu(I). Force-field calculations support enclosure of a C_{60} guest by simultaneous interaction with both calixarene units, and a favorable preorganization of the latter when Cu^+ is complex tetrahedrally by the two 2,2′-bipyridine moieties The C_{60}-binding constant in $CHCl_2CHCl_2$ was determinated by UV spectroscopy and is 39-fold enhanced by the allosteric effector Cu^+. An interesting change of guest selectivity is brought about by the allosteric effector: free **43** binds C_{70} 2.5 times better than C_{60}, but the C_{70}-binding constant is enhanced only 4-fold by Cu^+, so that (**43**)Cu^+ is a better receptor for C_{60} than for the larger fullerene C_{70}.

A cholic acid-based receptor **44**, with an allosteric tris(2-aminoethyl)amine (tren) site, was investigated by Schneider's group.[64] **44** alone does not interact with aromatic guests such as toluidino-2-naphthalenesulfonic acid in aqueous solution. On addition of Zn^{2+}, an umbrella-type conformation with a hydrophobic cavity is suggested to form by coordination of the metal ion to the tetradentate tren site. Binding of the naphthalene-type guest by (**44**)Zn^{2+} is detected by fluorerescence increase (compare receptor **20**), with a binding constant on the order of 4×10^4 M^{-1}.

2.2.2. Simple Anions as Allosteric Effectors

Smith and co-workers have communicated the heteroditopic receptor **45** which contains a bis(phenylboronic acid) site for the recognition of sugars, a diamide site for anion binding by H-bonding, and a ruthenium(II)−tris(2,2′-bipyridine) unit for fluorescence sensing of binding events.[65] Fructose does not appear to interact with **45** in an aqueous 10 mM $NaClO_4$ medium since in this case the fluorescence of the receptor remains unchanged. In contrast, an increase of fluorescence is observed in the presence of 10 mM NaH_2PO_4/Na_2HPO_4 buffer. This is interpreted as follows: recognition of phosphate (which is a much better H-bond acceptor than perchlorate) by the diamide site stabilizes a conformation suitable for fructose binding by the bis(boronic acid) site. A direct H-bonding interaction of bound HPO_4^{2-} and fructose could also contribute to the cooperative binding. **45** is also a good receptor for certain sugar phosphates. The system could also be interpreted as a heteroditopic receptor for fructose and phosphate, with an allosteric bpy site and Ru(II) as an effector. Data for the Ru(II)-free receptor, however, are not given in the reference.

2.2.3. Organic Molecules as Allosteric Effectors

Examples of "metal-free" allosteric systems that do not involve metal ions either as substrates/guests or as effectors are very rare. In an example reported by Pochini and co-workers, a calix[4]arene receptor **46** for tetramethylammonium salts displays different binding efficiencies, dependent on the counterion.[66] While the tetramethylammonium cation is thought to be deeply entrapped in the host cavity, the counterion appears to participate in the recognition process by hydrogen bonding to the four phenolic −OH groups of **46**. Binding studies were performed by ^{1}H NMR spectroscopy in $CDCl_3$. For example, the association constants for the tosylate and acetate are more than 10 times larger than for the "innocent" anion picrate, in which the carboxylate group is a poor H-bond acceptor.

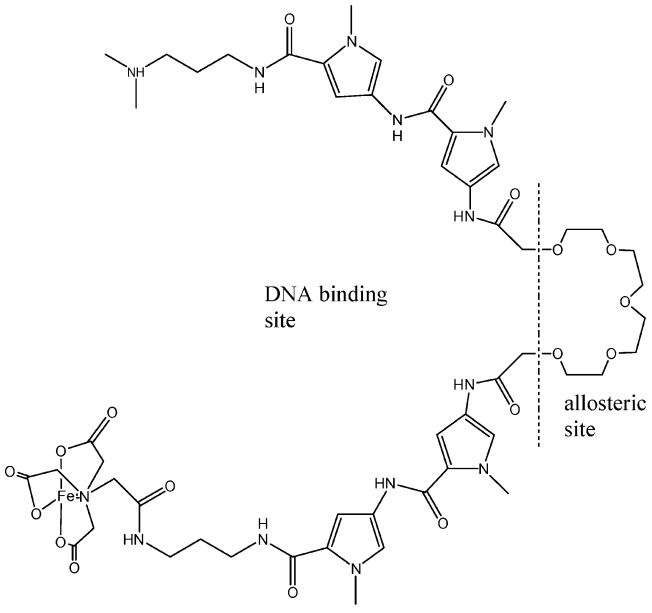
Kubik has prepared a cyclic peptide **47** based on L-glutamic acid and 3-aminobenzoic acid, and inves-

tigated simultaneous cation/anion complexation by this receptor by NMR spectroscopy.[67] A very large allosteric effect was quantified with the *n*-butyltrimethylammonium iodide/tosylate pair in $CDCl_3$. The association constant between (**47**)tosylate and the ammonium ion is 4×10^{-6} M^{-1}, and thus 10^4 times larger than that for the **47**/iodide combination. Tosylate is suggested to interact with **47** by H-bonding and to organize the receptor into a conformation with a shape suitable for efficient cation binding. Electrostatic cation/anion interaction within the ternary complex is thought to additionally contribute to enhanced cation affinity of (**47**)tosylate. Therefore, the large cation-binding constant of **47** may not be of pure allosteric (i.e., confomational) origin.

2.3. Allosteric Regulation of DNA Binders

Metal cations have significant effects on the transcription of prokaryotic and eukaryotic genes by interaction with the involved proteins. Prominent examples are the "zinc finger" proteins, for which Zn^{2+} is an essential cofactor since it imposes the active conformation of these proteins for their function as transcription factors.[68] Also certain antibiotics require metal ion cofactors to bind to DNA; for example, aureolic antibiotics are activated by Mg^{2+}.[69]

A metal-dependent, sequence-specific, double-stranded DNA binder **48** was described by Dervan in 1987.[70] In **48**, two oligo-pyrrolecarboxamide (netropsin) moieties are linked by a linear polyether chain. Netropsin is known as a sequence-specific ds-DNA minor groove binder. Additionally, an iron-chelating EDTA unit is attached terminally to the molecule in order to monitor sequence specific binding by concomitant cleavage of the DNA double strand. (EDTA)Fe(II) in the presence of dioxygen and a reductant (e.g., dithiothreitol) induces double-strand breaks in DNA by a radical mechanism.



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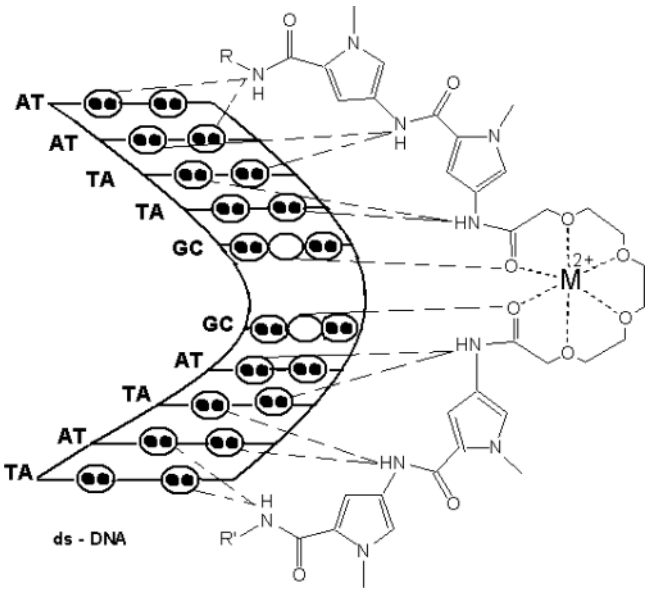
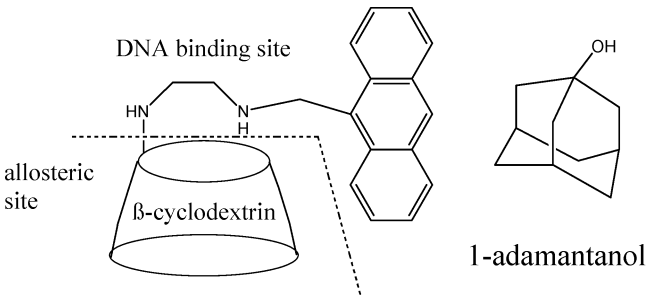
Scheme 2



A 517-base-pair restriction fragment was exposed to (**48**)Fe^{2+} at 37 °C (Scheme 2). Two AT-rich binding sites were identified. In the presence of alkaline earth metal ions Sr^{2+} and Ba^{2+} that coordinate to the polyether moiety and appear to organize the two oligopyrrole arms for efficient DNA binding, sequence-selective cleavage is very much improved (fragments were separated by gel electrophoresis and identified by autoradiography of the ^{32}P end-labeled DNA). It is not clear why the effect is not observed with other metal ions such as Cd^{2+}. A stabilizing interaction of Ba and Sr with the phosphate backbone of DNA is suggested. In that case, these metal ions would directly interact with the substrate as cofactors of the DNA binder and would not display a "clean" allosteric interaction.

Anthrylamines have potent DNA-intercalating properties. Ikeda and Schneider with co-workers have attached an anthrylamine to a β-cyclodextrin, compound **49**.[71] In aqueous solution, the anthryl (I) group is locked in the cyclodextrin (CD) cavity and is not available for intercalation into ds-DNA (Scheme 3). On addition of the strong intracavity complexer 1-adamantanol, removal of the anthryl group from the cavity is observed by NMR spectroscopy. While **49** alone does not intercalate into calf thymus DNA, intercalation is qualitatively detected by ^1H NMR spectroscopy (shifts and broadening of anthryl signals) in the presence of the 1-adamantanol guest (G).



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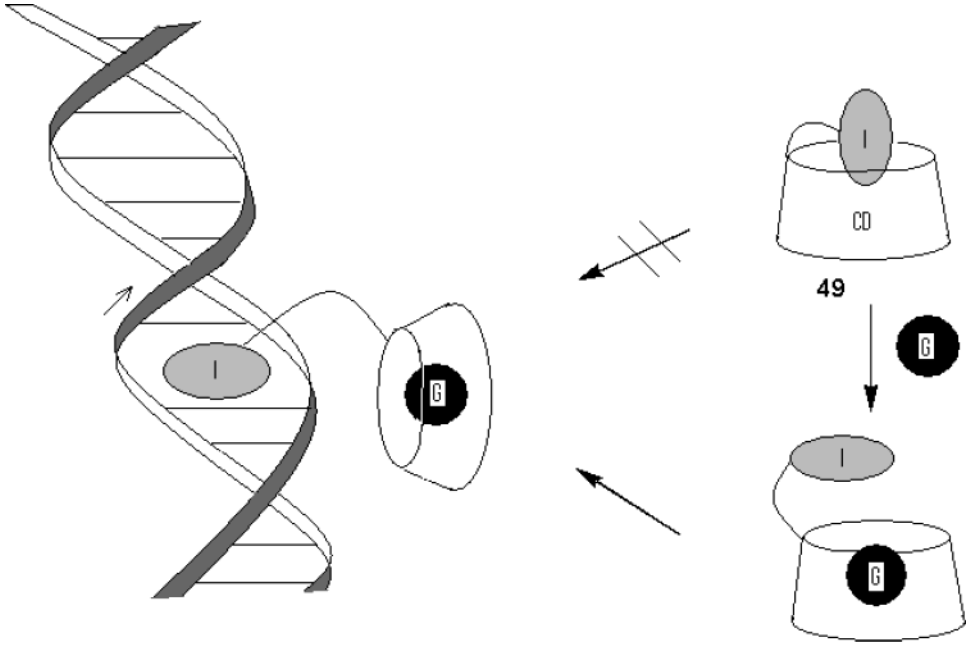
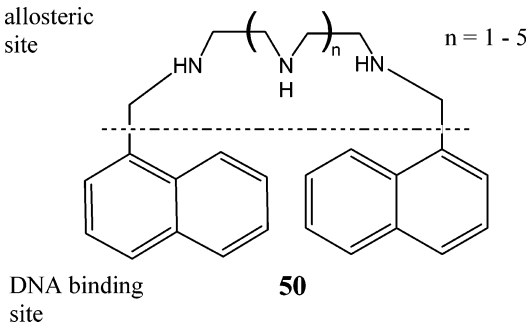
Scheme 3



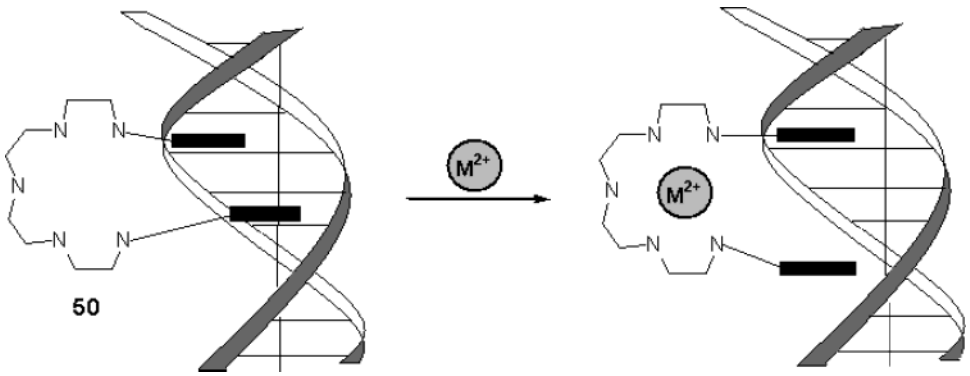
A DNA binder **50** which is inactivated by Cu^{2+} was desribed by Garcia-Espana, Luis, and Schneider.[72] A



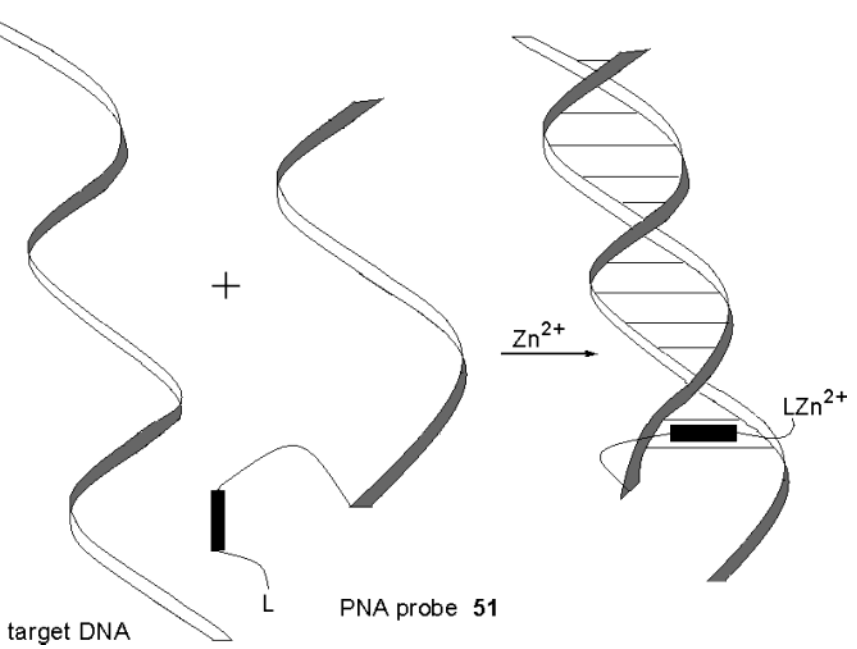
series of α,ω-dinaphthyl-substituted polyamines was prepared. Intercalation of the naphthyl groups into ds-DNA and ds-RNA is indicated by a strong decrease of melting temperature (T_m) of the nucleic acid double strands (e.g., $\Delta T_m = 24$ °C for $n = 5$). This effect is reduced significantly by Cu^{2+}, with a T_m increase up to 19 °C upon addition of the metal, and much more pronounced at 2 equiv of Cu per equiv of **50**. It is suggested that the conformation of, in particular, the dinuclear Cu^{2+} complex disfavors simultaneous intercalation of both naphthyl substituents into DNA (Scheme 4).

The control of sequence-specific nucleic acid binding by physiological metal ions is an attractive goal since metal ion levels can be both cell type and disease dependent. For example, nerve cells and some cancer types have elevated Zn^{2+} levels. An intracellular, metal-ion-dependent selective activation of antisense or antigene reagents would further improve their selectivity. Peptide nucleic acids (PNAs) are oligo-DNA analogues with potential as antisense or antigene reagents. We have recently conjugated a PNA 9mer with both an intercalator and a zinc chelating ligand, compound **51**. Sequence-specific binding to single-stranded DNA by **51** is very much enhanced by micromolar Zn^{2+} and ultimately "switched ON" by the metal ion (Scheme 5).[73] Electrostatic interactions of the dicationic Zn complex with polyanionic DNA backbone contribute to the increased target affinity, but this alone cannot explain the large effect of Zn ion on PNA/DNA duplex stability. We suggest that in free **51**, the naphthyl-based intercalator is "masked" by interaction the hydrophobic $(PyCH_2)_2N-$ moiety but becomes fully available for interaction with the PNA/DNA duplex

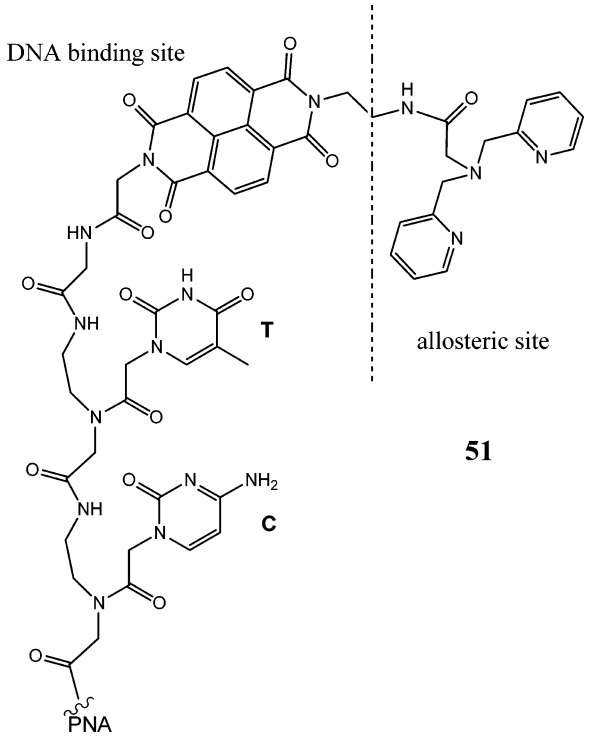
Scheme 4



Scheme 5



when Zn^{2+} complexation alters conformation and reduces hydrophobic character of the bis(picolyl)-amine unit.



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3. Allosteric Catalysts

Allosteric supramolecular catalysts in which the rate of the catalyzed chemical reaction is influenced by an allosteric effector represent the nonbiological counterpart to allosteric enzymes, but are much less well explored than allosteric supramolecular receptors. A requirement for a conformational response of the "active site" to the binding of the effector is a certain structural complexity, e.g., a bifunctional interaction with the substrate and a change in distance or relative orientation of the functional groups.

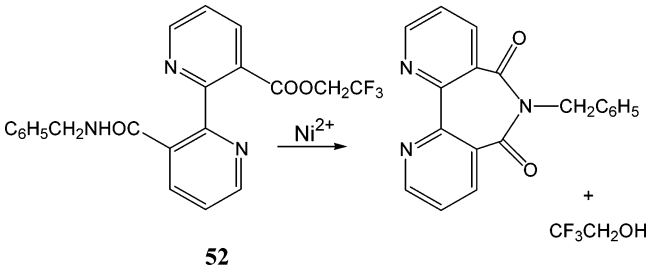
Before a discussion of systems with "true" catalytic behavior, examples of the allosteric control of stoichiometric intra- and intermolecular reactions will be given.

3.1. Allosteric Control of Reactivity

Control of intramolecular chemical reaction rates by conformational effects of metal ion coordination was described by Rebek in 1985.[74] In the 6,6′-substituted 2,2′-bipyridine **52**, cyclization to an imide by nucleophilic attack of the benzyl amide at the ester group is very slow at 45 °C. 2,2′-Bipyridines in general prefer a conformation with anti orientation of the pyridyl nitrogens, so that intramolecular reaction in **52** is disfavored. Complexation of 2,2′-bipyridines by Ni(II) stabilizes the syn conformation, brings the reactive groups in close proximity, and considerably accelerates the reaction (Scheme 6).

Tee and co-workers have carefully analyzed the kinetics of the cleavage of alkane carboxylate *p*-nitrophenyl esters by cyclodextrins **53** in alkaline aqueous solution, in the presence of various "potential inhibitors" such as alcohols, carboxylic acids, and sulfonic acids.[75,76] The cleavage reaction is described as a stoichiometric acyl transfer from the nitrophenyl ester substrate to a secondary OH group of cyclodex-

Scheme 6



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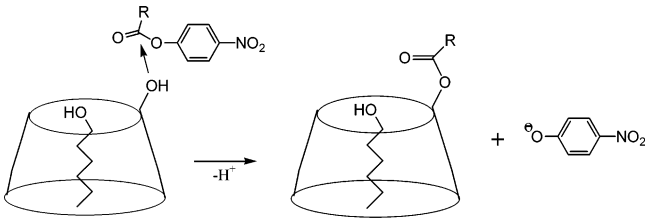
Scheme 7



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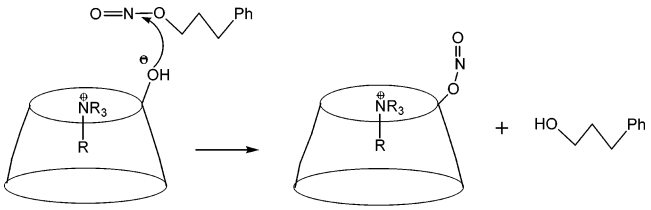
Scheme 8



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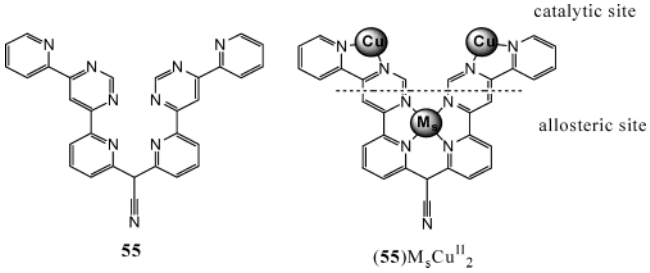
trin sugar. Besides the expected competitive inhibition, a rate enhancement (up to about 5-fold) was observed for certain additives (in particular alkyl alcohols) in the cleavage of *p*-nitrophenyl acetate by α- and *β*-cyclodextrins and, more pronounced, for longer chain alkanoate esters such as *p*-nitrophenyl hexanoate with *β*-cyclodextrin. The rate enhancement effects are interpreted as being due to the formation of a 1:1 cyclodextrin−alcohol inclusion complex which is more reactive toward the ester substrate. It was proposed that the substrate is located outside the cavity (which is blocked by the alkyl alcohol) in the transition state (Scheme 7). A direct interaction of the alcohol with the substrate or transition state by hydrophobic or H-bonding interactions was discussed. In this case, the alcohol would rather act as a cofactor than as an allosteric effector.

Related observations were described by Iglesias[77] for the *β*-cyclodextrin **54**-promoted cleavage of alkyl nitrites in alkaline aqueous solution. The reaction is described as a stoichiometric NO^+ transfer from the alkyl nitrite to a secondary hydroxyl group of the cyclodextrin, resulting in the formation of an alcohol and a cyclodextrin nitrite **(**Scheme 8). An about 3-fold increase of the cleavage rate is seen on addition of dodecyltrimethylammonium bromide which is again interpreted as being due to the formation of a reactive ternary complex (**54**)alkyl nitrite−ammonium ion on the basis of a kinetic analysis.

3.2. Allosteric Control of Catalysis

As early as 1977, Shinkai investigated the hydrolysis of carboxylic esters by histamine-containing methacrylic polymers.[78] The histamine−imidazole groups act as nucleophiles and cleave *p*-nitrophenyl hexanoate in water at pH 9. The reaction rate is enhanced up to 40-fold on addition of hydrophobic tetraalkylammonium salts, e.g., $[CH_3(CH_2)_7]_3NCH_3$-Cl. The effect is attributed to enhanced binding of the hydrophobic ester substrate to the polymer in the presence of hydrophobic ammonium ions. Kinetic data could be fitted to a mathematic model consistent with heterotropic allosteric behavior. In the absence of any structural information on the substrate-binding sites, it is difficult to distinguish allostery from a direct, cofactor-like interaction of the alkylammonium ion with either the ester substrate or the imidazole active sites.

We described in 2000[79] the first well-defined synthetic allosteric catalyst with turnover behavior. Catalyst design was based on the well-precedented catalytic cooperation of two metal ions in the cleavage of phosphate esters, which is a functional motif in both phosphoryl-transfer enzymes and their synthetic mimics.[80] We have prepared a polypyridyl ligand **55** that coordinates two catalytic or "functional" Cu^{2+} ions and has an additional specific site for the binding of an allosteric effector (a third "structural" metal ion, M_s). The effector influences the conformation of the



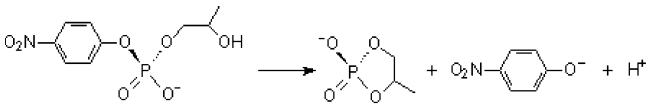
55 (**55**)$M_sCu^{II}_2$

complex and the preorganization of the two catalytic metal ions—a factor which determines the reactivity of the dinuclear metal site.

The reaction under investigation was the intramolecular cleavage in neutral water of the phosphodiester 2-(hydroxypropyl)-*p*-nitrophenyl phosphate (HPNP), which is a reactive RNA analogue with a nitrophenolate good leaving group (Scheme 9).

Trinuclear complexes have been prepared in situ that contain two functional Cu(II) ions and various structural metal ions M_s, and the kinetics of HPNP cleavage by these complexes has been analyzed. At least two turnovers were determined without reduction of catalyst activity. Remarkably, the nature of the structural metal ion M_s has a strong influence on the catalytic rate constant k_{cat} and the substrate-binding constant K_{HPNP}, both determined by Michaelis−Menten kinetics analysis (Table 2).[81,82] With M_s = Cu(II) or Co(III), k_{cat} is 70 times larger than with M_s = Pd(II).

Scheme 9



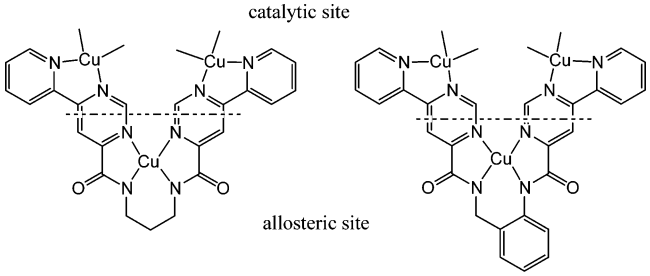
HPNP

Table 2. Catalytic Cleavage of HPNP by Catalyst (55)$M_sCu^{II}_2$: Values of K_{HPNP} (Formation Constant of Catalyst−HPNP Complex) and of k_{cat} (Rate Constant for the Cleavage of Catalyst-Bound HPNP)

M_s	K_{HPNP}, M^{-1}	k_{cat}, s^{-1}
Cu^{2+}	170	17.0×10^{-3}
Ni^{2+}	340	3.1×10^{-3}
Pd^{2+}	2600	0.2×10^{-3}
Co^{2+}	470	3.2×10^{-3}
Co^{3+}	820	15.0×10^{-3}

The activity of the complex in the absence of the allosteric metal M_s could not be studied because of the much stronger binding of metal ions by the tetradentate site than by the bidentate sites. The system rather mimics enzymes which are allosterically regulated by metal ions and in which the exchange of a metal ion by a different one affects catalytic activity. Such a behavior was described for alkaline phosphatases which have a dinuclear Zn^{2+} active site and are activated by an allosteric Mg^{2+} ion. Replacement of Mg^{2+} by other metal ions alters or even inhibits catalytic activity.[83−85]

Crystal structures of mononuclear complexes of **55** with Cu(II) and Co(III) confirm a significant influence of the allosteric metal ion on complex conformation. The "smaller" Co(III) ion, with M−N bonds distances 0.1 Å shorter than for Cu(II), brings the bidentate sites closer together so that a shorter Cu−Cu distance in (**55**)$Co^{III}Cu^{II}_2{}^{6+}$ is expected. However, this alone cannot explain the kinetic data given in Table 2, since the activities with M_s = Pd(II) and M_s = Cu(II) are very different, despite the rather similar M−N distances expected for the two metal ions. A study with related trinucleating ligands **56** and **57** suggests that reactivity is significantly dependent on the conformational flexibility of the complex.[86] The

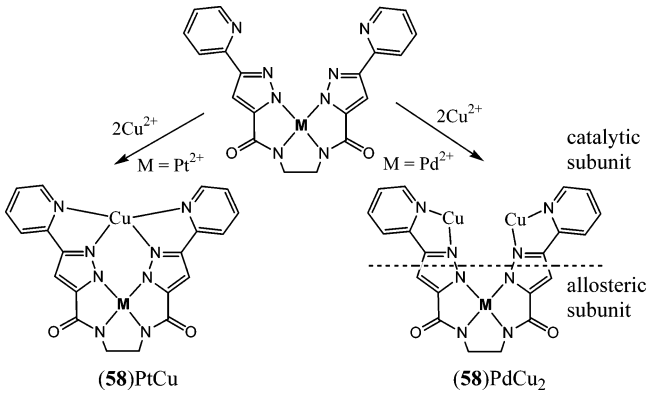


56 **57**

trinuclear Cu(II) complex of **56** has a 3 times higher k_{cat} value for HPNP cleavage than **(57)**Cu^{II}_3, in which ligand flexibility is slightly reduced by replacement of a $-CH_2CH_2-$ moiety by $-CH{=}CH-$. Also, in crystal structures of (**56**)Cu^{II}_3 complexes, containing Cu in both the allosteric and functional sites, the M−M distances of functional Cu ions range from 5.9 to 6.5 Å, and the coordination of allosteric Cu can be significantly distorted from square planar. Pd^{II} is much less susceptible to such distortions of the coordination plane than Cu^{II}. Such a reduced conformational flexibility—although potentially improving substrate binding—may keep the complex from adopting structural changes necessary for efficient stabilization of the transition state of phosphate ester cleavage.

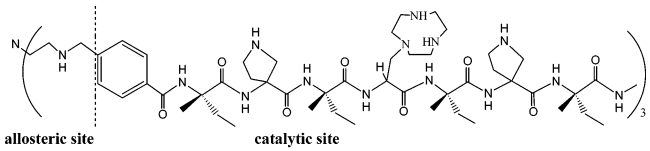
Complexes of ligand **58** feature a different type of allosteric control of catalytic activity.[87] The allosteric metal ion determines the nuclearity of the complex formed in the presence of an excess of copper. Two Cu(II) ions are incorporated if M = Pd(II) but only one Cu(II) if M = Pt(II). Since the bonding parameters of Pd(II) and Pt(II) complexes are usually very similar, this might be a consequence of a greater tolerance of Pd(II) for distortions of a square planar coordination polyhedron and widening of one of the

Scheme 10



N−Pd−N angles. (**58**)$PdCu_2$ is an efficient catalyst for HPNP, while (**58**)PtCu is inactive (Scheme 10).

A supramolecular allosteric catalyst based on a peptide template **59** and again on the cooperative action of several metal centers was reported by the groups of Scrimin and Göbel.[88] Three heptapeptide



59

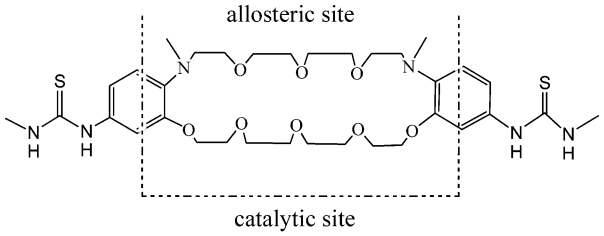
chains, each containing a macrocyclic triazacyclononane (tacn) unit, were N-terminally connected to a tris(2-aminoethyl)amine (tren) platform. Thus, **59** has four metal-binding sites: three identical tridentate tacn units, expected to provide catalytic sites, and a tetradentate tren site. Metal binding to the latter is suggested to induce a change from an open to a closed conformation in which the helical peptide chains are aligned in a parallel manner, and to improve the preorganization of the metal tacn sites for cooperative interaction with a substrate.

The catalytic behavior of the system was studied using the phosphoester substrate HPNP (Scheme 3). The tetrazinc(II) complex of **59**, in which Zn is bound to both the tacn and the tren sites, is an efficient catalyst for the cleavage of HPNP in neutral water. An allosteric behavior was deduced qualitatively from the sigmoidal dependence of cleavage rate on equivalents of zinc added to **59**. The Zn ion at the tren site could be replaced selectively by copper(II), as indicated by spectrophotometry, and this resulted in an about 3-fold reduction of catalytic efficiency of the system.

A different behavior of the zinc complex of **59** is observed when the substrate HPNP is replaced by an RNA oligonucleotide. The Zn^{2+}-promoted cleavage of RNA proceeds with participation of ribose 2′-OH group as an intramolecular nucleophile, similar to the case of HPNP cleavage. On addition of 1 or 2 equiv of Zn^{2+} to **59**, the cleavage rate is similar to that of a single Zn-complexed peptide branch. However, with addition of the third and fourth equivalents of Zn^{2+}, the activity decreases about 5-fold and is finally much lower than that of the monomeric subunit. This is indicative of a negative allostery induced by Zn interaction with the tren site. In contrast to HPNP,

the bulkier substrate RNA does not seem to be able to interact with the catalytic site in the "closed" conformation of the catalyst.

The phosphoesterase activity of **60**[89] is attributed to the interaction of the two thiourea units with the phosphodiester HPNP by H-bonding, as documented for related bis-guanidinium receptors that mimic phosphoesterase activity of staphylococcal nuclease.[90,91]

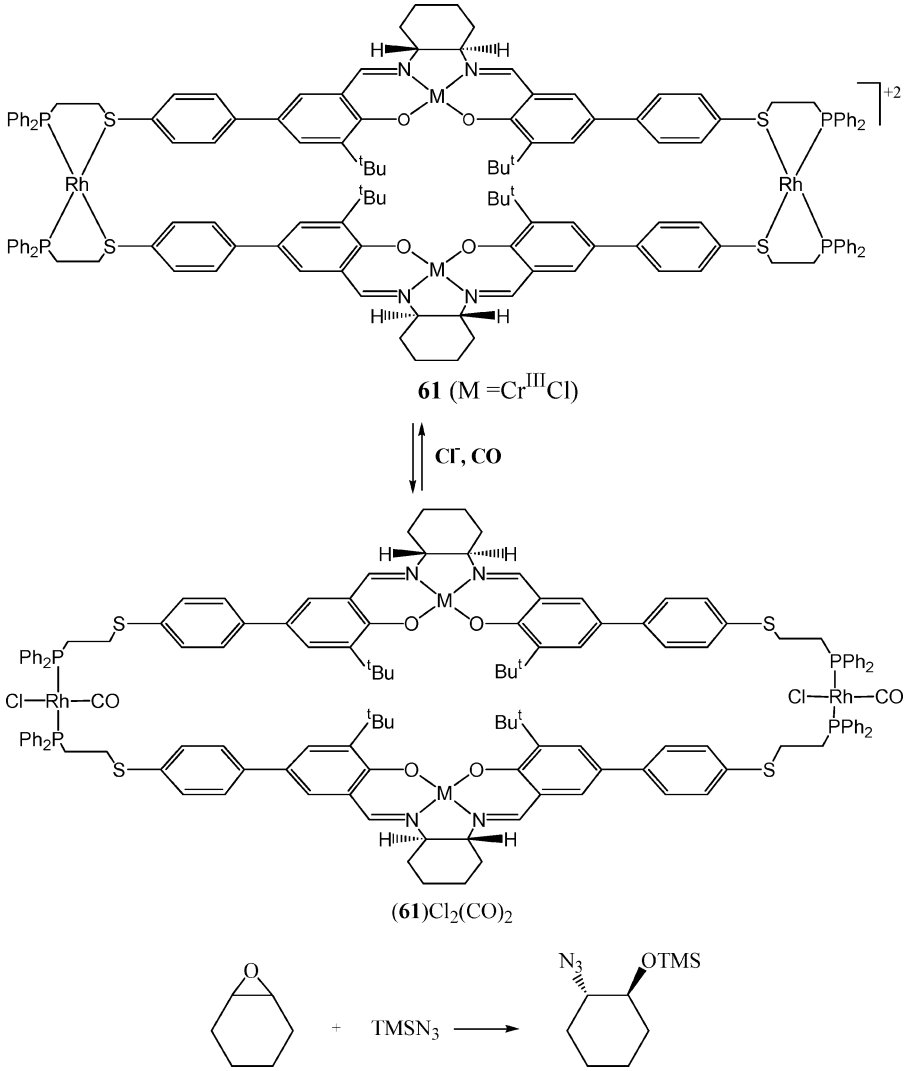


60

The activity of **60** alone for the cleavage of HPNP in acetonitrile and in the presence of triethylamine as a base is rather low. Alkali metal ions Na^+, K^+, and Cs^+, which are expected to interact with the crown ether, strongly accelerate the cleavage reaction. K^+ is the most efficient allosteric effector, with a 400-fold rate enhancement relative to **60** alone. Catalytic turnover was not demonstrated since experiments are performed with excess "catalyst" but reaction products are not expected to block the activity of **60**. On the basis of force-field calculations, a K^+-assisted organization of the two thiourea moieties, well suited for interaction with the phosphoester substrate by multiple H-bonding, is suggested.

A further example of an allosteric catalyst is a bis-(chromium salen) complex **61** for the ring-opening of epoxides, presented by Mirkin and co-workers.[92] Enantioselective epoxide ring-opening is a useful reaction in organic synthesis. Catalyst design was inspired by the observation that the Cr−salen-catalyzed ring-opening of epoxides by HN_3 involves a dimetallic intermediate.[93] A chiral, monomeric Cr complex was synthesized which has two additional potentially bidentate P,S sites for metal ion complexation. On addition of Rh(I), dimeric complex **61** is formed by self-assembly. A crystal structure of a corresponding Zn^{2+} complex (Zn replacing Cr) shows how the two salen−M units are held in close proximity in the self-assembled compound with a Zn−Zn distance of 5.2 Å. The self-assembled dimer is a much better catalyst than the monomeric precursor for the ring-opening of cyclohexene oxide by trimethylsilyl azide in benzonitrile at room temperature (Scheme

Scheme 11. Ring-Opening of Cyclohexene Oxide by Trimethylsilyl Azide ($TMSN_3$).

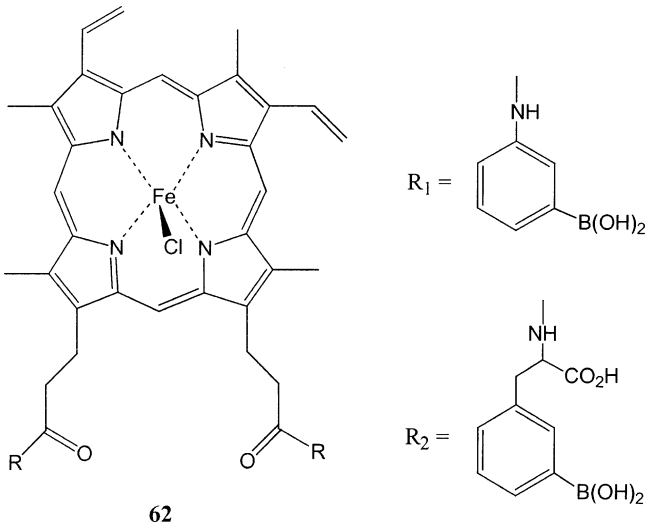


11). Also, the enantiomeric excess induced by the chiral 1,2-diaminocyclohexane moiety (*R*,*R*) is much higher (68% vs 12% for the monomer).

Allosteric regulation of the activity of this catalyst was achieved by addition of coligands Cl^- and CO, which act as effectors by interaction with the Rh(I) site. The thioether sulfur atoms are poor donors and are readily replaced by Cl^- and CO so that square-planar Rh(I) sites with trans-oriented P-donors are generated, as confirmed by ^{31}P NMR spectroscopy. This should create a much wider macrocyclic cavity and increase the Cr−Cr distance in in (**61**)$Cl_2(CO)_2$. Cl^- and CO are positive allosteric effectors since the catalytic efficiency in epoxide ring-opening by trimethylsilyl azide (TMS−N_3) is doubled. Unfortunately, the effect on enantiomeric excess could not be consequently studied due to insufficient solubility of the catalyst in solvents that support high enantiomeric excess for this specific reaction.

4. Semisynthetic Allosteric Enzymes

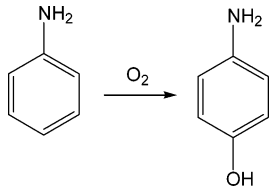
Artificial allosteric sites have been introduced into proteins and allow the modulation of catalytic properties by specific effectors. Hamachi and co-workers have prepared phenylboronic acid[94] and boronophenylalanine[95] appended myoglobins by attaching two such moieties to two side chains of the heme group **62**. Similar to the receptors in section 2.2.1, the



62

boronic acid conjugates of myoglobin bind sugars such as D-fructose. In the presence of D-fructose, the conjugates become more stable against denaturation. Also, the pK_a value of H_2O coordinated to heme Fe(III) is increased in the presence of fructose, indicating a modulation of the microenvironment of the active site. Like native myoglobin, the boronic acid appended myoglobins catalyze the hydroxylation of aniline to *p*-aminophenol (Scheme 12) in the presence of NADH and dioxygen. The net activity of this reaction is enhanced up to 7.7-fold by addition of D-fructose. While k_{cat} is increased about 3-fold for both the phenylboronic acid and boronophenylalanine derivatives, only in the former case the aniline-binding affinity is also enhanced by a factor of about 3. It was shown previously how the dioxygen storage

Scheme 12



capability is enhanced by allosteric sugar binding to phenylboronic acid−myoglobin conjugates.[95,96]

Ghadiri's group[97] has prepared an "inhibitor−DNA−enzyme" **63**. An endolytic metalloprotease was attached to a thiolated end of 24mer DNA by disulfide bond formation, while the opposite terminus was chemically modified during DNA synthesis with a phosphoramidite which represents a transition-state mimic and inhibitor to the enzyme active site. The flexibility of ss-DNA enables the phosphoamidite modification to act as an intramolecular competitive inhibitor of the enzyme and prevents hydrolysis of an oligopeptide. In the presence of a DNA oligomer which is complementary to the 24mer DNA target of **63**, the catalytic activity is switched ON (Scheme 13). This is a consequence of the formation of a rigid DNA/DNA duplex. The DNA oligomer may be considered as an allosteric effector which alters the conformation of **63** and favors the liberation of the inhibitor from the enzyme active site.

By introducing fluorescent reporter groups into the peptide substrate, the system could be applied to the detection of complementary DNA at 100 pM concentration within 3 min. Inhibitor−DNA−enzymes constitute a new class of reagents for the detection of label-free DNA sequences by chemical signal amplification.

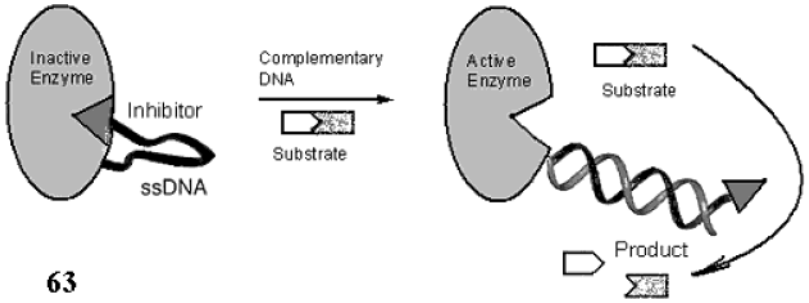
5. Summary and Perspectives

The development of supramolecular receptors and catalysts has been greatly influenced by biomimetic design principles. While a wide range of "simple" artificial host−guest systems demonstrate the principles of molecular recognition, the design of allosteric systems opens the possibility of controlling molecular function by external chemical input, as widely observed in biomolecular recognition and catalysis. Since first synthetic allosteric receptor was described in 1979, scientists have not only investigated the basic concepts of allostery in a supramolecular context but also identified a potential for applications.

Since the effector induces a conformational change of the active site, the design concepts of allosteric systems are similar to those of "molecular machines"[99] driven by chemical input, but with a smaller amplitude of molecular motion. A mutual stimulation of these two research fields is to be expected.

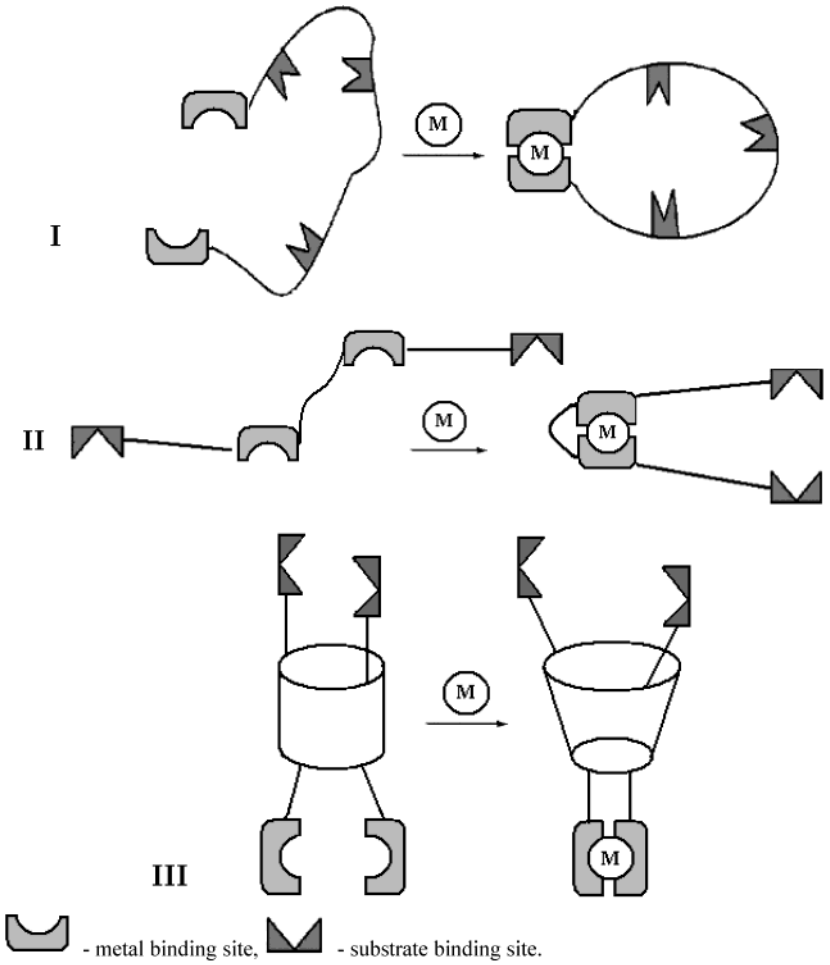
The chemistry of crown ethers[100] and calixarenes,[101,102] which often constitute the recognition sites, is of great relevance in the allosteric context. Coordination chemistry is involved in nearly all of the systems covered by this review. The well-predictable coordination behavior of metal ions with directed bonding facilitates the heterotropic design concept.

Scheme 13



63

Scheme 14



In the past 25 years, about 50 allosteric supramolecular receptors for metal ions and various organic guest molecules have been described. In most cases, a metal ion is the allosteric effector.

The receptors can be classified into three major groups:

(I) open or open-chain structures that are organized into cyclic structures (often crown ether type) on coordination of an allosteric metal by two chelating subunits located at the chain ends (2, 3, 6−9, 11, 12, 15, 17, 18, 20, 33−35, 40, 41);

(II) di- and tribrachial, pincer-like systems in which coordination of the allosteric metal ion affects the preorganization of functional groups interacting with the substrate (4, 5, 19, 23, (24), 25, 26, 28, 38, 39, 43, 44); and

(III) calixarenes or pseudocalixarenes in which coordination of the effector to the upper/lower rim affects the shape and size of the hydrophobic cavity, and/or the spatial organization of the functional groups that interact with the substrate (13, 14, 16, 21, 22, 27, 29, 30−32, 36, 37, 42, 46).

The three types of allosteric receptors are roughly sketched in Scheme 14. Few systems (45, 46, and 47) have been described which do not involve metal ions but in which an inorganic or organic anion is the allosteric effector, interacting with the receptor by H-bonding.

For many of the receptors, allosteric effects are large, i.e., the binding constants in the presence and absence of effector differ by 2 orders of magnitude or more, or binding has not been detected in one of the two states. This corresponds to an ON or OFF switching of the receptor property by the allosteric effector and accounts in particular for some of group I receptors and for group III calixarenes, having

spheric or hemispheric guest-binding sites, but to a lesser extent for the group II receptors. Allosteric effects appear to be more pronounced if the "interaction surface" between receptor and guest is large (spheric > cyclic > two-point recognition).

The allosteric effector influences not only the affinity for a specific substrate, but sometimes also the substrate selectivity pattern, as described below.

- Selectivity can be improved by the effector: The alkali ion discrimination of **2** is better in the presence of an allosteric Cu(I) ion.
- The effector can switch the selectivity toward two substrates: **43** is converted from a C_{70}-selective to a C_{60}-selective receptor by the allosteric metal.
- If the effector is a metal ion, variation of the metal can affect the selectivity: In **33**, replacement of allosteric Cu^{2+} by Zn^{2+} switches the substrate selectivity of the receptor, while in **37**, replacement of Li^+ by Na^+ results in a switching from positive to negative allostery.

Metal ions display a broad variety of ionic radii, coordination numbers, and geometries. Combination of a single receptor with a variety of allosteric metal ions improves to some extent the versatility of the receptor which otherwise requires laborious syntheses of tailor-made receptors with a desired individual selectivity pattern.

Allosteric receptors for biological target molecules open the possibility of blocking/activating the biological functions of the targets in response to the concentration of small molecules or ions which act as allosteric effectors. It is well documented that DNA or RNA binders can block (and sometimes activate) transcription or translation. It has been demonstrated in vitro how binding of nucleic acid targets by **48**, **49**, and **51** is switched ON by allosteric effectors.

Selective allosteric regulation of such compounds in vivo by ions or small molecules, which show significant cell-type or disease-specific variations in concentration, remains to be demonstrated.

Most recently, several examples of allosteric synthetic catalysts have been reported. The proof of concept is given by systems **55**−**60** that catalyze the cleavage of a phosphodiester by cooperative action of either two (three, respectively) metal ions or of two H-bond donor groups. The allosteric effector is a metal ion which controls the preorganization of the catalytic functionalities. A large influence on catalytic rate is observed in response to (up to 400-fold increase) or by variation of (up to 70-fold) the allosteric metal. Variation of the allosteric metal ion in **55**, along with its ionic radius and stereochemical preferences, enabled us to fine-tune the preorganization of the catalytic functionalities in the active site, with large effects on catalytic efficiency. Such sub-angstrom adjustments are otherwise difficult to achieve, and this has been identified as one of the problems that limit the success of supramolecular catalysis.[104]

A specific case of allosteric catalysis is represented by **58**: here, the allosteric metal ion controls the uptake of additional metal ions which are essential cafactors and thereby indirectly regulate catalysis (ON/OFF).

Another study using **61** is directed toward allosteric activity and selectivity control of a catalyst for a synthetically useful reaction.

A particularly attractive feature of allosteric synthetic catalysts is the possibility of signal amplification by catalytic turnover. By activating the catalyst for binding and conversion of the substrate, a single effector molecule can trigger the production of many product molecules, depending on the turnover number. Such systems have a potential for the highly sensitive detection of analytes (which act as effectors) by chemical signal amplification, as demonstrated by DNA sequence detection with the semisynthetic enzym-based catalyst **51**.

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