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Supramolecular complexations of natural products

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Complexations of natural products with synthetic receptors as well as the use of natural products as host compounds are reviewed, with an emphasis on possible practical uses or on biomedical significance. Applications such as separation, sensing, enzyme monitoring, and protection of natural drugs are first outlined. We then discuss examples of complexes with all important classes of natural compounds, such as amino acids, peptides, nucleosides/nucleotides, carbohydrates, catecholamines, flavonoids, terpenoids/steroids, alkaloids, antibiotics and toxins.

1. Introduction

The development of supramolecular chemistry has led to synthesis of host compounds for a manifold of substrates. Complexation of natural products with such artificial receptors is under active development and is of considerable theoretical and practical interest. Non-covalent interactions with biopolymers including *e.g.* enzymes or nucleic acids are the basis of most pharmacological activities, and can be studied with a variety of supramolecular model complexes, thus also providing insight into the design of drugs based on natural products. Interactions with proteins are, however, dealt with in detail in the framework of medicinal chemistry, and are not within the scope of this review. In terms of practical applications such complexes can be used for separation and purification, either by *e.g.* immobilization of suitable host compounds or by using them as selectors in chromatography/electrophoresis, or as components in membrane and related technologies. Characterization and quantitative determination can be supported by the use of colorimetric and in particular of fluorescence techniques with host compounds, which exhibit spectroscopic signals upon complexation. Another important application is the use of supramolecular complexes for protection, transport and targeting of bioactive natural compounds. Encapsulation in suitable hosts allows us to provide *e.g.* pH-selectivity, protection of natural drugs, or to manipulate redox properties. It is timely to highlight these developments based on the recent literature, with an

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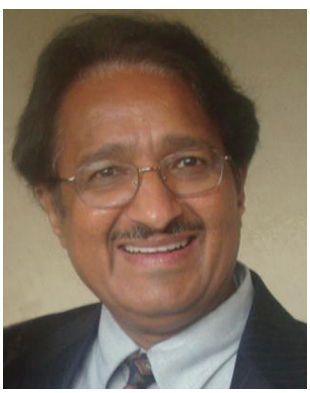
Hans-Jörg Schneider studied in Munich, Berlin and Tübingen, where he obtained his PhD degree in 1967. After two years as a postdoctoral researcher at the Univ. of California, San Diego, he worked on his habilitation and in 1972 he became Professor at the Universität des Saarlandes. His research concerned stereochemistry, mechanisms of organic reactions, NMR-spectroscopy, and later supramolecular chemistry, including receptor and enzyme mimics, more recently also molecular recognition in chemomechanical polymers. He is the author of over 260 publications, including several monographs and book contributions.



Pawan Agrawal

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emphasis on possible applications. Natural structures themselves or their derivatives can also be used very efficiently as host compounds, particularly in view of their chirality. The host compounds underlying the applications with different natural products and their predominating binding mechanism have been discussed in several textbooks and reviews.[1] Many natural compounds play an important role in the control of living systems; as a consequence their complexation is also of considerable biomedical interest.

2. Applications/methods

2.1 Separation/purification

Chromatographic separation with the help of supramolecular complexes can be achieved either by adding host compounds as selectors to the eluent, or, more often, by immobilization of receptors on the stationary phase.[2] A great advantage of using natural derivatives is their usual occurrence as optically pure enantiomers, which allows easy separation of racemic mixtures. Besides amino acids substituted with aromatic donor- or acceptor groups (so-called Pirkle-phases) as chiral selectors, macrocyclic glycopeptide antibiotics such as vancomycin (see Section 3) play an important role. Four chiral stationary phases for chromatography with vancomycin, ristocetin, teicoplanin, and the teicoplanin aglycone as selectors are commercialized under the trade name Chirobiotic by Astec and Supelco.[3] Separation of free α-amino acids or α-amino acid methyl esters was achieved early on with di-binaphthylo-22-crown-6 with attached atropisomeric (*R*,*R*)-1,1′-binaphthyl moieties, which were bound to cross-linked polystyrene-divinylbenzene beads.[4] The so-called affinity chromatography also allows to determine and in a practical way to screen the binding strength of natural compounds to a chosen receptor. Polymer inclusion membranes represent a particularly economical way to separate or purify by filtration also natural products, after the selector host is immobilized in plasticized membranes.[5] Separation by molecular imprinting *e.g.* is based on adding target compounds to oligomers or polymers which are then polymerized or crosslinked; after washing out the templating substances the polymers can then be used to separate also natural compounds from mixtures.[6]

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Anatoly K. Yatsimirsky has obtained PhD and DrSc degrees from Moscow State University, where he was Professor until 1992. Since 1992 he has been a Professor at the National Autonomous University of Mexico. His research interests include bioorganic chemistry and metal complex catalysis. He is the author of over 160 publications including a monograph on supramolecular chemistry and book contributions.

2.2 Sensing/concentration measurements

Detection and quantitative determination of an analyte requires a signalling event accompanying the analyte recognition by a receptor. Most often electrical or optical signalling is used, but fluorescence signaling attracts perhaps more attention because of its high sensitivity and a variety of possible mechanisms of coupling between recognition and signalling events.[7] A large number of sensors are based on a general 'fluorophore–spacer–receptor' structural organization, where typically the sensor is initially in an "off" state with the fluorophore quenched by photoinduced electron transfer (PET) from the lone electron pairs in the receptor site, and then it is switched "on" by the analyte (proton or Lewis acid) binding to the receptor.[8] Instead of chemical incorporation of a reporter group into the receptor one may apply an indicator displacement assay based on competition for the receptor binding site between the analyte and a fluorescent or chromogenic species possessing different optical properties in free and bound states.[9] Many natural compounds such as flavonoids or quinolinium alkaloids possess their own intense absorption or fluorescence, which can be significantly modified by inclusion in a host receptor and so generate a signal for analytical purposes. In the case of fluorescence signalling the observed optical effect often is a result of protection of the fluorophore from quenching by water by a hydrophobic microenvironment inside the host cavity, see *e.g.* ref. 10.

2.3 Enzyme monitoring

The indicator displacement method employing a synthetic macrocyclic receptor, which binds a fluorescent dye enhancing its fluorescence ("on" state), but possesses a higher affinity to a product of enzymatic reaction than to the educt, which displaces the dye quenching its fluorescence ("off" state), has been ingeniously adapted as a "supramolecular tandem assay" for label-free optical monitoring of enzymatic reactions such as peptide hydrolysis.[11] Fig. 1 illustrates the typical design and application of the method to monitoring of two enzymatic reactions producing two different types of signal *vs.* time profiles with cucurbit[7]uril (CB7) as the receptor molecule, and acridine orange (AO) as the indicator.

CB7 binds the protonated form of AO (pK_a 9.8) with a large binding constant of 2.9×10^5 M^{-1}, inducing a 3-fold increase in the fluorescence intensity of the dye. Peptides do not displace AO unless they possess an N-terminal hydrophobic amino acid such as phenylalanine or tryptophan, which specifically binds to CB7 as shown in the upper right corner in Fig. 1 with equilibrium constants of about 10^7 M^{-1}. Fig. 1a illustrates the most straightforward situation of peptide hydrolysis by an endopeptidase (thermolysin) specifically hydrolysing peptide bonds near hydrophobic amino acids. Cleavage of the peptide leads to a product with N-terminal phenylalanine, which displaces AO with a concomitant decrease in fluorescence (the graph on the right side of the figure shows time tracks obtained at different substrate concentrations). A different situation occurs when a peptide is hydrolysed by an exopeptidase, such as leucine amino peptidase (LAP), which cleaves consecutively amino acid residues of a peptide substrate from its N-terminus. In this case the fluorescence remains constant until the cleavage at *e.g.* phenylalanine

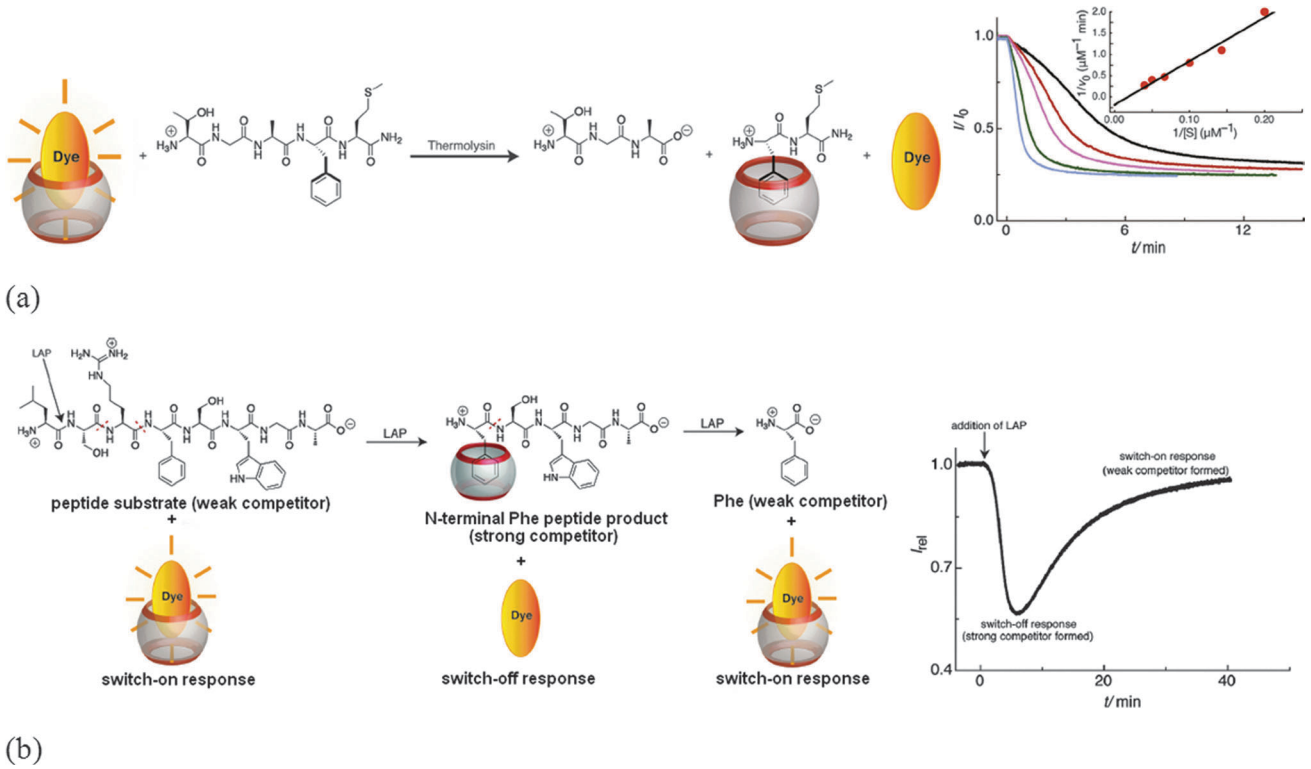
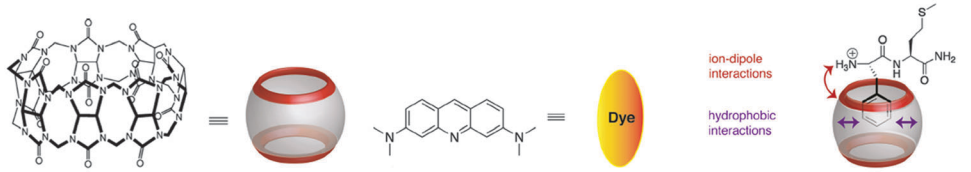


Fig. 1 Supramolecular tandem assay for thermolysin (a) and leucine amino peptidase (LAP) (b), explanations see text.[12] Reprinted with permission from *J. Am. Chem. Soc.* 2011, **133**, 7528. Copyright 2011 American Chemical Society.



cucurbit[7]uril, CB7 acridine orange (AO) binding mode of N-terminal Phe peptide residue

occurs, at this moment it goes down, but it is then restored after liberation of free phenylalanine, which does not have sufficient affinity to CB7 to compete with the dye (Fig. 1b). This approach is fairly general and may be adapted to different enzymes by varying the type of host and indicator molecules.[11,13]

2.4 Drug protection and targeting

Inclusion of redox-active compounds in suitable hosts can change significantly their redox potentials, as shown in particular with flavonols (see Section 4.5). Cyclodextrins bind and stabilize the natural antioxidant ascorbic acid and a combination of cyclodextrin and ascorbic acid acts as a ''secondary antioxidant''.[14] Shifts in redox potentials of natural compounds on inclusion in cyclodextrins and other hosts were reported, and employed also as a tool for electrochemical monitoring.[15] A similar mechanism of protection of phenolic compounds against *e.g.* enzymatic oxidation is responsible for prevention of juice browning by cyclodextrins.[14]

It should be noted that the protection mechanism by inclusion is not universal and supramolecular complexation can actually both decrease and increase the bioavailability of a natural compound. Thus, the biotransformation of steroids usually is improved in the presence of cyclodextrins apparently due to increased solubility.[16] However, it has been found that the rate of microbial oxidation of cholesterol strongly increases in the presence of methylated cyclodextrins, but it is completely inhibited in preliminary prepared cholesterol complexes with native cyclodextrins, which is indicative of importance of details of structures of host–guest complexes.[17] Manipulation of reactivity of natural compounds by inclusion *in vivo* obviously may produce some interference of host compounds with biocatalytic reactions. The subject was briefly addressed in a recent review.[18]

As a specific example for biomedical applications we describe the intriguing application of the host properties of vancomycin for promotion of opsonization (the process by which a pathogen, *e.g.*, a bacterium, is coated with an opsonin, typically an antibody, for binding and destruction by a

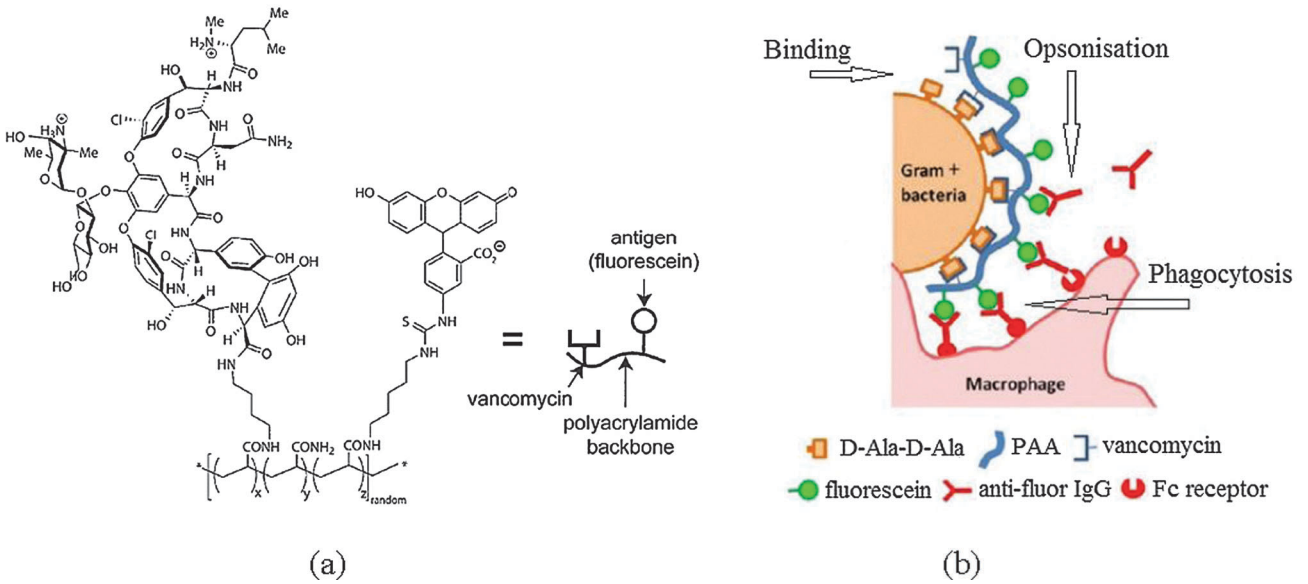


Fig. 2 (a) Vancomycin/fluorescein modified polyacrylamide chain[19] and (b) a cartoon illustrating the promoted opsonization and phagocytosis by polymer binding to a Gram-positive bacteria (adapted from ref. 20). Both reprinted with permission from Elsevier, 2006, 2011.

phagocyte) and phagocytosis of Gram-positive bacteria by a bifunctional polyacrylamide receptor illustrated in Fig. 2.[19]

Polyacrylamide was modified with vancomycin molecules, which acted as the specific binder of peptides terminated in

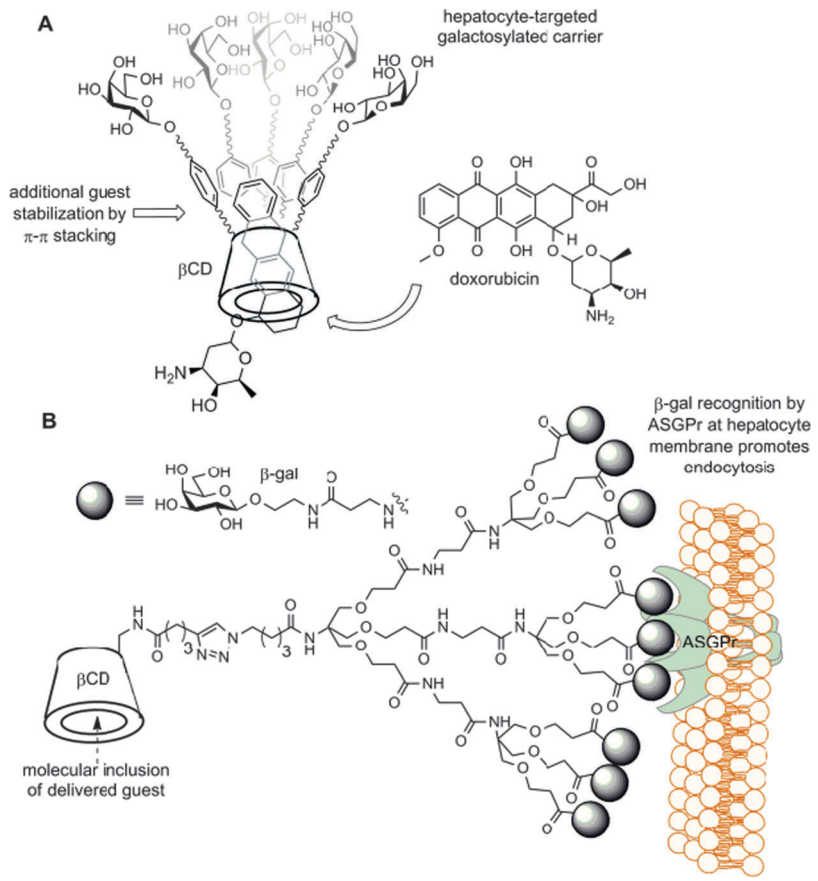


Fig. 3 (A) Cyclodextrins with heptagalactose antennae and phenyl units complex by efficiently π-stacking doxorubicin;[21] (B) glycodendritic β cyclodextrin may be used for hepatocyte-targeting.[22] Figure from C. Ortiz Mellet, J. M. García Fernández and J. M. Benito in: *Supramolecular Systems in Biomedical Fields* RSC, Cambridge, H.-J. Schneider, Ed., 2013, with permission.

D–Ala–D–Ala on the bacterial cell wall and with the fluorescein groups, which allowed the imaging of binding of a polymer to the surfaces of bacteria by fluorescence, and attracted anti-fluorescein antibodies (IgG) promoting opsonisation of the bacteria. Finally macrophages bind to the opsonized bacteria through interactions with the Fc region of the bound IgG's. Flow cytometry revealed that polymer-labeled *S. aureus* and *S. pneumoniae* were opsonized by anti-fluorescein antibodies and underwent phagocytosis much more efficiently than were untreated bacteria.

Another recent application is the targeting of cell membrane receptors with the help of saccharide antennae on cyclodextrin. Doxorubicin, an anthracyclinic intercalating anti-tumor drug, which closely resembles the natural product daunomycin, can thus be shuttled to membranes using hyperbranched galactosylated cyclodextrins with different spacer lengths and numbers of galactose arms.[21] Galactosylated cyclodextrin dendrimers allow specific delivery and uptake of doxorubicin into liver cells.[22] (Fig. 3).

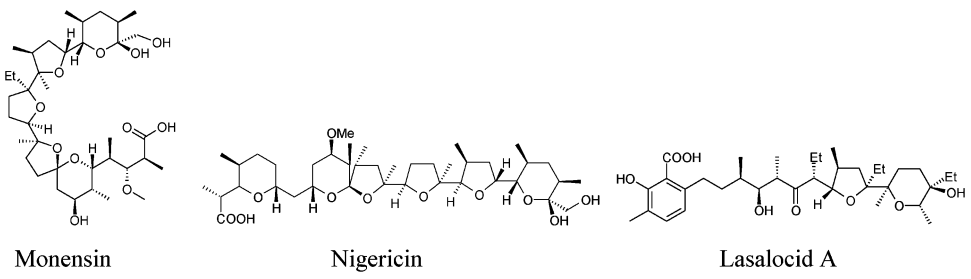
3. Natural compounds as hosts

Many natural low-molecular weight compounds that function as antibiotics or have other biological activities possess structural elements such as specific arrangements of charged or donor–acceptor groups, cavities or clefts, allowing them to act as host molecules for recognition of various guests. Some of these compounds, such as biological ionophores and glycopeptide antibiotics really use the classical host–guest complexation mechanism for their biological action, but in other cases the ability of natural compounds to bind guests of different types is not directly related to their biological functions. Since many of these compounds are commercially available and inexpensive, they find practical applications as host molecules in analysis and separation, especially chiral separation, achieved due to the presence of often several chiral centers in their structures.

Particularly popular natural compounds with receptor properties are cyclodextrins currently produced industrially by enzymatic degradation of starch. They are discussed in numerous books and reviews[2,3] and several examples of complexation by cyclodextrins will be described below in this paper.

Ionophores are synthesized by microorganisms and have antibiotic properties by disrupting *trans*-membrane ion concentration gradients. They may be relatively large molecules like the polypeptide gramicidin, which forms a *trans*-membrane channel for ion transport or smaller molecules acting as mobile ion carriers. Ionophores of this last type were a subject of intensive studies in 1960–1980 and served in many aspects as reference compounds for synthetic ionophores, which laid down the fundamentals of modern supramolecular chemistry. They were also used for development of first neutral carrier-based alkali metal ion-selective electrodes.[24] Thermodynamic parameters of cations binding to natural ionophores can be found in several reviews.[25] Siderophores, an important group of ionophores specifically designed for transport of iron ions are covered in several recent reviews, *e.g.* ref. 26. It is worth mentioning that synthetic ionophores reach and sometimes even surpass affinity and selectivity of natural ionophores toward their biological targets.[27] As an example Fig. 4 shows comparative profiles of stability constants for alkali cations binding by valinomycin and some calixcrowns in methanol. Also some artificial siderophores, *e.g.* a tripodal trihydroxamate ligand TRENDROX with a tris(2-aminoethyl)amine top[28] are more efficient than the respective biological ligands.

In biological systems ionophores selectively transport alkali and alkaline earth metal ions, but they may also have large



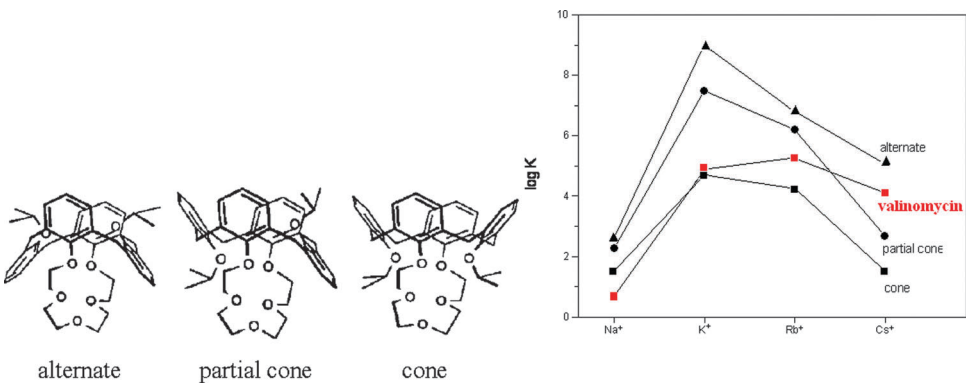


Fig. 4 Selectivity of alkali cation binding by calixcrowns and valinomycin in methanol. Reprinted with permission from F. Arnaud-Neu (personal communication).

affinities to other cations. Thus the binding constants for Pb^{2+} to nigericin and monensin anions surpass those for biological cations by four orders of magnitude, which allow using these antibiotics for treatment of lead poisoning.[29] Extensive studies including both inner-sphere and outer-sphere complexation of cations were performed with lasalocid A, a small natural ionophore containing a salicylic acid fragment.[30] Chirality of natural ionophores makes them potentially useful receptors for recognition of chiral ammonium ions, *e.g.* amino acid esters.[31]

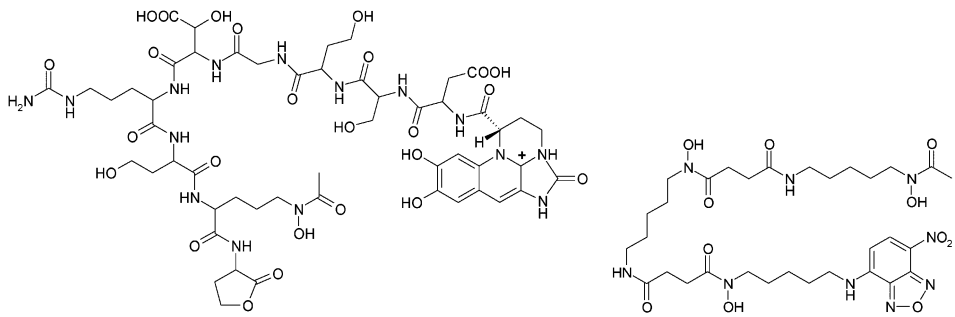
Naturally-occurring fluorescent siderophores such as azotobactin δ (a pyoverdin type siderophore) as well as fluorescent-labeled non-fluorescent siderophores such as desferrioxamine have been employed for the fluorometric detection of Fe(III).[32]

The ferric-siderophore complexes interact with specific bacterial cell membrane receptors with association constants of up to 10^8–10^9 M^{-1}, providing a possibility for detection of pathogens. Different strategies have been developed, one of which is illustrated in Fig. 5.[33] A Ga^{3+} complex of pyoverdin, a siderophore, that *Pseudomonas aeruginosa* must bind to obtain iron, was conjugated with BSA and attached to the surface of a gold-plated glass chip. Then a solution of fluorescent-labeled bacteria was applied to the chip and captured bacteria were detected using a microscope. The method allows specific detection of *P. aeruginosa* down to as small number of bacteria as 100 per mL.

There are over a hundred cross-linked polymacrocyclic heptapeptides with sugar substituents in different positions known as glycopeptide antibiotics. They act by specifically binding to polypeptide intermediates terminating with the sequence -D-Ala-D-Ala-COOH and prevent the transpeptidation reaction necessary for the synthesis of peptidoglycan thus inhibiting biosynthesis of the bacterial cell wall. The highest affinity is observed with Ac_2-L-Lys-D-Ala-D-Ala. The complexation is remarkably enantioselective: substitution of the terminal D-Ala with L-Ala eliminates the binding completely. The binding interactions involve a set of hydrogen bonds shown in Fig. 6a and hydrophobic contacts between methyl groups of the guest molecule and aromatic rings of the antibiotic.[34]

Bacteria resistant to this antibiotic use the intermediate peptide which contains -D-Ala-D-Lac terminus instead of -D-Ala-D-Ala terminus and this simple substitution of O for NH (see Fig. 6a, the group marked with red asterisk) does not prevent the biosynthesis of peptidoglican, but reduces affinity to vancomycin by more than three orders of magnitude (Fig. 6, bottom).[36] This effect was attributed to the lack of one hydrogen bonding interaction and a repulsion between lone pairs of lactate oxygen and the carbonyl oxygen of a vancomycin peptide group.[36] This model was recently supported by analysis of the crystal structure of the vancomycin complex with Ac_2-L-Lys-D-Ala-D-Lac (Fig. 6a), which showed increased N–O distances in two NH–O hydrogen bonds in the complex with an O/N mutated peptide and a short O–O distance with the lactate oxygen.[35] One way to overcome the problem consists of chemical modification of the antibiotic converting the amide oxygen (marked red in Fig. 6a) in the imine group (Fig. 6b).[37] The modified antibiotic binds Ac_2-L-Lys-D-Ala-D-Ala less tightly than the native one, but still with high affinity, which is similar for both normal and mutant ligands (Fig. 6, bottom). It is proposed that the imine group conserves the ability to serve as a hydrogen bond acceptor toward the NH group of the normal peptide and also can serve as a hydrogen bond donor towards the oxygen atom of the mutant ligand, especially because it may be expected to partially exist in the protonated form, as illustrated in Fig. 6b.

Since the first demonstration in 1994 of the potential use of macrocyclic antibiotics as chiral selectors in analysis,[38] glycopeptide antibiotics have been successfully applied for enantiomer separations by liquid chromatography as recognition components of chiral



Azotobactin δ

Desferrioxamine-fluorophore conjugate

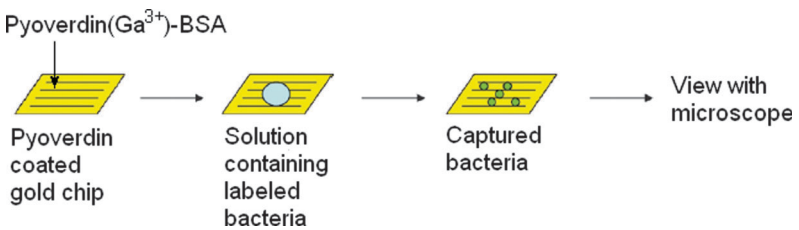


Fig. 5 Schematic diagram illustrating a siderophore-based sensor for *Pseudomonas aeruginosa*.[33] Reprinted with permission from D. D. Doorneweerd, W. A. Henne, R. G. Reifenberger, and P. S. Low, *Langmuir* 2010, **26**, 15424. Copyright 2010 American Chemical Society.

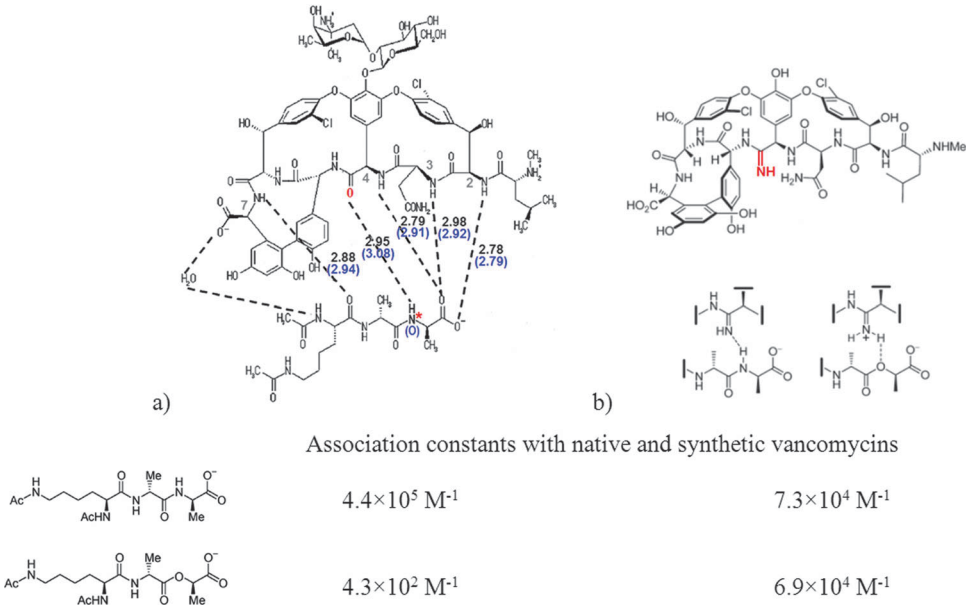
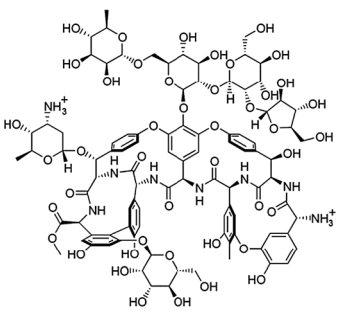
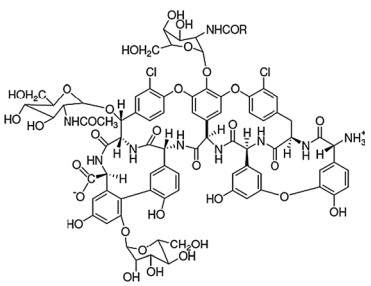


Fig. 6 (a) The interaction between vancomycin and peptide ligand Ac_2-L-Lys-D-Ala-D-Ala (adapted from ref. 35). Black numbers are N–O distances in the complex with peptide, and blue numbers are the respective distances in the complex with mutant lactate ligand. (b) Synthetic vancomycin aglycon with equal affinity to normal and mutant ligands and proposed respective binding modes.

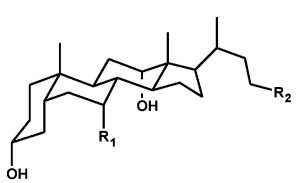
stationary phases,[39] and by capillary electrophoresis as soluble chiral selectors.[40] Vancomycin, ristocetin, teicoplanin and the teicoplanin aglycone are employed as selectors.

The structures of analytes, which can be successfully resolved with antibiotics, such as ketoprofene, methotrexate, mandelic acid, *etc.*, have very little in common with structures of specifically bound peptides. They often contain a carboxylate group, which probably binds to the antibiotic site occupied by the terminal peptide carboxylate, but since also phosphates as well as neutral compounds can be resolved, the presence of a carboxylate group seems not to be generally necessary. Typically better resolved are compounds bearing a large aromatic fragment, which suggests that principal interactions responsible for enantioseparation may involve stacking and hydrophobic binding of analytes with aromatic rings of antibiotics. No studies with non-peptide substrates, which could clarify the nature of enantioselectivity for practically important analytes, were reported yet.


Ristocetin A


Teicoplanin

Bile salts (Fig. 7) are studied both as host and guest molecules. They have chiral rigid structures and serve as important building blocks for the synthesis of both cyclic and



R_1= OH, R_2= COONa	sodium cholate
R_1= H, R_2= COONa	sodium deoxycholate
R_1= OH, R_2= $CONHCH_2CH_2SO_3Na$	sodium taurocholate
R_1= H, R_2= $CONHCH_2CH_2SO_3Na$	sodium taurodeoxycholate

Fig. 7 Most common bile salts.

acyclic hosts.[41] In living systems they solubilize apolar compounds, in particular cholesterol and fat-soluble vitamins.

Natural bile acids are employed for enantioseparation of racemates of various classes of organic compounds by enantioselective inclusion complexation in the solid state.[42,43] The separation is generally highly efficient with an enantiomeric excess often reaching 99%. The host–guest interaction within the chiral cavity is so strong that it can force the flexible guest to adopt a chiral conformation.[44]

Bile salts form micellar aggregates in the concentration range 1–10 mM, operating as soluble hosts by enantioselective inclusion of guests in these aggregates, which allows enantiodiscrimination in micellar electrokinetic chromatography.[40] Several studies of host properties of bile salt micelles by using spectroscopic probes were reported, which allowed to characterize sites of guest localizations inside micelles, see *e.g.* ref. 45. The micellar properties of bile salts are significantly different from those of common surfactants possessing long flexible hydrocarbon chains, in particular their aggregation is driven not only by hydrophobic interactions, but also by hydrogen binding.[46]

Cinchona alkaloids have been used for chiral separations since long time ago and until now have been perhaps the most popular chiral building blocks for preparation of chiral catalysts and reactants.[47] Recently they attracted considerable attention also as chiral selectors for liquid chromatography and electrophoresis[48] and as chiral solvating agents for NMR spectroscopy.[49] This stimulated physicochemical studies of host properties of cinchona alkaloids as a basis for their applications in separation and analysis.

Thermodynamic parameters for association between model chiral amino acid derivatives (R)- and (S)-**DNB-Leu** as guests and quinidine (**QD**), quinine (**QN**) and their carbamates (**t-BuCQD** and **t-BuCQN**) as hosts were determined by spectroscopic and calorimetric titrations in MeOH.[50] The study was completed with determination of the crystal structure as well as solution structure of a similar complex (see below) by NMR and theoretical calculations providing a comprehensive picture of involved intermolecular interactions.

The purpose of using carbamates was to make receptors structurally closer to their analytically employed immobilized forms prepared by attachment of alkaloid to a carrier *via* a carbamate bond. The results are summarized in Table 1. The thermodynamic analysis shows that the association in all cases is an enthalpy-driven process, accompanied by unfavorable entropic contributions. The exothermic character of association was attributed to the attractive van der Waals interactions, hydrogen bonding and π–π stacking interactions, whereas the negative entropy was considered as a normal entropic loss generated in an association process.

Table 1 Logarithms of association constants and thermodynamic parameters (kJ mol^{-1}) for binding of (R)-and (S)-**DNB-Leu** to the chiral selectors **t-BuCQD** and **t-BuCQN**, quinine and quinidine in methanol at 25 °C[50]

	(S)-**DNB-Leu**			(R)-**DNB-Leu**		
Host	log K	ΔH^0	$T\Delta S^0$	log K	ΔH^0	$T\Delta S^0$
t-BuCQN	3.57	−33	−13	2.34	−27	−14
t-BuCQD	3.00	−30	−13	3.95	−37	−14
QN	4.04	−38	−15	4.08	−36	−13
QD	4.00	−30	−7	4.11	−29	−6

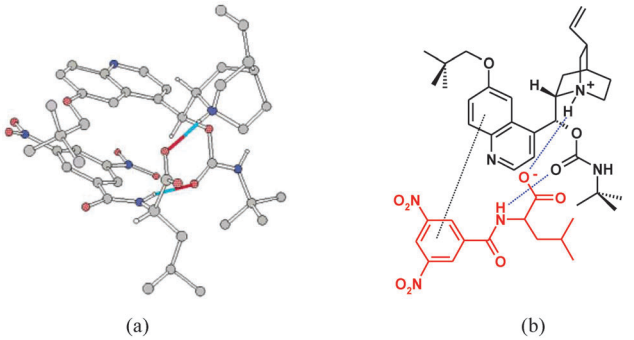
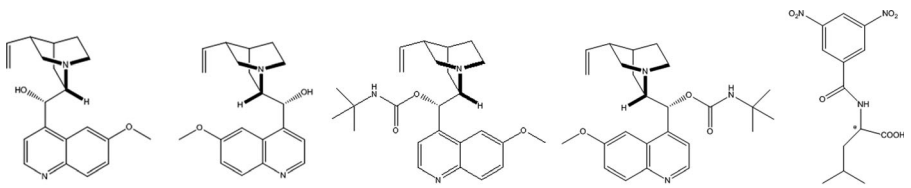


Fig. 8 (a) Single crystal structure of a complex of (S)-**DNB-Leu** with a cinchona-based chiral selector; (b) chemical structures of the guest and selector with dashed lines indicating principal attractive pair-wise interactions inferred from the crystal structure and solution studies. Reprinted with permission from Lah J, Maier NM, Lindner W, Vesnaver G. *J. Phys. Chem.*, *B*, 2001, **105**, 1670. Copyright (2001) American Chemical Society.

Structural studies of the complex of (S)-**DNB-Leu** with a similar selector (Fig. 8) confirmed the proposed set of attractive pair-wise interactions involving stacking between the dinitrophenyl group of the guest and quinoline ring of the host, an ionic hydrogen bond between the protonated amino group of the host and the deprotonated carboxylic group of the guest obtained by proton transfer to the quinuclidine nitrogen and an amide type hydrogen bond between the *N*-acyl group of the amino acid and the carbamate group of the receptor.[48a]



The most unexpected result of this study is that unmodified alkaloids bind the guest enantiomers more tightly than their carbamate derivatives, but with a complete lack of enantioselectivity (Table 1). On the other hand, the direction and magnitude of enantioselectivity observed in solution complexation with **t-BuCQD** and **t-BuCQN** coincided with those observed in chromatographic separation experiments with the same chiral selectors in immobilized form. The latter suggests that the possible interaction of analytes with a carrier does not contribute to observed selectivity.

A possible effect of the carbamate group is creation of some steric restrictions and/or additional solvophobic interactions with the guest molecule forcing it to adopt a more rigidly fixed conformation on the binding site. Strong sensitivity to the type and position of substituents was also reported for enantiodiscrimination by cinchona derivatives employed as chiral solvating agents for NMR spectroscopy.[49]

Other classes of natural macrocyclic compounds occasionally employed as hosts involve rifamycins, cyclic polypeptide antibiotics and bisbenzylisoquinoline alkaloids.[51] Amongst acyclic natural compounds, aminoglycosides were used as hosts for anionic species such as nucleotides, and were employed as chiral selectors in capillary electrophoresis. Kanamycin was shown to be as effective as many synthetic hosts in terms of affinity and selectivity in complexes with ATP, CTP and GTP, with *e.g.* lg $K = 7.08$ for ATP, 10 times higher than with GTP.[52]

4. Examples for complexation with different classes of natural compounds

4.1 Amino acids and peptides

Complexation of aminoacids with optical signalling can be achieved for instance with the dicopper host **1**, which allows glutamate recognition with rhodamine as a displacement indicator.[53] The copper-containing complex **2** allows chiral recognition with Val, Phe, Trp, or Leu, using pyrocatechol as an indicator, with binding constants of up to 10^5 M^{-1} and selectivity ratios K_D/K_L of between 1.7 and 2.6.[54] Host **3** allows fluorescence detection of ω-aminoacids as well as of drugs such as GABA or of L-carnitine down to 10^{-6} M solution.[55]

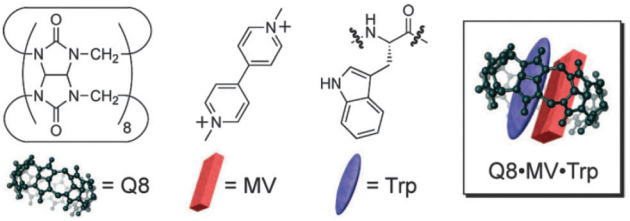
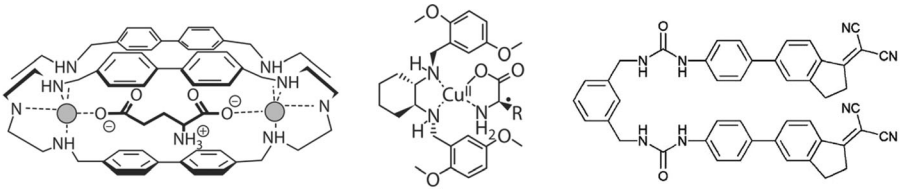


Fig. 9 Ternary complex of cucurbit[8]uril with methylviologen MV and the aminoacid Trp. Reprinted with permission from *J. Amer. Chem. Soc.* 2005, **127**, 14511. Copyright 2005 American Chemical Society.

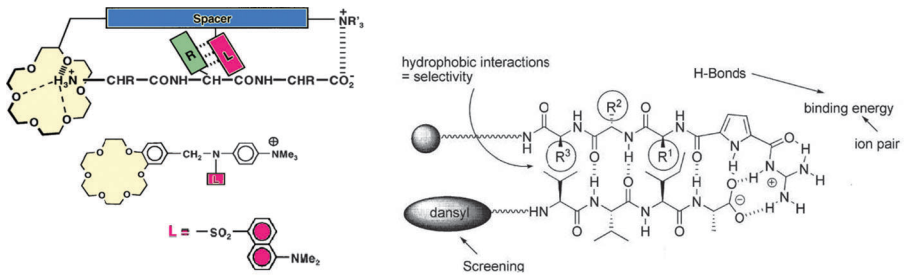
In view of the biological importance, hundreds of artificial receptors have been developed for peptides.[1,57] A general principle for length and sequence-selective complexation of peptides is shown with structure **4**; here one places host units for the $^+NH_3$ and COO^- termini at the ends of a spacer, which also bears recognition substituents at suitable positions for interaction with side chains; for instance the K value for Gly-Phe-Gly is 1700 M^{-1}, whereas Gly-Gly-Phe or Phe-Gly-Gly as well as *e.g.* Gly-Gly-Ala bind 10 times less tightly.[1g] Several examples, often with protected peptides, have shown how larger affinities can be reached with *e.g.* guanidinium groups for the carboxylate binding, and additional hydrophobic or hydrogen bond contributions.[57] Cucurbit[8]uril in combination with methylviologen (MV) can be used as described for amino acid detection (Fig. 9) also for peptides; tripeptides bind with *e.g.* log $K = 3.5$ for Gly-Gly-Trp, 4.3 for GlyTrpGly, or 5.1 for TrpGlyGly; the limitation is that aromatic residues are necessary.[56] The smaller cavity of cucurbit[7]uril (CB[7]) allows without co-complexation with MV direct selection of peptides. Only dipeptides with aromatic residue at the N-terminus interact strongly



1 **2** **3**

Cucurbit[8]uril in combination with methylviologen as a guest has been used for the detection of both amino acids and peptides (Fig. 9). Aromatic amino acids bind with log K values of 3.3 (Tyr), 3.7 (Phe), or 4.6 (Trp).[56]

with CB[7] with $K(M^{-1})$ values of 3.0×10^7 (PheGly), 3.6×10^6 (TyrGly), 5.6×10^5 (TrpGly), but only 1.3×10^3 (GlyPhe), 2.0×10^2 (GlyTyr) and 2.8×10^2 (GlyTrp). The reason for the strong complexation of peptides with aryls at the N-terminus is the



4 **5**

strong hydrogen bond between the $-^{+}NH_3$ group of the guest and the CB carbonyl oxygens together with aryl inclusion.[58]

Combinatorial searches for a suitable receptor of the tetrapeptide Ac-Val-Val-Ile-Ala-OH, which represents the C-terminal sequence of the amyloid peptide, led to the guanidiniocarbonyl pyrrole receptor **5**; here hydrophobic interactions secure selectivity, ion pairing and hydrogen bonds the affinity.[57]

Artificial hosts for all kind of positively charged bioanalytes, including basic amino acids, neurotransmitters, *etc.* have been aptly reviewed.[59]

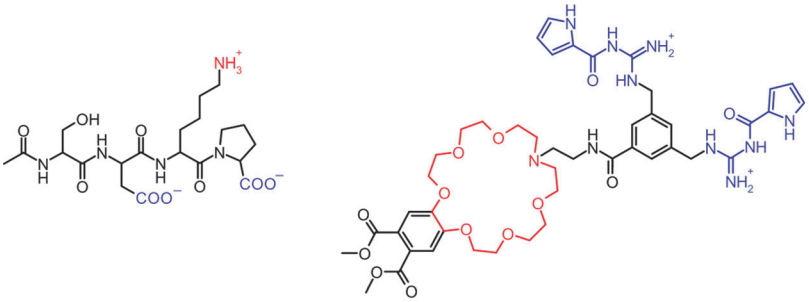
The bis-guanidinium crown ether **7** binds the naturally occurring hemoregulatory peptide Ac-Ser-Asp-Lys-Pro (**6**), which has anti-inflammatory and antifibrotic properties, with $K = 7 \times 10^3$ M^{-1} exhibiting a fluorescence emission increase; shorter or other tetrapeptides show no or significantly reduced affinity. Fig. 10 shows the possible combination of hydrogen bonding and ion pairing in the complex.[60]

Sulfonato-calixarenes such as **8** form strong complexes with basic amino acids, with K values for Arg and Lys of 1520 and 740 M^{-1} at pH 8; aliphatic or aromatic amino acids are bound with K only between 15 and 65 M^{-1}.[61]

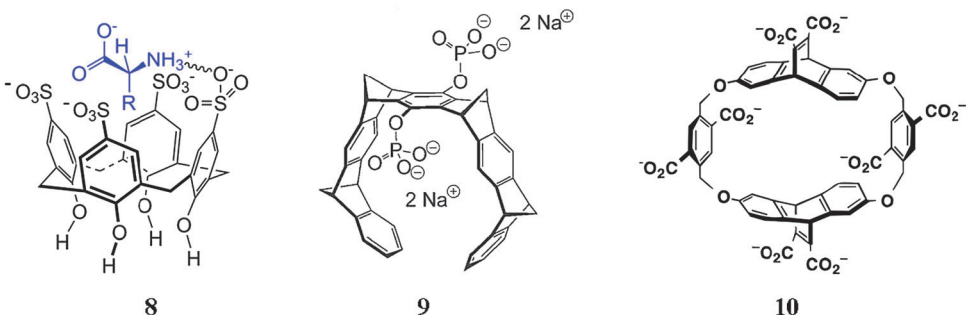
The molecular tweezer **9** forms a complex with lysine with $K_d \approx 20$ μM, binds 10-times less tightly with arginine, and very little with most other cationic bio-molecules. The selectivity, which also allows sensing location of lysine in peptides, results from threading the alkyl chain of Lys through the cavity of **9**, allowing hydrophobic interactions with host sidewalls, and simultaneous ion pairing with the anionic bridgehead groups of **9**.[62]

Tweezers such as **9** were found to be useful to find a lead compound which is capable of inhibiting the aggregation and toxicity of multiple amyloidogenic proteins by binding to Lys residues and disrupting hydrophobic and electrostatic interactions, which are responsible for nucleation, oligomerization, and fibril elongation in such proteins. Binding of the tweezer **9** to the Lys residues in the amyloid β-protein occurs at the earliest stages of assembly, and is found to be non-toxic at the level used for interference.[63]

Arginine is strongly bound in aqueous media to the cyclophane **10** which allows many salt bridges to be formed. Arginine shows a higher affinity than lysine, due to cation–π interactions for which the planar cationic arginine site is well suited.[64]



6 **7**



8 **9** **10**

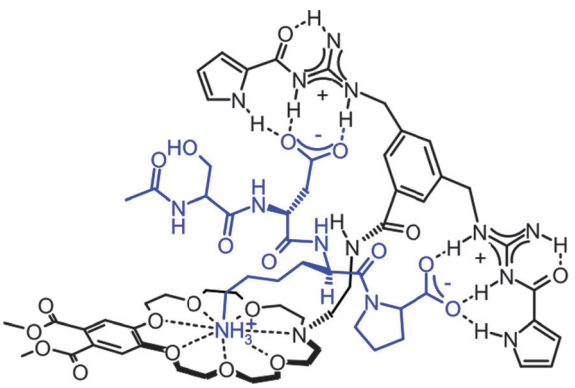


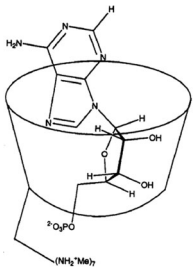
Fig. 10 Possible combination of hydrogen bonding and ion pairing in the complex between a bis-guanidinium crown ether and tetrapeptide Ac-Ser-Asp-Lys-Pro. Reprinted from ref. 60 with permission from Elsevier, 2010.

4.2 Nucleotides, nucleosides

Numerous host compounds have been designed for nucleotides with complexes dominated by ion pairing between the phosphate residues and cationic sites in the receptor.[65] With the cyclodextrin **11** bearing seven ammonium side chains the binding constants for

ATP can reach up to $K = 10^{10}$ M^{-1} if calculated for full protonation and deprotonation, respectively; in buffer at medium ionic strength the value is $K = 10^6$ M^{-1}; and decreases as expected for all ion-pairing dominated complexes with ADP and AMP.[1g] Host **11** as well as the host **12** exhibit only small base selectivity, although the latter is equipped with stacking acridine units. These lead to significant fluorescence enhancement with ATP and NADPH, with $K = 3 \times 10^8$ M^{-1}, with some contribution of base stacking.[66]

The hosts **13**[67] and **14**[68] show a strong preference for guanine, with **13** one observes *e.g.* $K = 57\,000$ M^{-1} for GTP in



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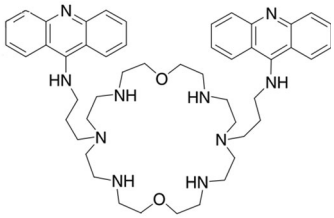


12

comparison to $K = 15\,000$ M^{-1} for ATP. Host **15** is one of the few receptors selective for uridine, likely due to a UTP/UDP promoted strengthening by the Zn^{2+} coordination.[69]

High base selectivity can be achieved with an aptamer, which is a hydrogen-bonding single stranded oligonucleotide and can recognize the adenosine group in ATP specifically (Fig. 11). Recently such an anti-adenosine aptamer was labeled with a fluorescent group and used as the labeled receptor

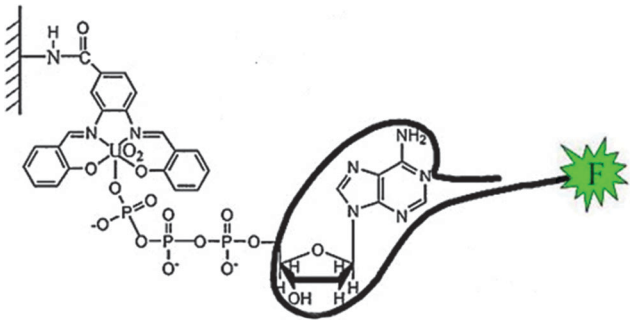
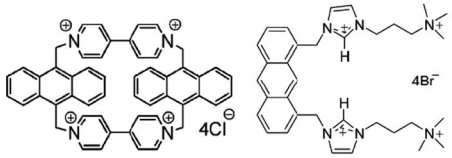


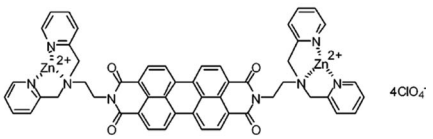
Fig. 11 Recognition of uranyl–salophen–bound ATP with an aptamer bearing a fluorescence label F.

and 4200 for ADP.[71] This is due to smaller chain length with AMP, which allows preferential stacking of adenine with the receptor units. The large π-surface of porphyrin leads with tweezer **17** even to an equally strong association with electroneutral nucleosides, with *e.g.* log $K = 4.8$ for adenosin and log $K = 4.5$ for ATP.[72]

The zinc(II)–cyclen complex **18** with pendant acridine groups shows nucleoside affinities with log $K = 7.2$ for thymine dT, and log $K = 6.9$ for uracil U, consistent with the basicities of the conjugate nucleobase at the N(3) position.[73] The Zn(II)–cyclen complex lacking the acridine moiety was found to form an about 100 times weaker complex, the difference of $\Delta\Delta G = 10$ kJ mol^{-1} is due to stacking with the nucleobase. Interaction of the Zn–cyclen–acridine complex with poly(dT) occurs with T deprotonation and stacking with $K_{app} = 3.6 \times 10^4$ M^{-1}. Studies with double-stranded poly(A) and poly(U) indicated that all Zn(II)–cyclen complexes penetrate the core of the helix with binding to the N(3)-deprotonated



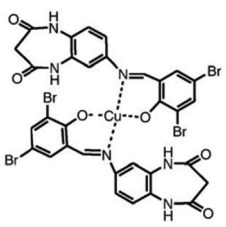
13 **14**



15

of ATP, which was bound first with a uranyl–salophen complex, immobilized on the surface of amino-silica gel particles.[70] The detection limit for ATP was 0.037 nmol mL^{-1}, and GTP, CTP and UTP did not exhibit any fluorescence.

Receptor **16** shows an unusual preference for AMP, with K (M^{-1}) values of 40 000 for the monophosphate, but 1200 for ATP



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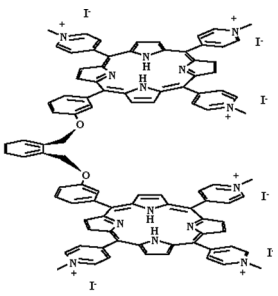
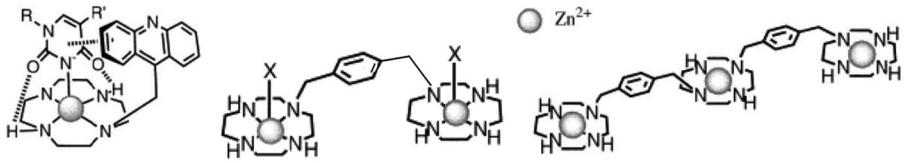


17

uracil bases, with subsequent selective denaturation of ds nucleic acid. DNase footprinting experiments showed that the Zn(II)–cyclen–acridinyl binds only to the thymine groups and separates the A–T pairs so that the separated adenine partners are more exposed to the nuclease action. The complex is also able to inhibit the binding of human TATA binding protein to the so-called TATA box, which is an AT-rich DNA sequence located at 25–30 base upstream from the transcriptional start sites; it is an essential element of the promoter for eukaryotic RNA polymerase, playing a key role in regulating the overall level of transcription. The concentration required for 50% inhibition (IC_{50}) of the TBP–DNA complex formation was 15 nM; related zinc(II) complexes were shown to work even at 0.4 nM. The complex **19** was shown to hold some promise for prevention of formation of complexes of key viral RNA with proteins; this plays a role in transcription of the HIV-1 genome which is facilitated by a HIV-1 regulatory protein. Footprinting analysis using micrococcal nuclease revealed the UUU bulge in RNA to be strongly protected by **19** in a TAR model



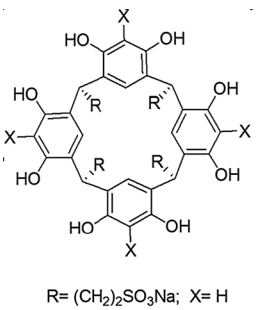
18 **19** **20**

sequence, the *trans*-activation responsive RNA element. With the tris Zn–cyclen derivative **20** the K_d value for the TAR_{33}–Tat complexation and the IC_{50} value of **20** was as low as 15 nM, which may offer a new strategy to fight AIDS. In comparison, aminoglycoside antibiotics such as neomycin show inhibition values (IC_{50}) for TAR RNA–Tat protein complex formation of around 1 μM.

4.3 Carbohydrates

Most artificial receptors for carbohydrates rely on covalent boronic ester formation and not on non-covalent interactions; they are reviewed elsewhere[74] and are not discussed here. The particularly weak intermolecular forces exerted by carbohydrates make the detection of carbohydrates by supramolecular complexation a difficult task.[75] In protic media hydrogen bonding is usually too weak, for which reason one relies *e.g.* on extraction of saccharides out of water into a lipophilic phase such as chloroform, for instance with the resorcarene derivative **21**.[76] Sugar acids can be complexed with cationic receptors such as **22**; binding constants are pH-dependent and amount to 6×10^3 with *e.g.* galacturonic acid or to 25×10^3 with glucose-1-phosphate.[77]

Effective complexation of neutral carbohydrates in aqueous medium needs special efforts to compete with bulk water for solvation. Receptor **23** binds 1-O-methyl-*b*-D-cellobiose with an association constant of approximately 900 M^{-1}, with an affinity



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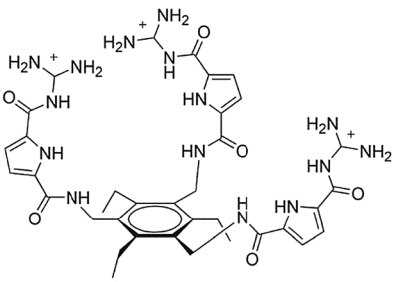
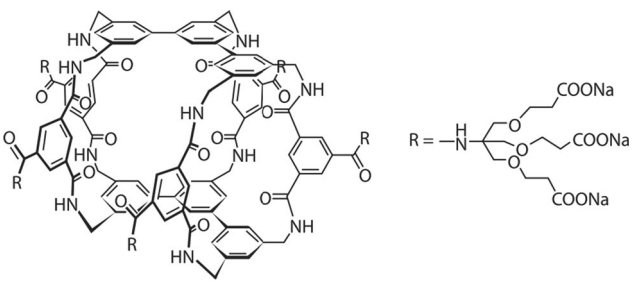


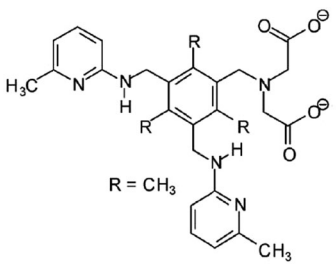
22

and even selectivity similar to that of natural lectins; glucose, however, is still complexed with only $K = 60$ M^{-1}.[78]



23

The dicarboxylate receptor **24** binds cellobiose with an association constant of 305 M^{-1} in a 1 : 1 complex, and with additional association constant of 66 M^{-1} for the binding of a second sugar molecule. Obviously the carboxylates in **24** act as enforced hydrogen bond acceptors even in the highly competitive aqueous medium (H_2O/D_2O 93 : 7 v/v). Much smaller affinities were observed with *e.g.* methyl β-D-glucopyranoside.[75c] A porphyrin derivative with four bile acid substituents is claimed to complex *e.g.* glucose with lg $K = 5.7$,[79] presumably



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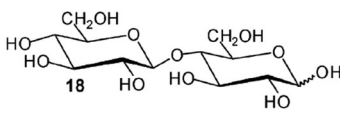
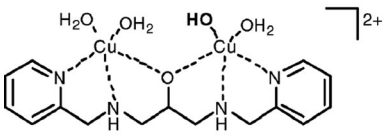


Cellobiose

due to the hydrophobic environment built up by the large steroidal groups.

An attractive possibility is to use the relatively strong affinity of metal ions towards saccharides, although usually under rather basic conditions. With the dinuclear copper complex **25** the apparent binding constants with unprotected disaccharides are 10^3 to 10^4 M^{-1};[80] with the lanthanide complex **26** one finds increased fluorescence with saccharides in mM concentration.[81]



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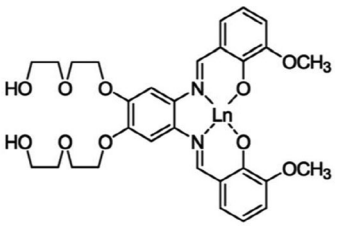


26

4.4 Catecholamines

Catecholamines (epinephrine, norepinephrine, and dopamine) act as neurotransmitters and hormones, playing an important role in the body's physiological response to stress (Chart 1). Compounds often employed as reference guests in studies of their recognition are DOPA, a precursor of dopamine in its biosynthesis and phenethylamine or tyramine, structurally related amines lacking the catecholate diol moiety. Recognition of catecholamines by synthetic receptors in water was reviewed[82] and the subject was also covered in an extensive review on recognition of organic ammonium ions in solution.[59]

Fig. 12 illustrates the binding pattern of noradrenaline in the natural β-adrenergic receptor[83] and possible types of binding interactions with synthetic receptors.[82] As one can see the difficult to achieve hydrogen bonding interactions with the diol fragment of the guest can be substituted in synthetic receptors by efficiently reversible boronate ester formation.

Combinations of a boronic acid recognition site with an anionic site for ammonium recognition and possible stacking interactions involve receptors **27**[84] and **28**.[85] The proposed binding mode for **27** is illustrated in **29**.[85]

The major interaction with these receptors is due to boronate ester formation as is evident from a small difference in affinity toward catecholamines and simple non-substituted pyrocatechol. An interesting feature of the receptor **28**, absent in other synthetic receptors, is its ability to discriminate dopamine and adrenaline in favour of the former with a factor of up to 10 in the binding constant.

A multipoint recognition of cationic forms of catecholamines *via* only non-covalent interactions was reported with receptor **30** in non-polar medium.[86] Binding constants of up to 5×10^4 M^{-1} for dopamine and adrenaline surpass by an order of magnitude those for phenethylamine and tyramine and by two orders of magnitude that for *N*-methylated phenethylamine in 2% MeCN/98% $CHCl_3$, but the affinity strongly decreases already in 10% MeCN/90% $CHCl_3$.

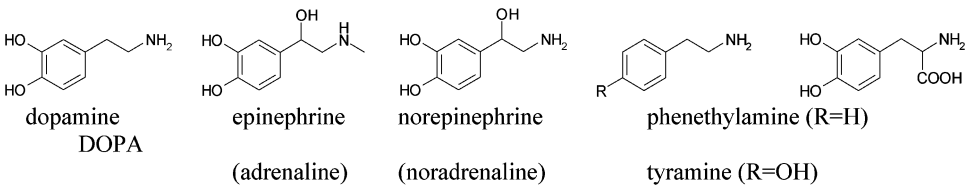


Chart 1 Catecholamines and related compounds.

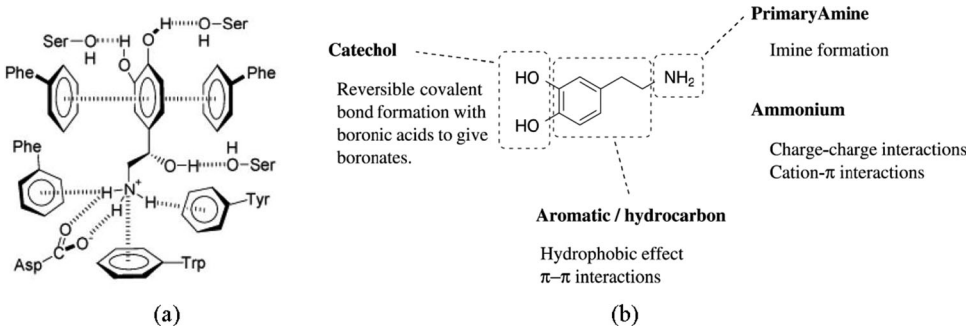
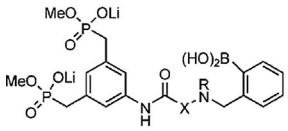


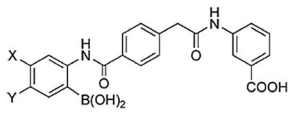
Fig. 12 Combination of ionic, cation–π, π–π interactions and boronate ester formation for catecholamone recognition. (a) Binding pattern of noradrenaline in the natural β-adrenergic receptor;[83] (b) possible types of binding interactions with synthetic receptors.[82]



1–3

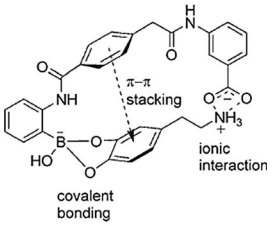
1 : X = -C6H4-CH2-; R = H
2 : X = -(CH2)3- ; R = H
3 : X = -(CH2)3- ; R = Me

27



1a: X=H, Y=H;
1b: X=CN, Y=H;
1c: X=H, Y=F;
1d: X=CONH2, Y=H

28



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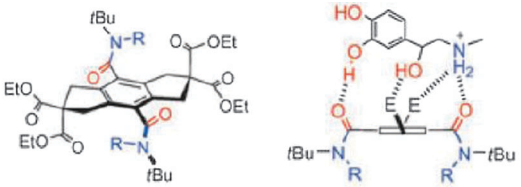

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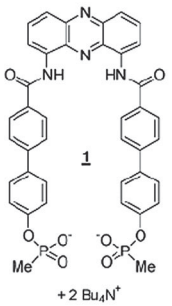

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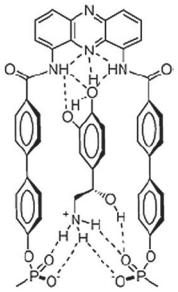

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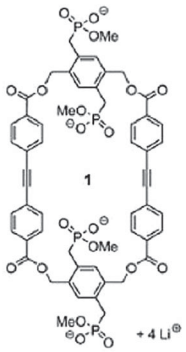


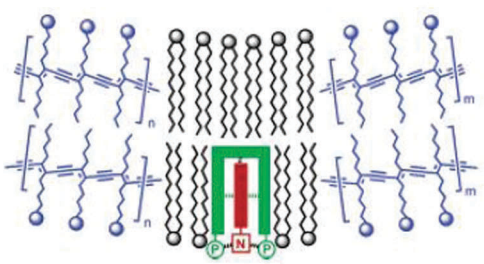
33

Cleft type receptor **31** was designed to recognize catecholamines by a combination of interactions similar to those in the natural receptor *cf.* **32** and Fig. 7a.[83] The host selectively binds noradrenaline with the binding constant 1800 M^{-1} in methanol. A tetraanionic macrocycle **33** has similar affinity to noradrenaline (association constant of 1250 M^{-1}), but in water.[87] Both 1 : 1 and 2 : 1 (guest : host) complexes are formed and the receptor tends to auto-associate with micelles above 3×10^{-4} M with concomitant improvement in the recognition properties, which are further improved on inclusion of the receptor into a lipid monolayer. In a subsequent paper the authors have achieved a specific fluorescent detection of noradrenaline with host **31** incorporated into phospholipid/polydiacetylene vesicles shown schematically in **34**.[88]

The highest binding constant of protonated dopamine in water close to $K = 10^6$ M^{-1} was reported for a monoprotonated form of a bicyclic receptor **35**[89] and explained by encapsulation of dopamine in the macrocyclic cage.

4.5 Flavonoids

Flavonoids, a group of polyphenolic compounds, constitute a diverse class of natural products, usually derived from a parent diarylpropane (C6–C3–C-6) skeleton; they represent a valuable



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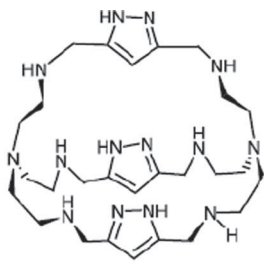

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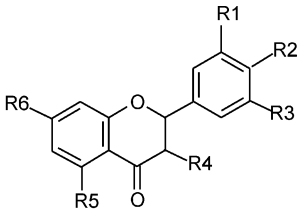
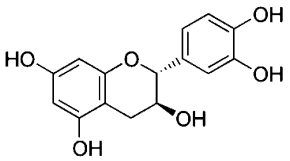


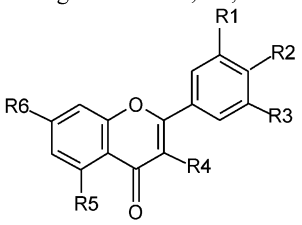


Flavanone R1, R2, R3, R4, R5, R6 = H

Catechin

Naringenin R1, R3, R4 = H; R2, R5, R6 = OH

Naringin R1, R3, R4=H; R2, R5 = OH; R6 = O-rhamnoglucoside



Luteolin R3,R4 =H; R1, R2 ,R5, R6 = OH

Kaempferol R1, R3= H; R2, R4, R5, R6 = OH

Myricetin R1, R2 , R3, R4, R5, R6 = OH

Chart 2 Some typical flavonoid structures used in complexation studies.

resource for many pharmaceutical applications (Chart 2). Flavonoids scavenge reactive oxygen species (ROS) and this activity has often been credited for their reported health benefits.

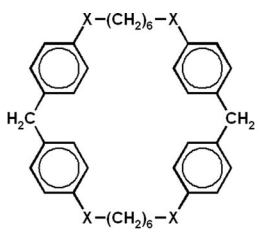
The flavonoids luteolin, kaempferol, and myricetin (Chart 2) have antioxidant effects or free radical scavenging properties on a wide range of oxidative-stress associated pathologies. Their complexation with sulfobutylether-β-cyclodextrin affects positively the solubility, bioavailability and antioxidant activity compared with the free flavonoids.[90] Complexation with methyl-cyclodextrin allows application of the cupric reducing antioxidant capacity (CUPRAC) assay irrespective of the lipophilicity of polyphenolics and flavonoids.[91] The antioxidant activity of tea catechins inclusion complex with native and derivatized-β-CD has been studied by means of the ORAC-fluorescein (ORAC-FL) and the ORAC-pyrogallol red (ORAC-PGR) assay. The difference between ORAC-PGR values for the same tea catechin shows that the inclusion structures should be different.[92]

The abundant flavonoid aglycone, naringenin, which is responsible for the bitter taste in grapefruits, suffers from low oral bioavailability, limiting its clinical potential for the treatment of dyslipidemia, diabetes, and HCV infection. Hydroxypropoyl-β-cyclodextrin (HPβCD) increases the solubility of naringenin by over 400-fold, and its transport across a Caco-2 model of the gut epithelium by 11-fold. Stereospecific resolutions of naringenin, its glycoside naringin, and catechin were accomplished by LC-ESI-MS/MS on a β-CD-bonded stationary phase.[93]

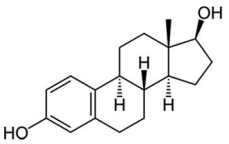
4.6 Terpenoids/steroids

Many terpenoids and in particular steroids exhibit significant biological activities; also for this reason they were often studied in combination with suitable host compounds. Terpenoids are notoriously hydrophobic compounds, and their complexation in hydrophilic hosts offers a pharmaceutically interesting way to solubilize them. Early attempts with the water-soluble azoniacyclophane **36** showed in 50% aqueous methanol with the hormone beta-estradiol a binding constant of 25 M^{-1}, which increased to 250 M^{-1} with the corresponding anion as a result of additional ion pairing between the steroidal anion and the cyclophane nitrogen centers. Extrapolation to pure water as medium predicts association constants of up to 10^5 M^{-1} in neat water; NMR spectra indicate the inclusion of the steroidal A/B rings within the cavity of **36**.[94]

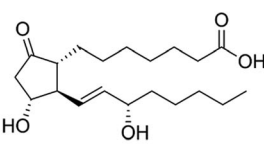
Dendritic cyclophanes (dendrophanes) of type **37** (R = OH) of first to third generations bind the steroids testosterone and progesterone in 50% aqueous methanol with association constants of about 1000 M^{-1}.[95] The stability of the complexes is not affected by the size of the host, but is significantly reduced for



36 (X= $^{+}NMe_2$)

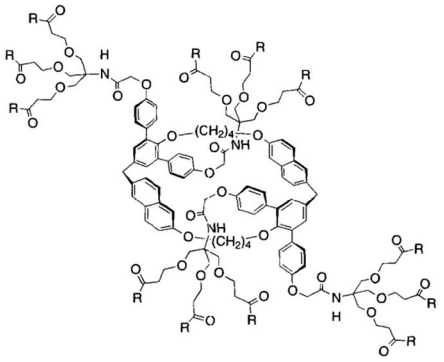


Estradiol



prostaglandin E1

steroids like cortisone and hydrocortisone bearing hydrophilic substituents.



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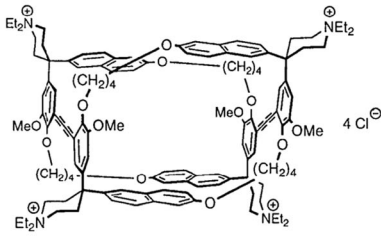


38

The complexation between the double-decker cyclophane **38** and a series of 30 steroids was investigated in CD_3OD by ^{1}H-NMR titrations.[96] The geometries of the complexes, in which the substrates are axially included in the receptor cavity, were established and correlations of stability constants varying in the range 1.3×10^2–3×10^3 M^{-1} with the lipophilicity of receptors were discussed.

Cyclodextrins modified by attached nucleobases were employed for enantioselective recognition of camphor and borneol in water.[97] Native β-CD binds (+)-camphor with an association constant of 8300 M^{-1} and an enantioselectivity $K(+)/K(-)$ = 1.66, but with ade-β-CD the association constant increases to 48 000 M^{-1} and $K(+)/K(-)$ to 3.7 (Chart 3). Binding of borneol with native β-CD is practically non-enantioselective with an association constant of about 18 000 M^{-1}, with ade-β-CD, however, its affinity is *ca.* 4-fold higher and the enantioselectivity increases to 1.7 in favor of (−)-borneol. The largest enantioselectivity of 3.5 also in favor of (−)-borneol is observed with ura-β-CD, which binds (+)-borneol with the same affinity as the native β-CD. The observed effects are rationalized in terms of formation of inclusion complexes where self-included nucleobase substituents of the host which remain inside the CD cavity together with included guest, thus providing additional hydrogen bonding to the guest (Fig. 13).

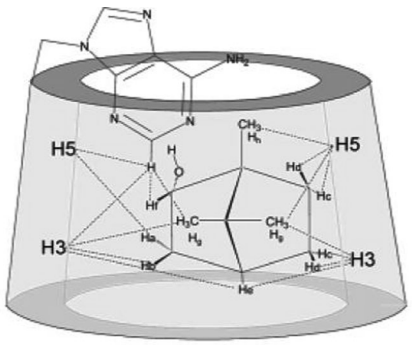
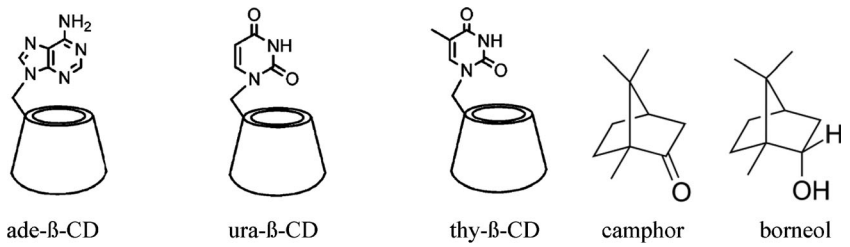


Fig. 13 Complex of cyclodextrin with the covalently attached nucleobase and borneol, with hydrogen bonds indicated as dashed lines. Reprinted with permission from *J. Phys. Chem. B*, 2007, **111**, 12211. Copyright 2007 American Chemical Society.

The complexation of bile salts (see Fig. 6) by modified mono- and bis-cyclodextrins was studied rather extensively. L/D-Tryptophan-modified β-cyclodextrins were employed as fluorescence sensors for bile salts such as deoxycholate (Fig. 14).[98] Trp-β-CD is more fluorescent than free tryptophane and addition of bile salts induces quenching. This behavior is consistent with intramolecular inclusion of the indolyl moiety of the amino acid into a more hydrophobic environment of the cyclodextrin in the initial state and subsequent expulsion of this moiety back into an aqueous environment upon complexation with the guest. The binding constants of between 1000 and 5000 M^{-1} differ just by a factor of 2 or less from those measured with free β-cyclodextrin, but show opposite changes for L- and D-Trp derivatives. A detailed study using 2-dimensional NMR allowed the authors to propose the mechanism of interaction illustrated in Fig. 14.[98]

The complexation of large hydrophobic steroid molecules with unsubstituted cyclodextrins usually involves spontaneous formation of encapsulated 2 : 1 host : guest complexes.[99]



ade-ß-CD ura-ß-CD thy-ß-CD camphor borneol

Chart 3 Cyclodextrins with attached nucleobases and structures of complexed monoterpenes.

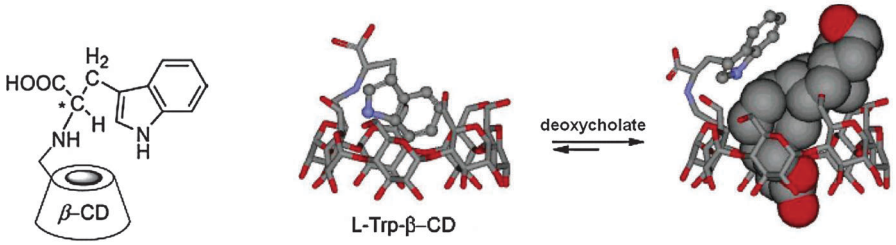
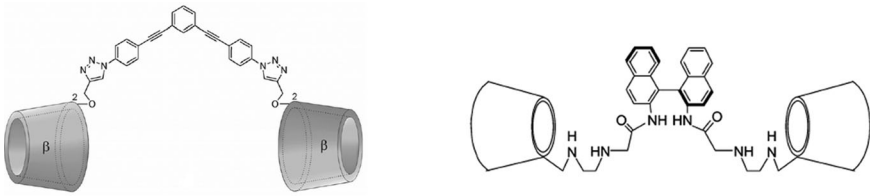


Fig. 14 Trp-β-CD and conformations of the host and its inclusion complex with deoxycholic acid (see Fig. 6). Reprinted with permission from *J. Org. Chem.*, 2005, **70**, 8703. Copyright 2005 American Chemical Society.



39 **40**

Also steroid-induced highly efficient encapsulation was observed with a deep cavity resorcinarene host.[100] In view of this one may expect bridged bis-cyclodextrins to show generally higher affinities to bile acids due to a possible chelate effect, but this approach is not always successful. Examples of such receptors are **39**[101] and **40**.[102] Receptor **39** binds sodium cholate and deoxycholate with high stability constants of up to 1×10^5 M^{-1} and serves as a ratiometric fluorescence sensor for these bile acids. On the other hand receptor **40** binds two guest molecules to each cyclodextrin unit independently with stability constants of up to only 10^4 M^{-1}. The receptor suffers also from extensive self-inclusion of the aromatic spacer in one of the cyclodextrin units.

Cyclodextrins or less toxic derivatives such as 2-hydropropyl-β-cyclodextrin are used most often as complexing agents for hydrophobic drugs including also terpenoids.[103] Thus, prostaglandin E1, which has vasodilatory, anti-inflammatory, anti-aggregatory, and anti-proliferative properties, was tried in combination with microsphere encapsulation for pulmonary delivery of prostaglandin E1 for treatment of pulmonary arterial hypertension, observing a significant increase in drug release *in vitro*, and a prolonged in circulation availability of PGE1 *in vivo*.[104]

Chiral capsules obtained from glycoluril derived structures (Fig. 15) bind pinanes with a selectivity of *e.g.* 50% de, due to the chirality stemming from hydrogen-bonded dimerization of the non-chiral starting monomers (Fig. 15). The affinity of similar capsules with pinanediol reaches *e.g.* K = 800 M^{-1}. The measurements must be done, however, in solvents such as *p*-xylene, which are too large to fill the capsule. Comparison of the capsule volumes and the guest volumes shows generally a filling factor of only about 50%.[105]

4.7 Alkaloids

Alkaloids are a group of nitrogen containing natural compounds of enormous chemical diversity and variable biological activity, produced by plants as a defense against herbivores and pathogens. Studies of host–guest complexes with alkaloids as guests are undertaken for development and/or improvement of analytical procedures for their determination and for improvement of their

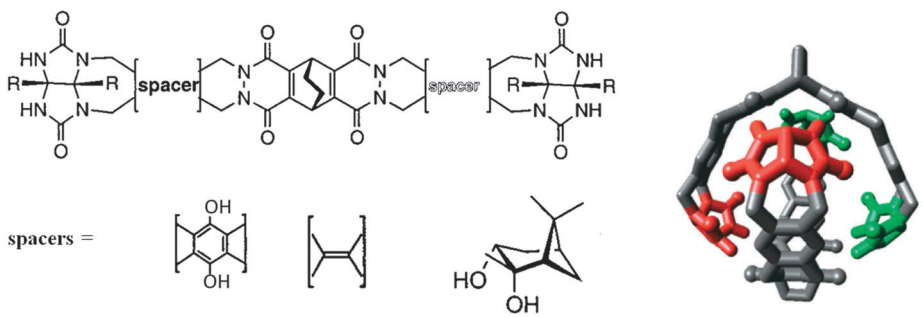


Fig. 15 Chiral capsules obtained from glycoluril, and dihydroxypinane structures. Reprinted with permission from *J. Amer. Chem. Soc.*, 2001, **123**, 5213. Copyright 2001 American Chemical Society.

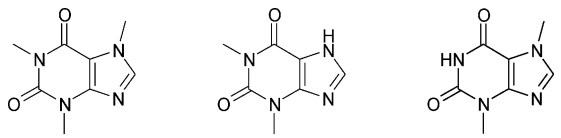


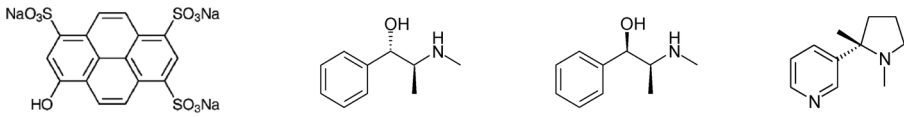
Chart 4 Xanthine alkaloids.

solubility and bioavailability. Such studies involve mostly native or modified cyclodextrins as hosts. An extensive compilation of complex formation constants for these and some other hosts with alkaloids can be found in ref. 106. Significant attention was devoted to caffeine detection. Caffeine belongs to a group of xanthine alkaloids (xanthenes, Chart 4) and is the only drug which is present in widely consumed foods and beverages either as a natural component or as an additive.

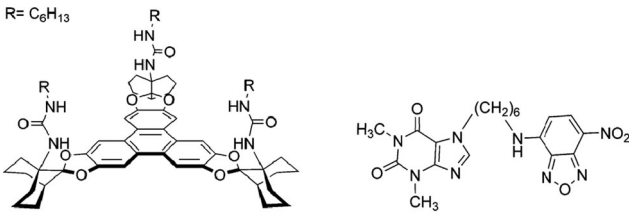
The first efficient receptor acting in water was a tetracationic Zn(II) porphyrin–peptide conjugate (Fig. 16).[109] The binding mode of caffeine involves stacking interaction with the porphyrin plane and coordination to the metal ion (Fig. 16). Binding constants in basic aqueous solutions are of the order of 5×10^3 M^{-1}.

Another approach is based on known capacity of caffeine to associate with polyphenols *via* π-stacking interactions. A commercially available fluorescence dye, 8-hydroxypyrene-1,3,6-trisulfonate (HPTS), binds to caffeine in neutral aqueous solutions with the association constant of 245 M^{-1} and its binding is accompanied by strong fluorescence quenching of HPTS allowing us to detect less than 1 mM caffeine with good selectivity.[110]

Because of their basic nature alkaloids are present in neutral aqueous solutions mostly as protonated cationic species, which



Selective recognition of caffeine can be achieved by hydrogen-bonding receptors in non-aqueous media.[107] The combination of host **41** with an indicator **42**, which competes with caffeine complexation with **41**, yields a practicable sensor for caffeine: if **42** is bound to **41** it quenches the fluorescence of the triphenylene fluorophore in **41**, displacement of the indicator **42** by caffeine leads to a fluorescence signal increase by a factor of up to four. After extraction from aqueous beverages with methylenchloride caffeine measurements are this way straightforward and sensitive over a wide concentration range.[108]



makes them appropriate guests for cucurbiturils (see Fig. 1). Thus, an indicator displacement assay for pseudoephedrine with CB7 as a host and some azobenzenes as indicators was developed, although with rather low selectivity: binding constants for caffeine, pseudoephedrine, ephedrine, dopamine and nicotine vary in limits from 4×10^5 M^{-1} for the most tightly bound pseudoephedrine to 2×10^4 M^{-1} for the less tightly bound dopamine.[111] The recognition properties of cucurbiturils were studied thoroughly with isoquinoline alkaloids, such as berberine or sanguinarine, compounds, which can target DNA and RNA.[112]

Berberine forms a very stable complex with CB7 in water (log $K = 6.2$), accompanied by a 500-fold increase in the fluorescence intensity of the alkaloid.[10a] Molecular modeling predicts a partial inclusion of the guest into the host cavity with the dimethoxyisoquinolinium moiety inside the cavity and the ammonium centre located near the host carbonyl groups. This structure was confirmed by 1H-NMR studies. The increased fluorescence of the bound alkaloid was attributed to the low

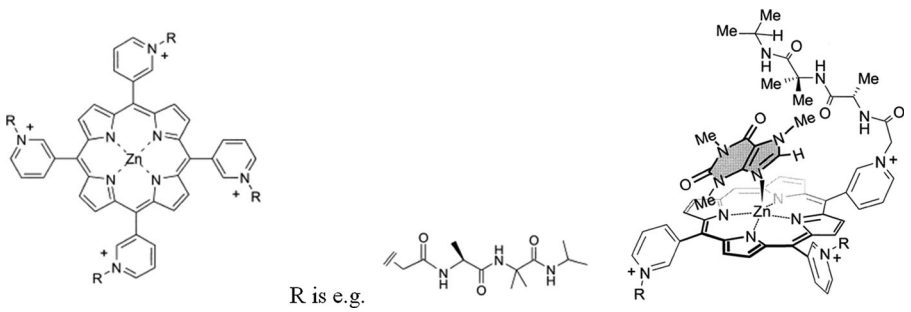
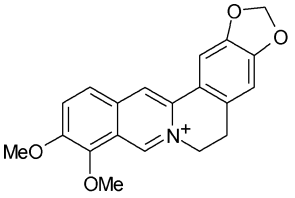
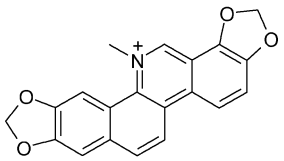


Fig. 16 Tetracationic Zn(II) porphyrin–peptide conjugate and its complex with caffeine.



Berberine



Sanguinarine

polar microenvironment of the host interior. This sensitivity of the berberine fluorescence to the medium was used also for testing micelle formation by bile acids.[10b]

A detailed NMR study of the interaction of sanguinarine with CB7 revealed formation of two types of complexes: weak non-specific associates, which are in fast equilibrium with free molecules, and slowly formed in NMR time scale inclusion complexes of 1 : 1 and 1 : 2 guest : host stoichiometries (Fig. 17).[113] The first association constant is very large (log K = 6.2) and exceeds by *ca.* 1000 times the second one. Complexation-induced changes in the absorption spectrum of sanguinarine are small, but the fluorescence intensity increases by 20 times with a concomitant shift in the emission maximum from 604 to 556 nm. The excited-state lifetime increases from 2.3 ns for the free alkaloid to 17.5 ns for the bound one.

Biologically important polyamines are not considered as alkaloids, but have an obvious similarity with them. Development of a synthetic receptor for tight and selective binding of spermine capable of competing with DNA is of interest as an alternative approach to spermine targeting anticancer agents. A macrocyclic receptor which binds spermine inside the cavity (Fig. 18) with a dissociation constant of 22 nM, lower than that for DNA, was prepared by using a dynamic combinatorial library obtained by oxidation of the mixture of mercaptobenzoic acids.[114] The ability of the receptor to remove DNA-bound spermine was confirmed by demonstration of the change of left-handed DNA conformation induced by spermine binding to DNA back to its right-handed form upon addition of the receptor.

Sensing of some alkaloids, which by their strong physiological action are drugs of abuse (cocaine, morphine, codeine, ephedrine, nicotine), is an important practical task. These compounds are rather small molecules with a few polar groups and a limited hydrophobic surface, which makes it difficult to design an appropriate efficiently acting host molecule for their recognition. Significant progress was achieved here by using molecularly imprinted polymers[115] (for general review on separation and screening of biological compounds using molecularly imprinted polymers see ref. 116) and aptamers,[117] receptors, which allow creation of a specific binding site by a selection process. For example, an optical fluorescence-based sensor for cocaine was prepared by molecular imprinting of a polymer containing as a fluorescent functional monomer acrylamide N-substituted with fluorescein.[118] The signaling is based on ion-pairing of the fluorescein carboxylate group with the protonated form of the basic cocaine molecule, which induces an increase in the fluorescence intensity. The sensor allows detection of 2 μM cocaine in the

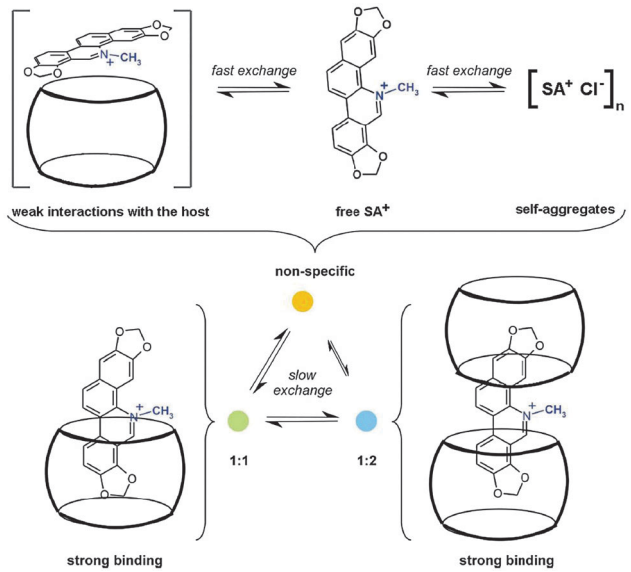


Fig. 17 Binding modes of sanguinarine with cucurbit[7]uril. Reproduced from ref. 113 with permission from The Royal Society of Chemistry.

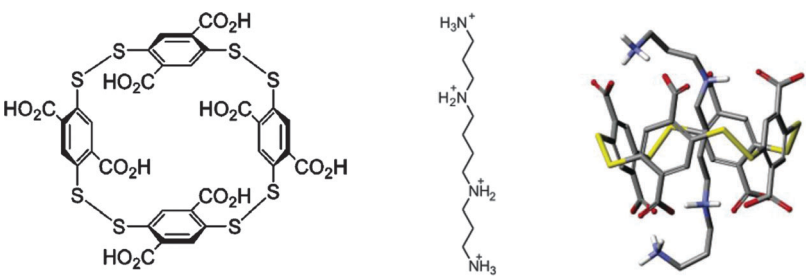


Fig. 18 A macrocyclic receptor which binds spermine inside the cavity. Reprinted with permission from *J. Amer. Chem. Soc.*, 2006, **128**, 10253. Copyright 2006 American Chemical Society.

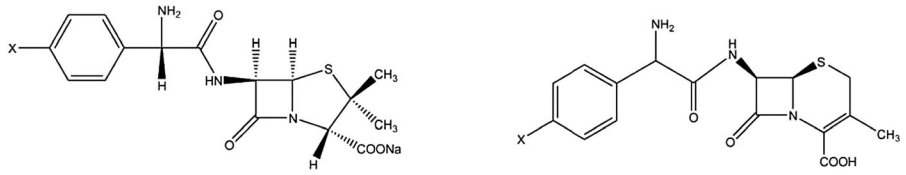


Chart 5 Penicillins and cephalosporins.

presence of some typically interfering substances such as codeine, amphetamine, ketamine, or buprenorphine.

An example of an aptamer-based cocaine sensor is shown in Fig. 20.[119] Two aptamer sub-units are labelled with a CdSe/ZnS quantum dot (QD) and a fluorescent dye, respectively, and a subsequent self-assembling process induced by cocaine binding brings the QD and the dye in close proximity sufficient for observation of the fluorescence resonance energy transfer (FRET). This allows the detection of 1 μM cocaine.

4.8 Antibiotics and toxins

Hydrogels derived from β-cyclodextrin are suitable for release of antibiotic drugs, as shown with rifampin, novobiocin and vancomycin. This way one can obtain longer and more linear release than with purely diffusion-based mechanisms, as evident from comparison with dextrose gels.[120]

NMR studies revealed that penicillins (Chart 5) bind to cyclodextrins with constants of up to 200 M^{-1}, whereas cephalosporins are not complexed. The degradation of the penicillin ampicillin by β-lactamase is significantly slower in the presence of a carboxylated cyclodextrin.[121]

Natural paralytic shellfish toxins or poisons such as saxitoxin can be detected directly in fish extracts *e.g.* by the host in Chart 6 at 40 μM limit, moreover by fluorescence emission in the visible range; they can thus replace mouse tests.[122] The guanidinium residue interacts with crown and arene parts of the host; upon complexation the PET from the crown to the fluorophore is not quenched anymore as the oxygen molecular orbitals are used for hydrogen bonds.

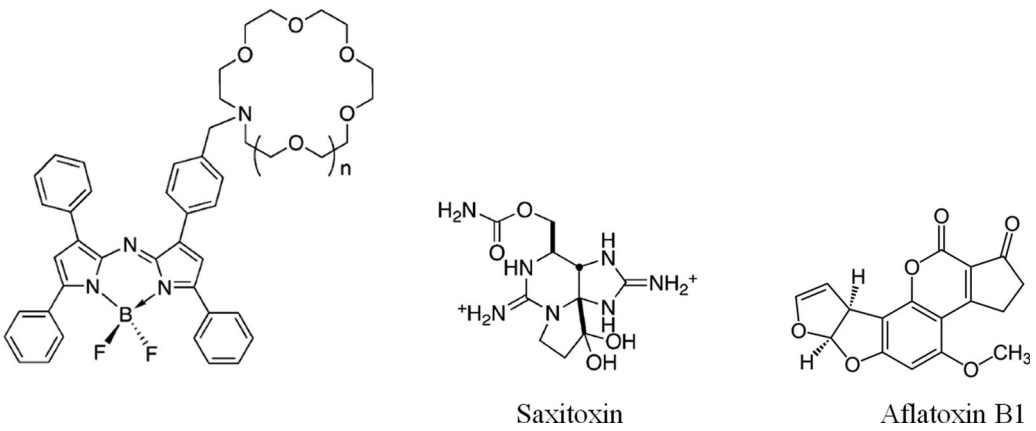


Chart 6 Toxins aflatoxin and saxitoxin, and host for saxitoxin.

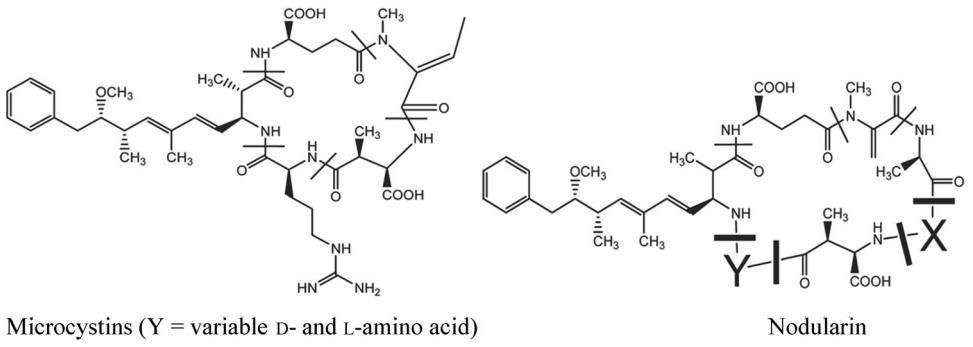


Chart 7 Cyanotoxins.

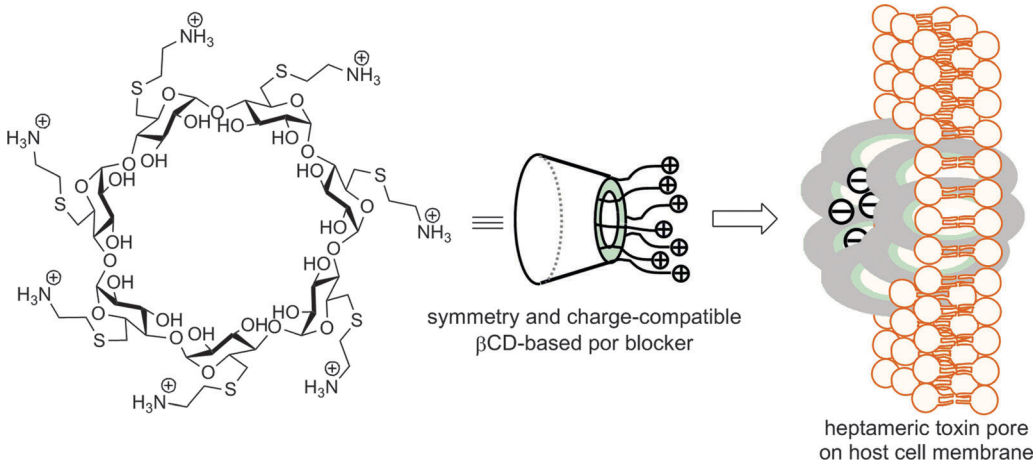


Fig. 19 High-affinity blockage of transmembrane channels with β-cyclodextrin-heptaamino derivatives as an anti-anthrax toxin strategy.[125] Figure from C. Ortiz Mellet, J. M. García Fernández and J. M. Benito in: *Supramolecular Systems in Biomedical Fields* RSC, Cambridge, H.-J. Schneider, Ed., 2013, with permission.

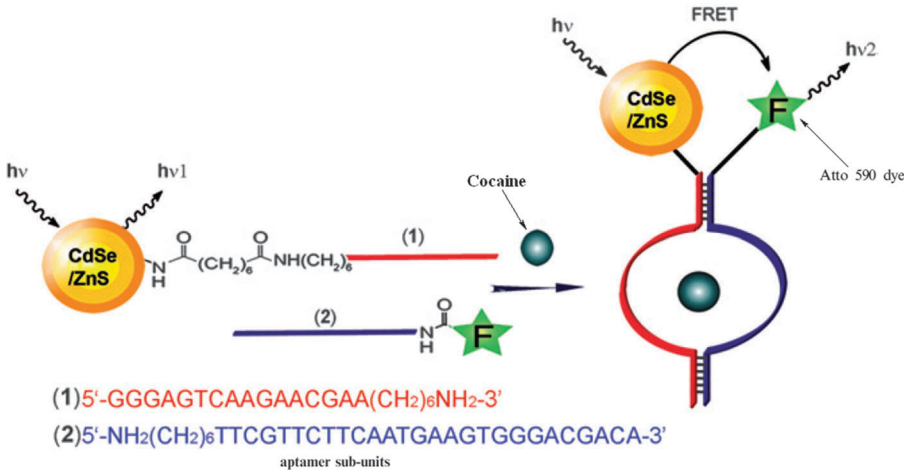


Fig. 20 Optical cocaine sensing based on FRET induced by self-assembly of two aptamer sub-units in the presence of analyte.[119] Reproduced from ref. 119 with permission from The Royal Society of Chemistry.

Aflatoxins are frequently occurring mutagenic, carcinogenic and teratogenic products from fungi in phytogenous foodstuff like bread. Based on reaction with 2-hydroxypropyl-β-cyclodextrin in solution they can be traced by strong fluorescence intensity, with an increase of the sensitivity by an order of magnitude in comparison to surfactants used otherwise.[123] Cyanotoxins such as microcystins and nodularin (Chart 7) are toxic compounds produced by cyanobacteria from algae. They bear lipophilic chains containing diene and benzene units, which by NMR analyses were shown to complex within cyclodextrin cavities. The complexation constants with β-CD reach up to 1155 M^{-1}, holding promise for detoxification of *e.g.* drinking water.[124]

Anthrax toxins, which contain several peptides, can be targeted using highly charged β-cyclodextrin-heptaamino derivatives for high-affinity blockage of transmembrane channels[125] (Fig. 19). This anti-toxin strategy has been successfully tested with mice and infectious bacteria, and hold promise in view of the possible use of these toxins as biological weapons.

5. Conclusions

Nature offers a rich reservoir of bioactive compounds, often occurring in complex mixtures. Supramolecular chemistry can help to analyze and to separate these compounds, to use them in suitable form for medicinal applications, and to detoxify dangerous ingredients. Complexation of natural compounds can provide insight into significant interaction mechanisms of biological systems. The use of natural compounds and their derivatives as synthetic receptors holds much promise, in particular in view of their chiral properties, which can provide for efficient enantioselective separations. Given the unlimited number and variety of both natural compounds and synthetic host structures there are virtually endless possibilities to explore new applications.

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