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Cation-Induced Pesticide Binding and Release by a Functionalized Calix[4]arene Molecular Host

Li Luo, Xiaoyan Zhang, Ningmei Feng, Demei Tian, Hongtao Deng & Haibing Li

Key Laboratory of Pesticide and Chemical Biology (CCNU), Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079 (P. R. China).

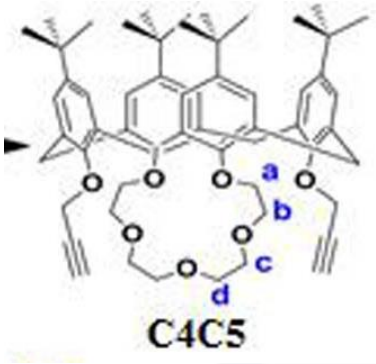
Ion-controlled switchable progress is very important in many biological behaviors. Here, we reported K^+-controlled switch, this switch system exhibited excellent carbaryl (G) binding/release by fluorescent (FL), ultraviolet-visible (UV) spectrums and ^1H NMR spectroscopy. More importantly, the K^+-controlled G binding/release switch based on C4C5 not only in the solution, but also on the surface, promising for the application for the pesticide controlled release.

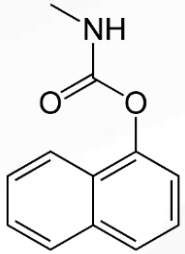
Lezione 12

- Applicazioni dei calixareni

Ion-controlled switchable process is very important in many biological behaviors, for example, metal ion directed protein folding and self-assembly, Ca2+ induced contraction or relaxation of the human heart and the Na1 stimulated nerve impulses.

Recently, those in which the binding substrate could be ‘‘on’’ or ‘‘off’’ by ion, have attracted great interest in host-guest complex, due to their extensive potential application not only in the construction of artificial molecular machines but also in the development of sensing and controllable drug delivery system.





Carbaryl (G)

Carbaryl (G) is a pesticide which have naphthalene group can interact with calixarene through π-π stacking.
Crown ether was employed to selectively bind K^+ in aqueous solution. It was known that 18C6 is a very strong sequestering agent for potassium ion, which has strong binding affinity toward K^+ . Therefore, we designed a wettable responsive switch based on K+-controlled calix-crown binding/release G.
All were shown in the figure 1b. As a consequence of its outstanding properties, the K+-controlled switch has formed attractive application in many fields, especially on a silicon surface.

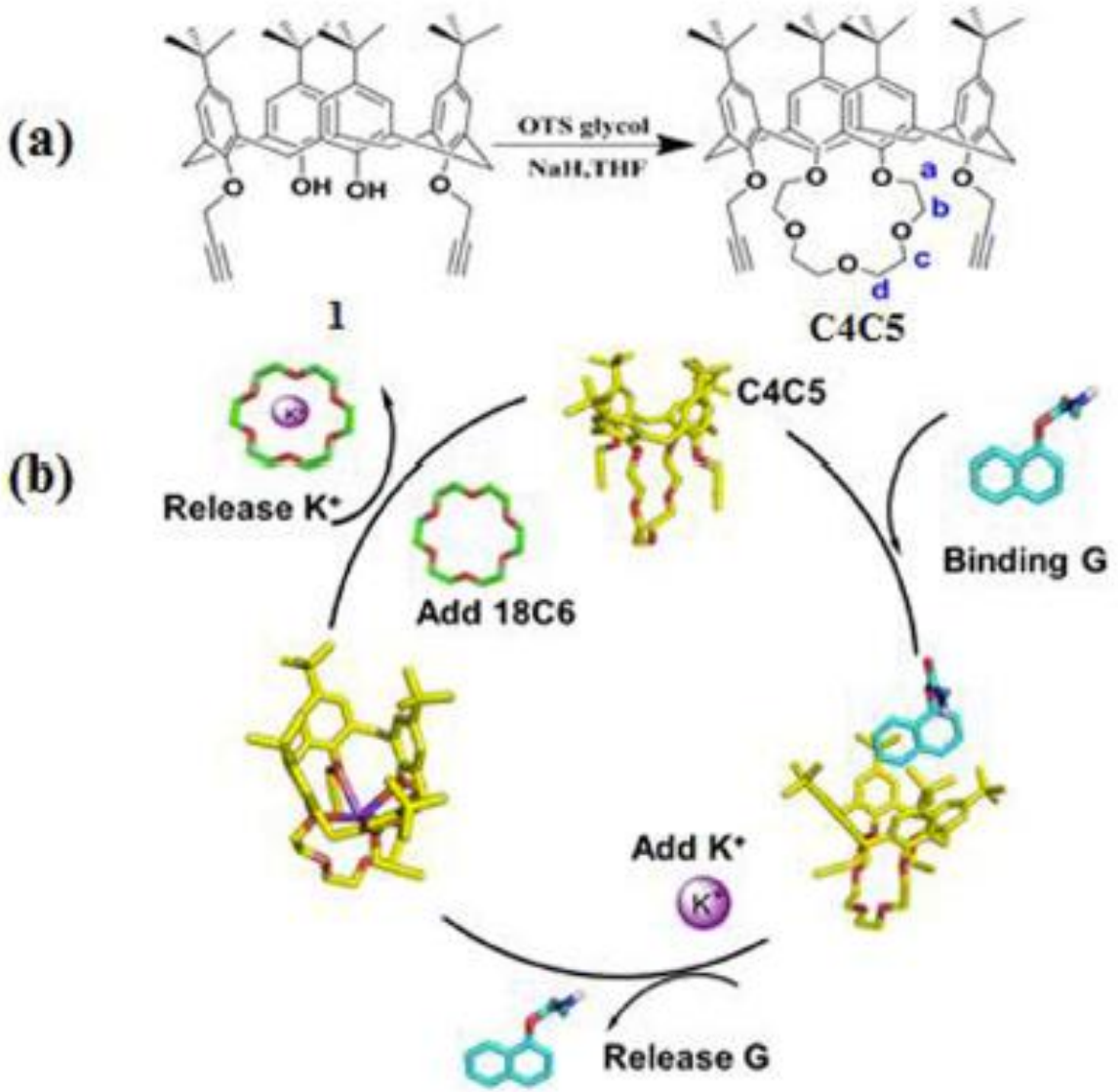
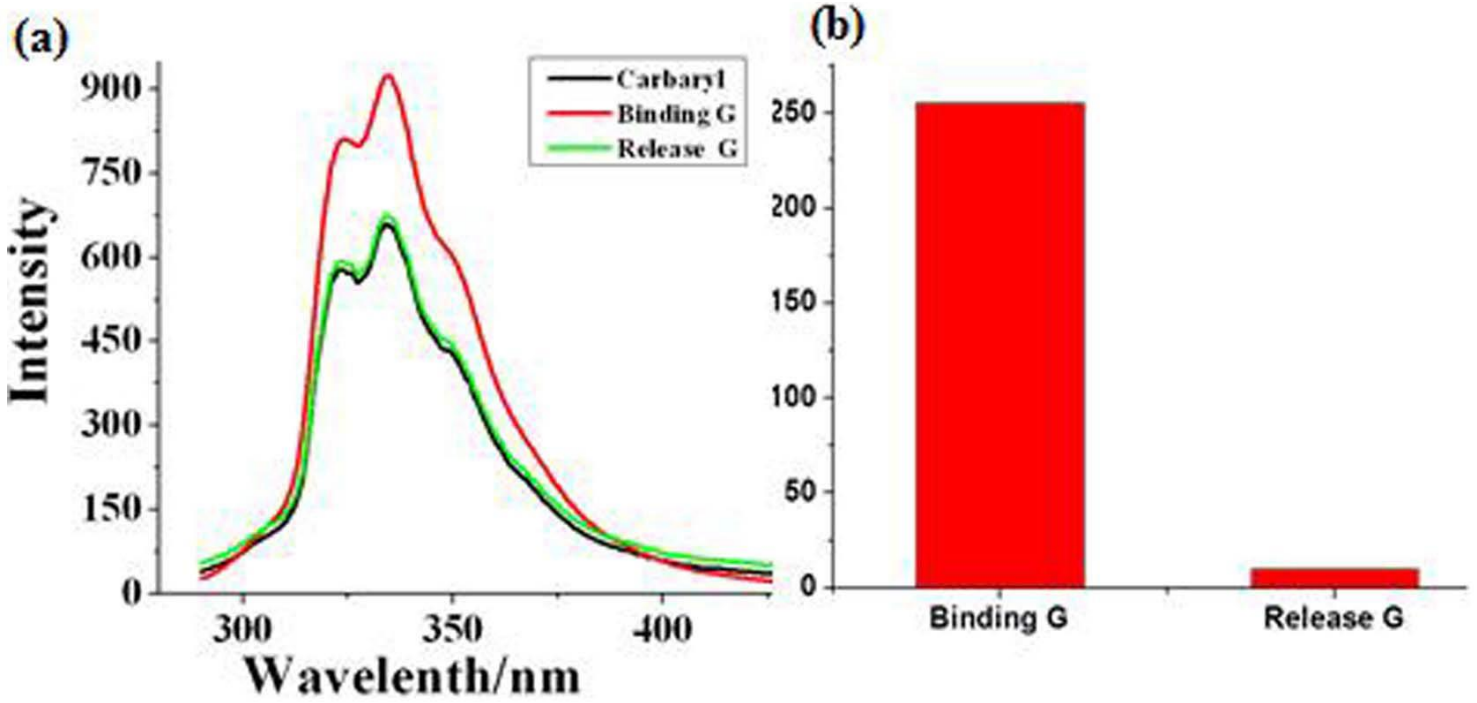


Figure 1 | (a) The synthesis of C4C5. (b) The scheme of K^+-controlled binding/release G.



The fluorescence of G (1.0 x 10^{-5} M, 2.0 mL) enhanced with the addition of the C4C5 (1.0 x 10^{-3} M, 20 μL) in CH3CN because of their interaction.

To further study of the C4C5 with G carried out by K, the same equiv. of K was added for the interaction with [C4C51G] which showed fluorescence recovered.

In order to illustrate the details of formation of [C4C51G] in the solution, the binding stoichiometry of the complex formed between C4C5 and G was 151 from Job's plot by ultraviolet-visible (UV) spectrum, which had a peak at 277.9 nm with a molar fraction of 0.5 (Supplementary Fig. S5)

To obtain insights into the mechanism, NMR experiments were carried out, 6.0 mM C4C5 and 1.0 equiv. of G and K+ in CD3CN, as showed in Fig. 3.

The protons of the napthalene in the G underwent upfield shift of 0.05 ppm in the presence of C4C5. The result also indicated that the [C4C51G] complex was successfully formed.

When K+ added, the protons of naphthalene underwent an **upfield** shift of 0.05 ppm and recovered to original chemical shift, **moreover, them protons of crown have underwent the protons of crown ether** unit of C4C5 Ha, Hb, Hc and Hd underwent downfield shift of 0.04 ppm,

0.18 ppm, 0.14 ppm and 0.17 ppm respectively.

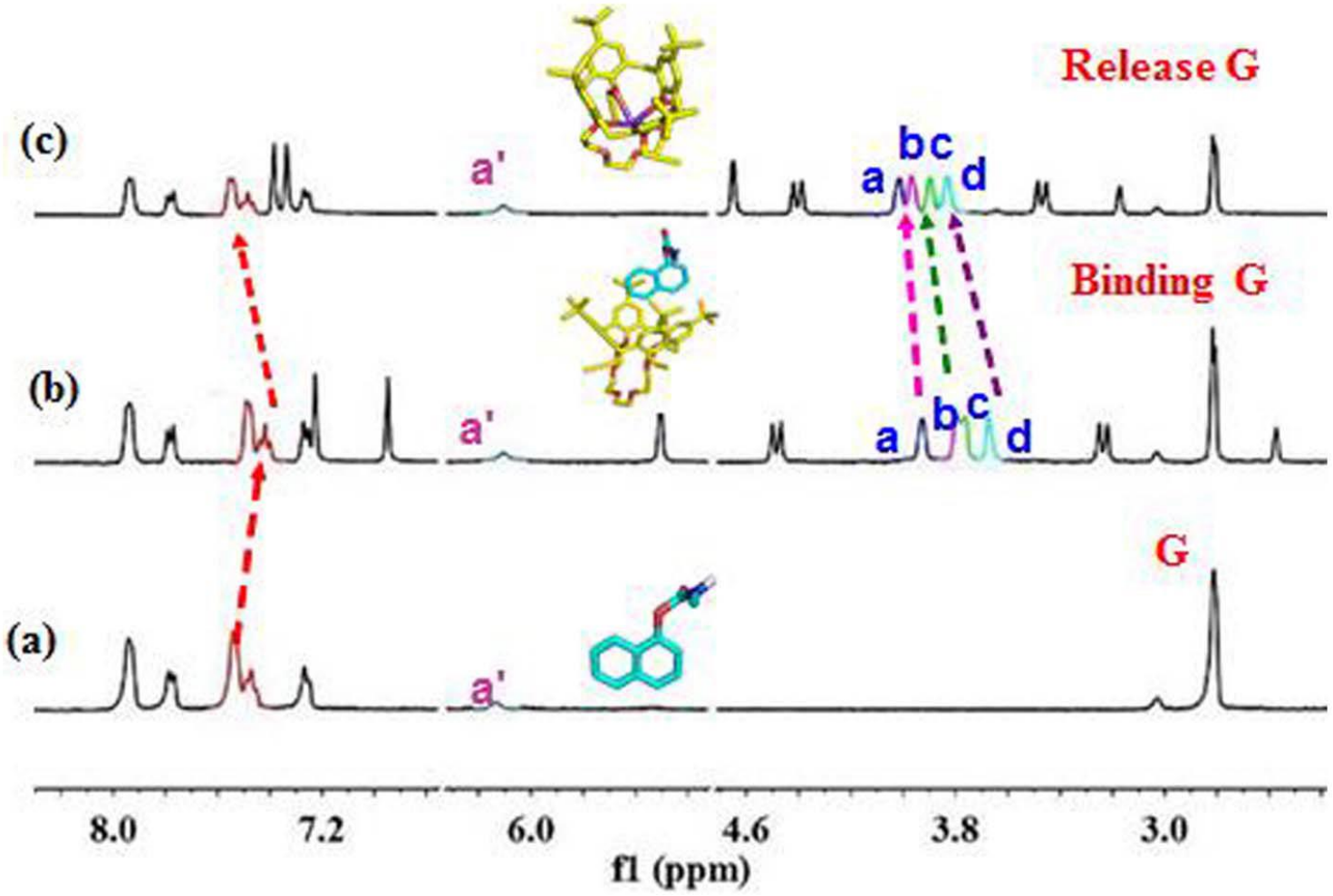
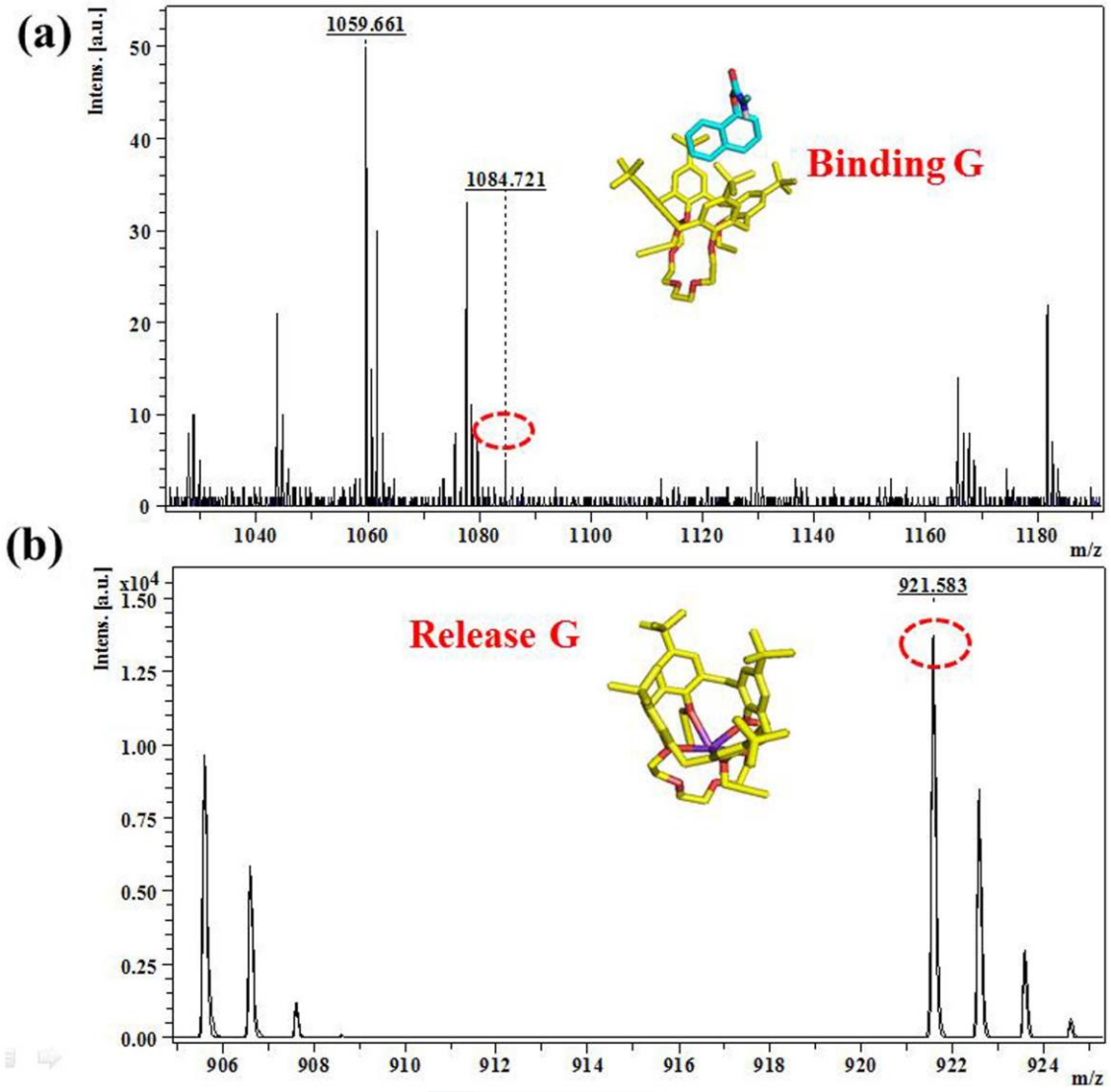
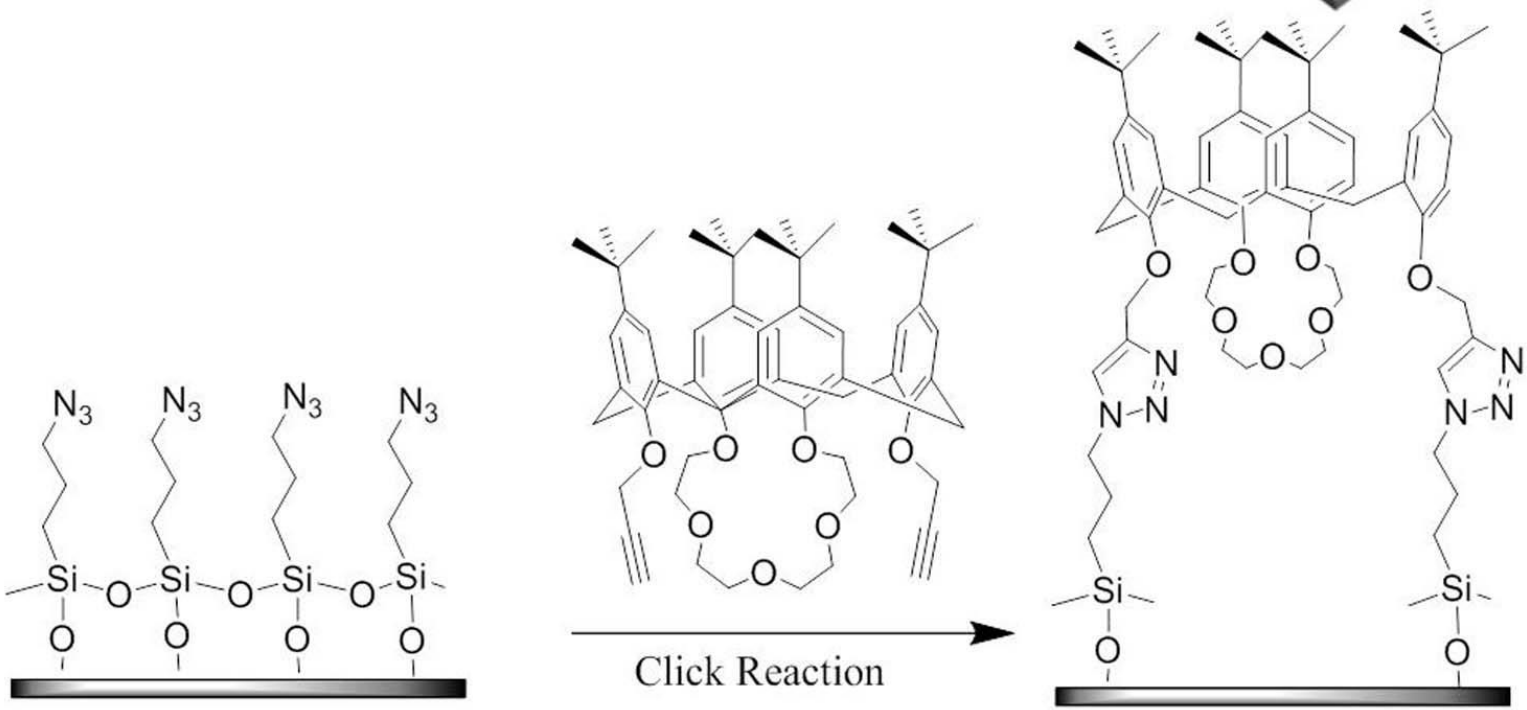


Figure 3 | (a) Partial 1HNMRspectroscopy ofG (6.0 mMeach,CD3CN, 400 MHz, 298 K). (b) Partial 1HNMRspectroscopy of C4C5 reacted with G. (c)
1H NMR spectroscopy of K1 and C4C5, which indicated G release from the C4C5 by K1 interacted with crown ring with C4C5.

Binding followed by ESI-MS spectrometry



Conjugation to surface through click-chemistry



Review

Metal binding calixarenes with potential biomimetic and biomedical applications

Daniel T. Schühle [a,1], Joop A. Peters [a,**], Jürgen Schatz [b,*]

[a] *Department of Biotechnology, Biocatalysis and Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands*
[b] *Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Henkestrasse 42, 91054 Erlangen, Germany*

- The calixarenes are interesting scaffold to mimic protein functions:
- ENZYMES
- ION CHANNELS

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Ion transport thorugh cellular membrane

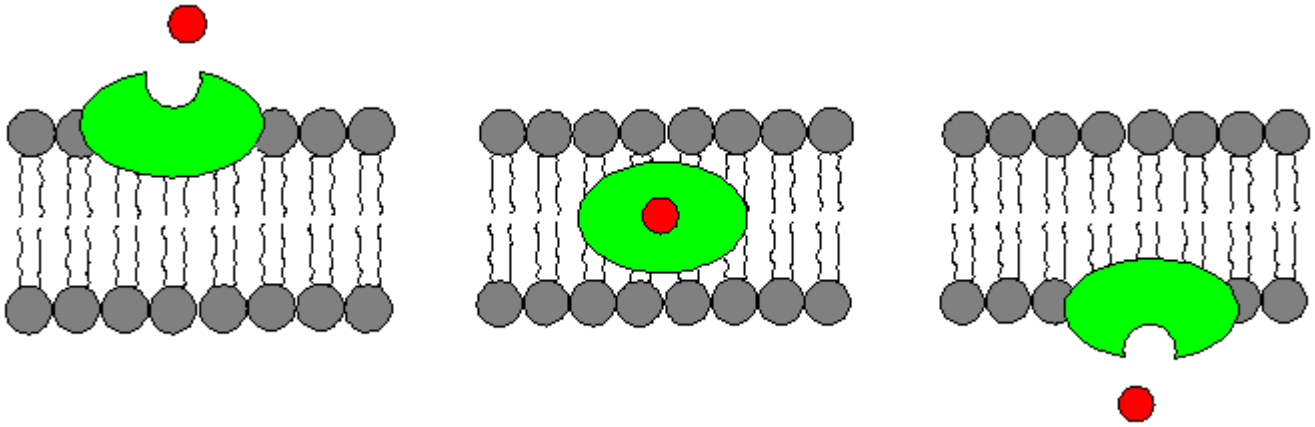
The intra- and extracellular concentrations of physiologically relevant metal ions usually differ. The transport of inorganic cations between intra- and extracellular compartments occurs mainly through two mechanisms:

(i) ionophore mediated transport by which a chelator binds the metal ion and transports it through the lipophilic cell membrane and

(ii) formation of transmembrane channels with selectivity for a certain metal ion.

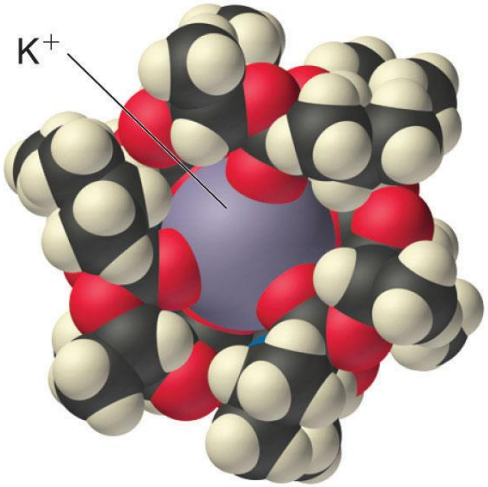
Whereas ion shuttles can transport cations with a rate of 10^4 ions per second, channels can transport ions about 10,000 times faster. As a result, there is considerably more interest in channels than in the development of new ionophores. In general, these channels provide a hydrophilic path inside the hydrophobic cell membrane.

Metal ionophores

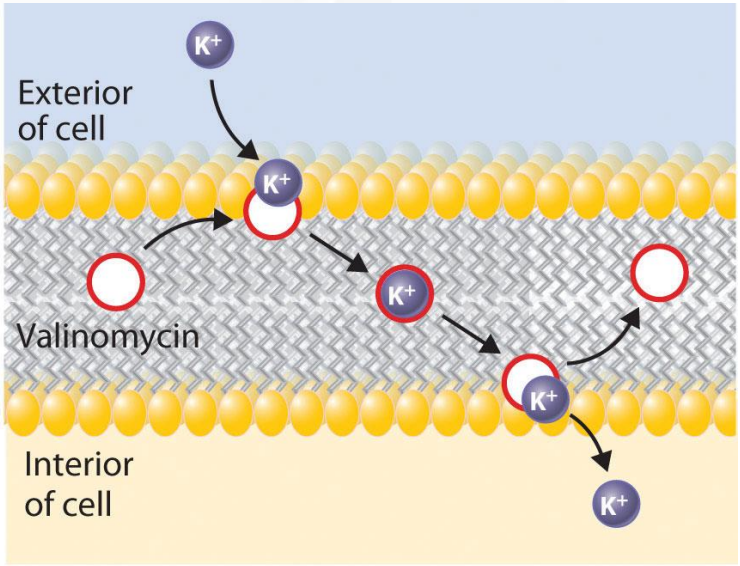


IONOPHORES ARE MOLECULES THAT FACILITATE ION PASSAGE IN OR OUT OF CELL MEMBRANES.

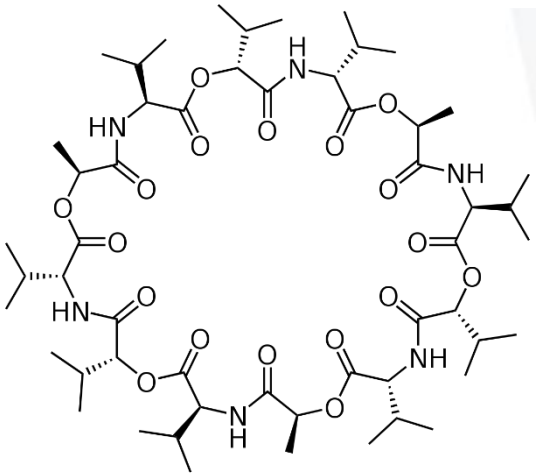
Valinomicin is an ion transporter (ionophore)



(a) K^+–valinomycin complex

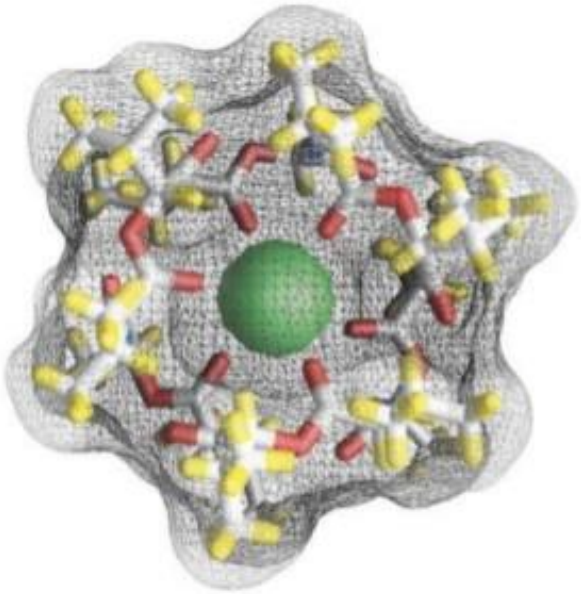


(b) Transport of K^+ across a membrane



valinomycin

Ionophores



Eg.

Oligomycin

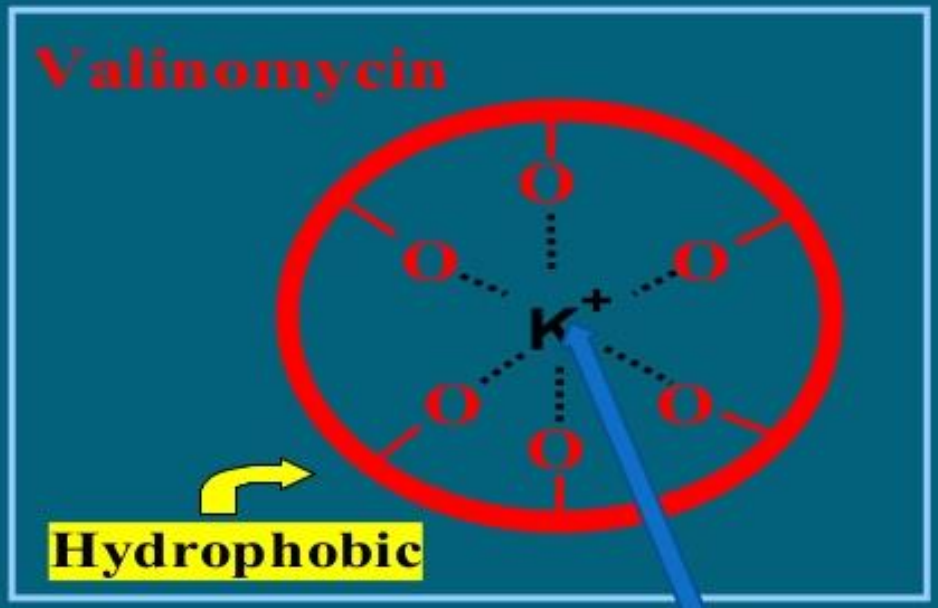
Uncoupler - transports the H+ ions , breaks the ETC.

Valinomycin

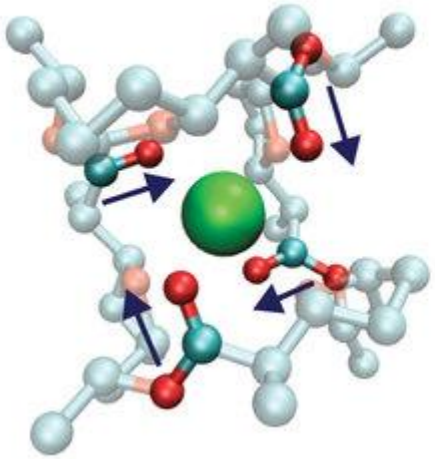
Masks the K+ ions, transports it across the membranes. Thereby breaks the electrochemical gradient across the membranes.

Lehninger's Textbook of Biochemistry, 5th ed

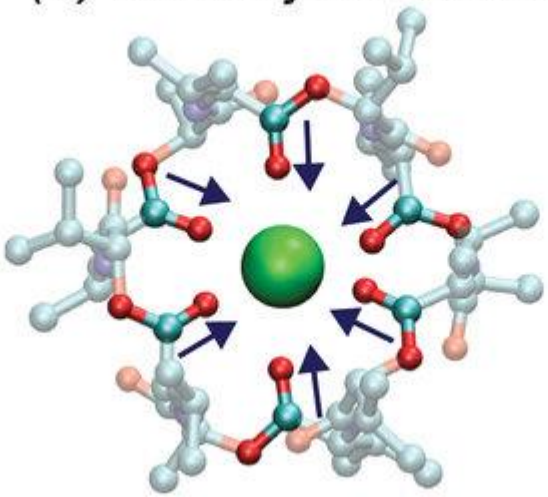
representation



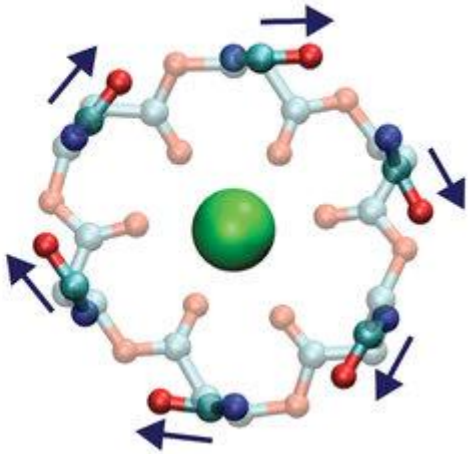
(A) Nonactin - Esters



(B) Valinomycin - Esters

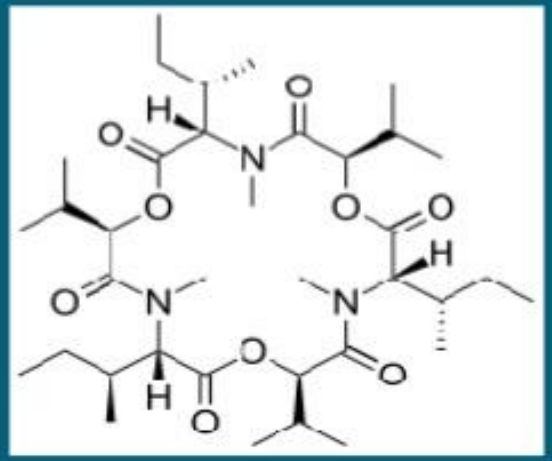


(C) Valinomycin - Amides



8. Enniatins

- These are mixture of depsipeptides that bind and transfer ammonium ion across the membrane



Ionophores have antibacterial but also anticancer activity

Ionophore	Transported Ion	Cancer Type	Target CSCs	Reference
Nigericin	K^+, H^+	Several	Yes	[4–6]
Salinomycin	K^+, Ca^{2+}	Several	Yes	[7–9]
Obatoclax	HCO_3^-, Cl^-	Several	Yes	[10–15]
Gramicidin	H^+, Na^+, K^+	Renal cell carcinoma,	Not known	[16,17]
Ionomycin	Ca^{2+}	Breast	Not known	[18,19]
Monensin	Na^+, H^+	Glioblastoma, Bladder	Not known	[20–22]
Valinomycin	K^+	Ovarian, Colorectal,	Likely	[3,23,24]
Lasalocid	K^+, Na^+, Ca^{2+},	Prostate	Not known	[25]
Enniatin	Mg^{2+}	Colon, Ovarian Prostate	Not known	[26,27]
Beauvericin	NH_4^+, Ca^{2+}, Ba^{2+}	Cervical, Colorectal Hepatoma, Lung	Not known	[28,29]

Anticancer activity of natural ionophores

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cancers

MDPI

Review

Ionophores: Potential Use as Anticancer Drugs and Chemosensitizers

Vivek Kaushik [1], Juan Sebastian Yakisich [1], Anil Kumar [2], Neelam Azad [1] and Anand K. V. Iyer [1,*]

[1] Department of Pharmaceutical Sciences, School of Pharmacy, Hampton University, Hampton, VA 23668, USA; vivek.kaushik@hamptonu.edu (V.K.); juan.yakisich@hamptonu.edu (J.S.Y.); neelam.azad@hamptonu.edu (N.A.)

[2] Great Plains Health, North Platte, NE 69101, USA; kumara@gphealth.org

* Correspondence: anand.iyer@hamptonu.edu; Tel.: +1-757-728-6768

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Only water, oxygen and carbon dioxide freely move across plasma membrane. Cells overcome these transport issues by devising mechanisms for facilitated diffusion as well as active transport of ions and molecules across membrane.

Facilitated transport involves diffusion of ions towards concentration gradient mediated by proteins which form water filled ion channels across the membrane. These ion channels are gated and can be opened and closed based on the cellular requirements.

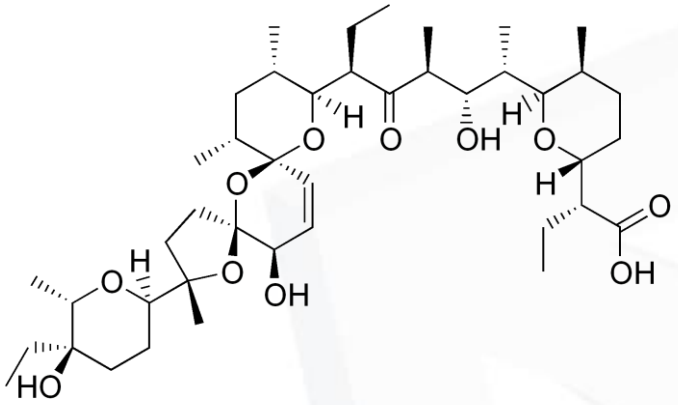
The most common types of gated ion channels are ligand-gated, mechanically gated, voltage-gated, and light-gated [1]. In active transport ions or molecules are transported against the concentration gradient with the help of transporter proteins using energy from the ATP. Na+/K+ ATPase, H+/K+ ATPase, Ca2+ ATPase, ABC transporters are a few examples of active transporters

Aberrant expression and/or functioning of ion channels and ion pumps in cancer cells establish a unique ion homeostasis which is specifically advantageous to cancer cells.

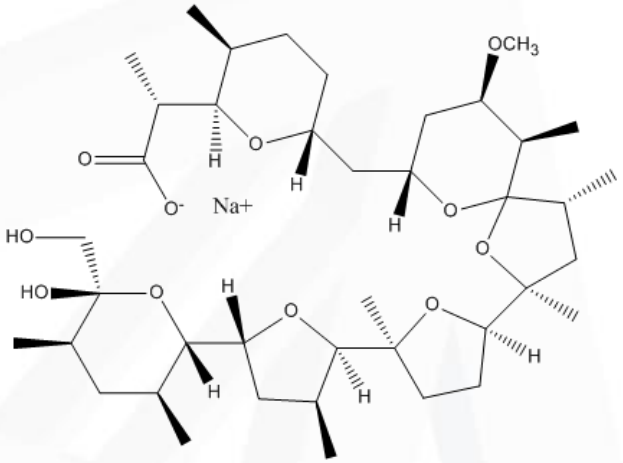
Maintenance of this ion homeostasis is of great interest to cancer cells.

this ion homeostasis is targeted to develop novel therapeutic interventions for cancer treatment and discuss role of ionophores as anticancer drugs.

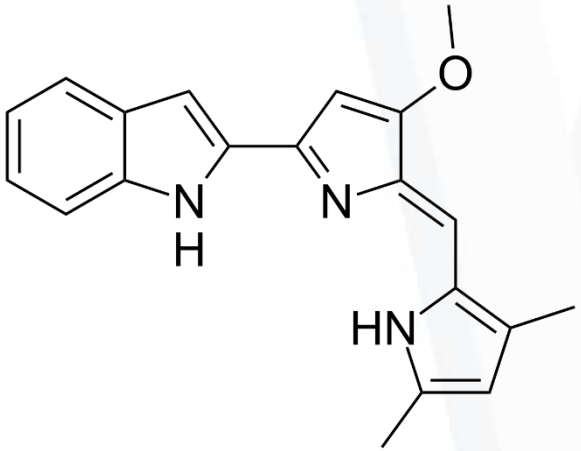
Ionophores are a class of compounds which have been successfully employed to eliminate cancer by manipulating ion balance in cancer. Salinomycin (SAL), Nigericin (NIG) and Obatoclax (OBT) as these ionophores have shown well-documented potent anticancer activity against cancer stem-like cells as well as promising use as chemosensitizer.



salinomycin

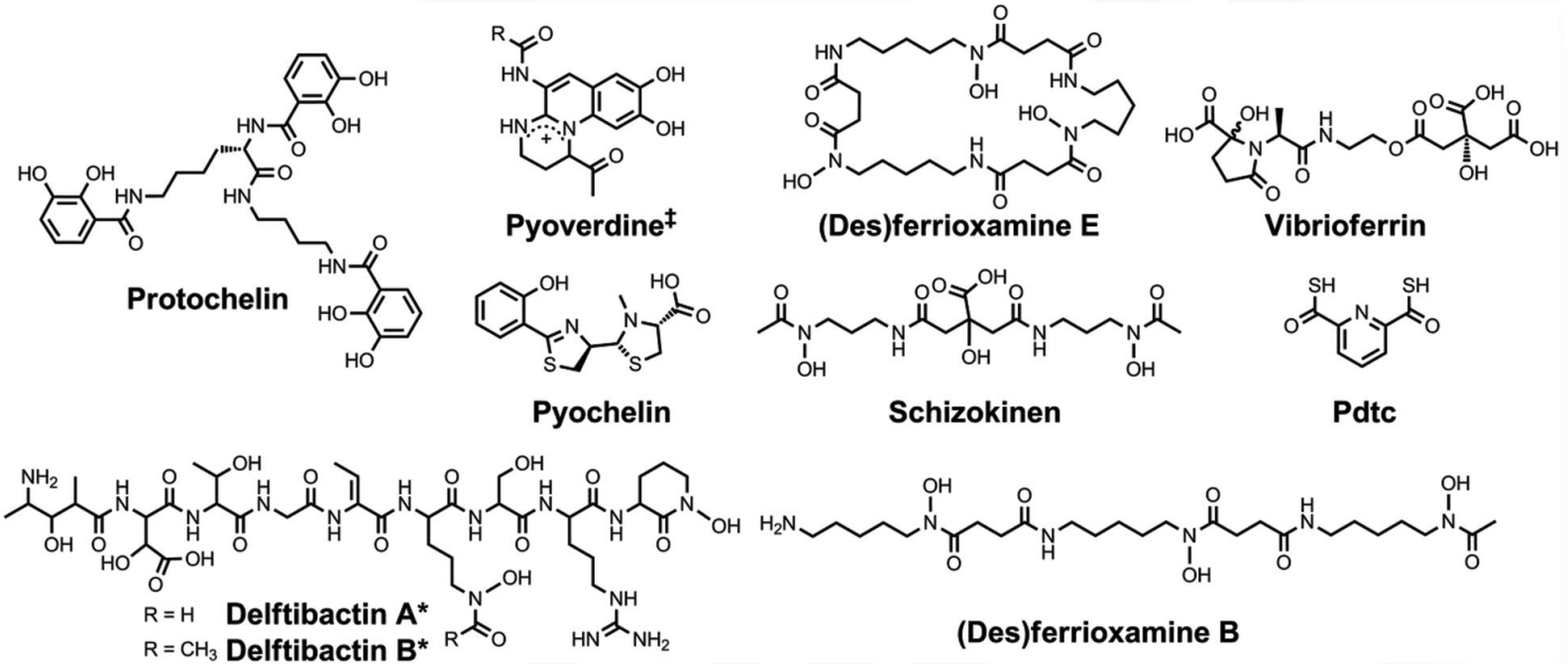


Nigerimycin sodium salt



Obatoclax (synthetic)

Bacterial siderophores



Bacteria need iron from environment as a nutrient and produce ion transporters called siderophores

Ion carriers and ion channels

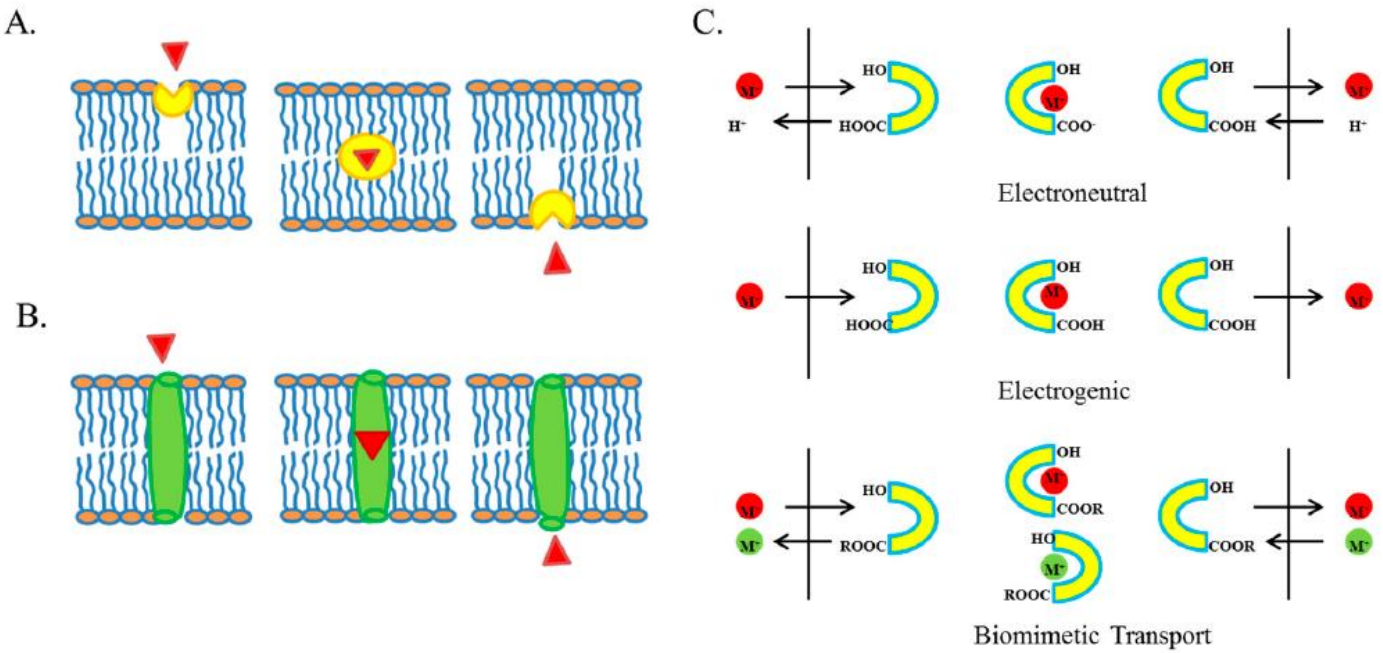
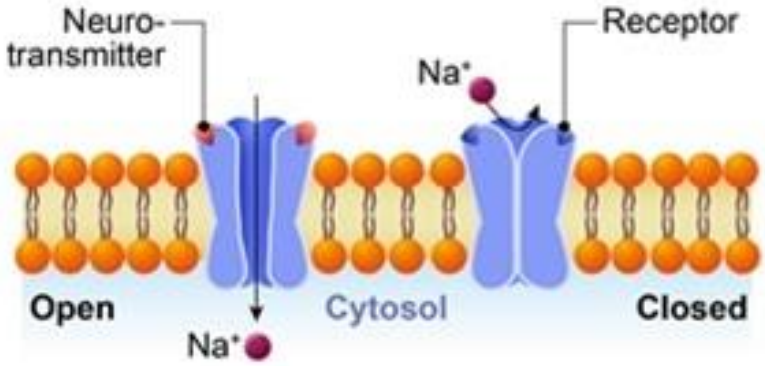


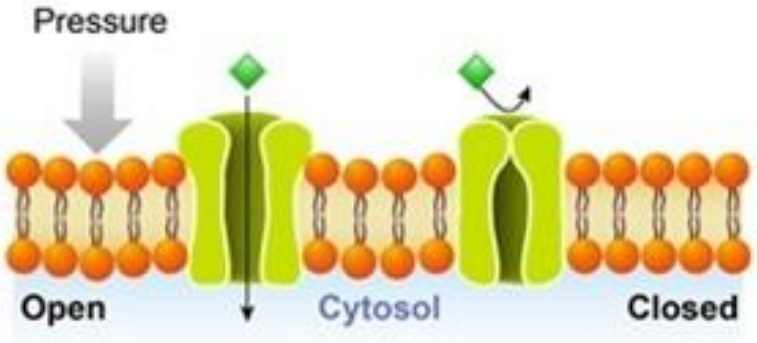
Figure 1. Ionophore mediated ion transport across the membrane. (**A**) Small ionophores "ion carriers" bind with ion, shield it from lipophilic interior of membrane, transport it across the membrane and release it other side of membrane. (**B**) Large ionophores form "ion channels" across the membrane and transport ions through these channels. These channels have a hydrophilic interior which assist in transport of ions while its lipophilic exterior shield ions from repulsive interior of membrane. (**C**) Polyether ionophores carry ions across membrane by electroneutral, electrogenic and biomimetic methods based on the microenvironmental conditions and structure of ionophore. Panel C was modified from [71].

ION CHANNEL

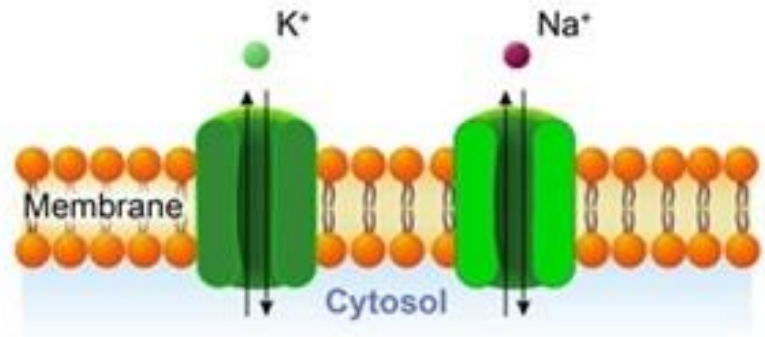
Ligand-gated



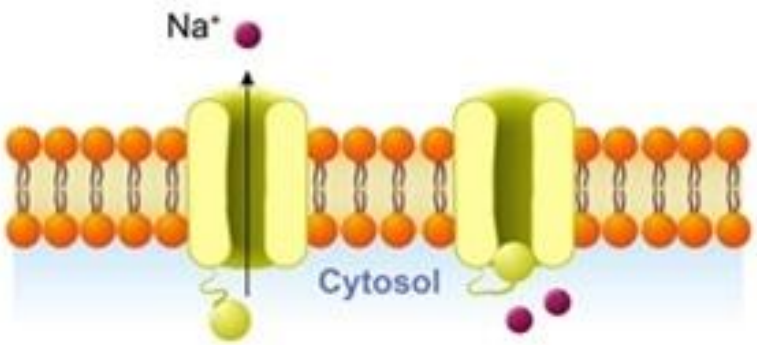
Mechanically-gated



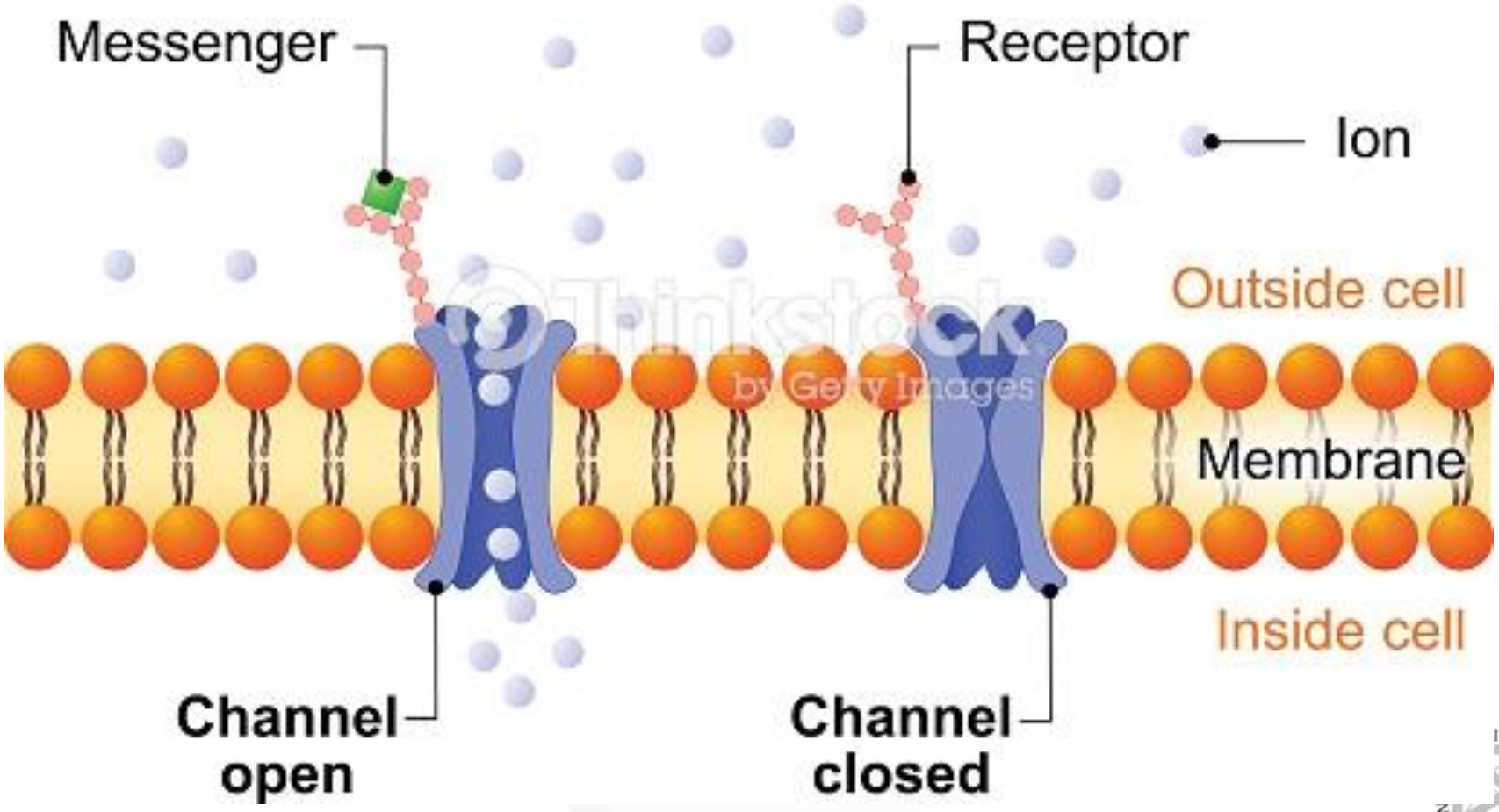
Always open



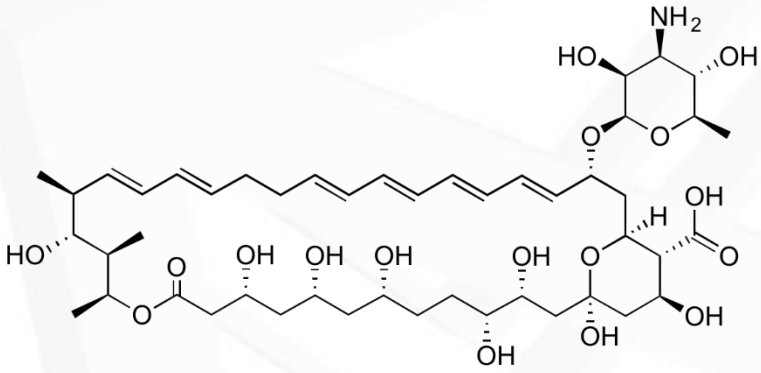
Voltage-gated

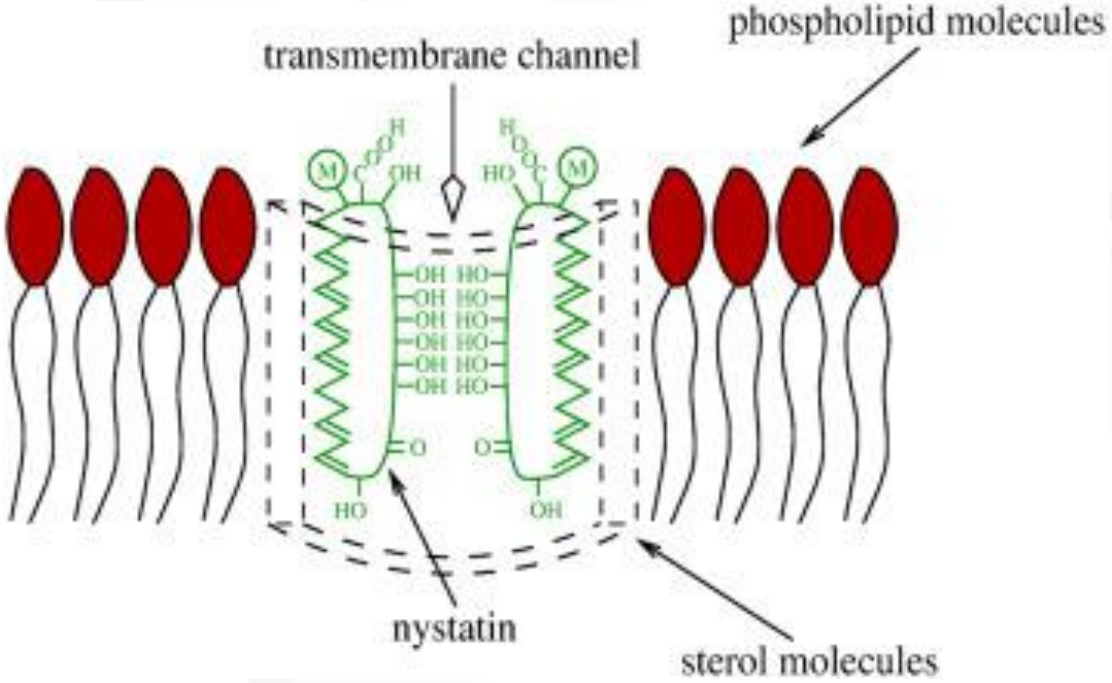


Ligand-gated ion channel



The channels (pores) forming ionophore nystatin





Pore-forming toxins (PFT)

- PFTs function to perforate membranes of host cells, predominantly the plasma membrane but also intracellular organelle membranes .
- They are classically hypothesized to do so in order to directly kill target cells, for intracellular delivery of other bacterial or external factors, to release nutrients, or for phagosomal escape in the case of intracellularly acting PFTs.
- Loss of their PFTs generally causes pathogenic bacteria to be less virulent or completely avirulent.

Nystatin properties

1 Nystatin forms pores in many types of lipid bilayer membranes, including all cell membranes tested.

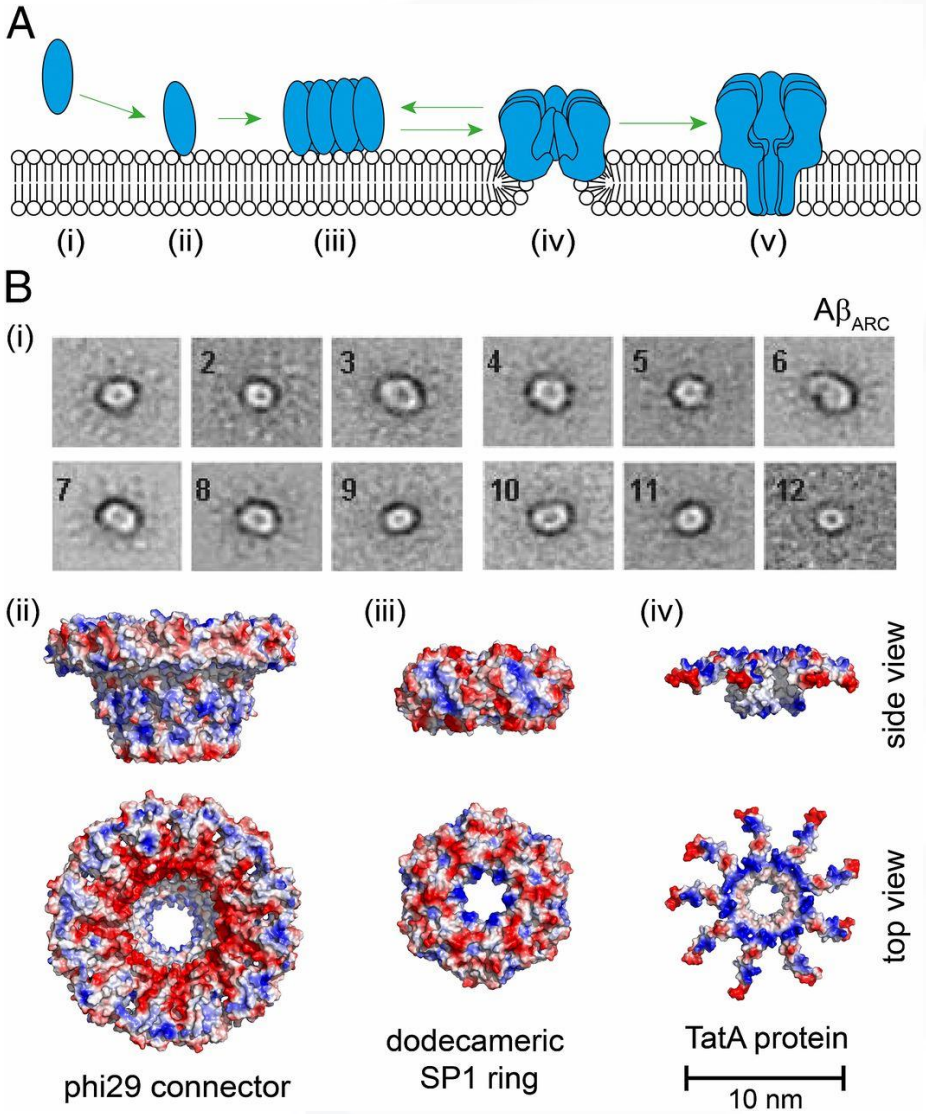
2. Nystatin pores are selective for monovalent ions. Sodium, potassium, lithium, cesium, and chloride are all permeant, although cations are more permeant than anions. Calcium, magnesium, and all other multivalent ions tested are impermeant.

3. Nystatin pores show little voltage dependence.

4. Nystatin applied to the outside surface of the plasma membrane does not enter the cytoplasm.

5. Molecules with a diameter greater than ~0.8 nm and a molecular weight greater than ~200 (most intracellular biochemicals and metabolites) do not permeate nystatin pores.

PFTs: perfect supramolecular architectures



Na and K ion transport across membranes

Especially systems that are able to transport the biologically most relevant alkali metal cations, Na+ and K+, selectively through membranes are of great interest.

The ratio of the concentrations of Na+ and K+ in- and outside cells are important for e.g. the proper functioning of signal transduction in nerves.

An anti-tubercolotic calixarene

Reported in 1955, macrocyclon, a calixarene derivative with tert-butyl or tert-octyl groups on the upper rim and polyethyleneglycol functions at the lower rim, has anti-tuberculous effects [16].

Nowadays, there is speculation that the anti-tuberculous and anti-inflammatory properties of these compounds might be due to their possible function as ion channels [17].

In general, suitable amphiphilic calixarenes can be incorporated into biological membranes and then serve as artificial ion channels [18–20].

This type of calixarene may also be used for selective binding of metal ions.

There are strong indications that, as a result of the size and the hydrophobicity of the calix[4]arene backbone, the potassium aquo-ion is partly dehydrated prior to its transport through the cavitytowards metal binding functions on the lower rim [15].

Natural ion channels have this dehydration capability as well and, consequently, this property is of importance for a successful mimic.

- In principle, calixarenes can form ion channels through membranes in two different ways. Cone conformers are likely to form channels with the cavity located at either the outer or the inner surface of the membranes whereas 1,3-alternate conformers probably form channels with the calixarene cavity located inside the membrane.

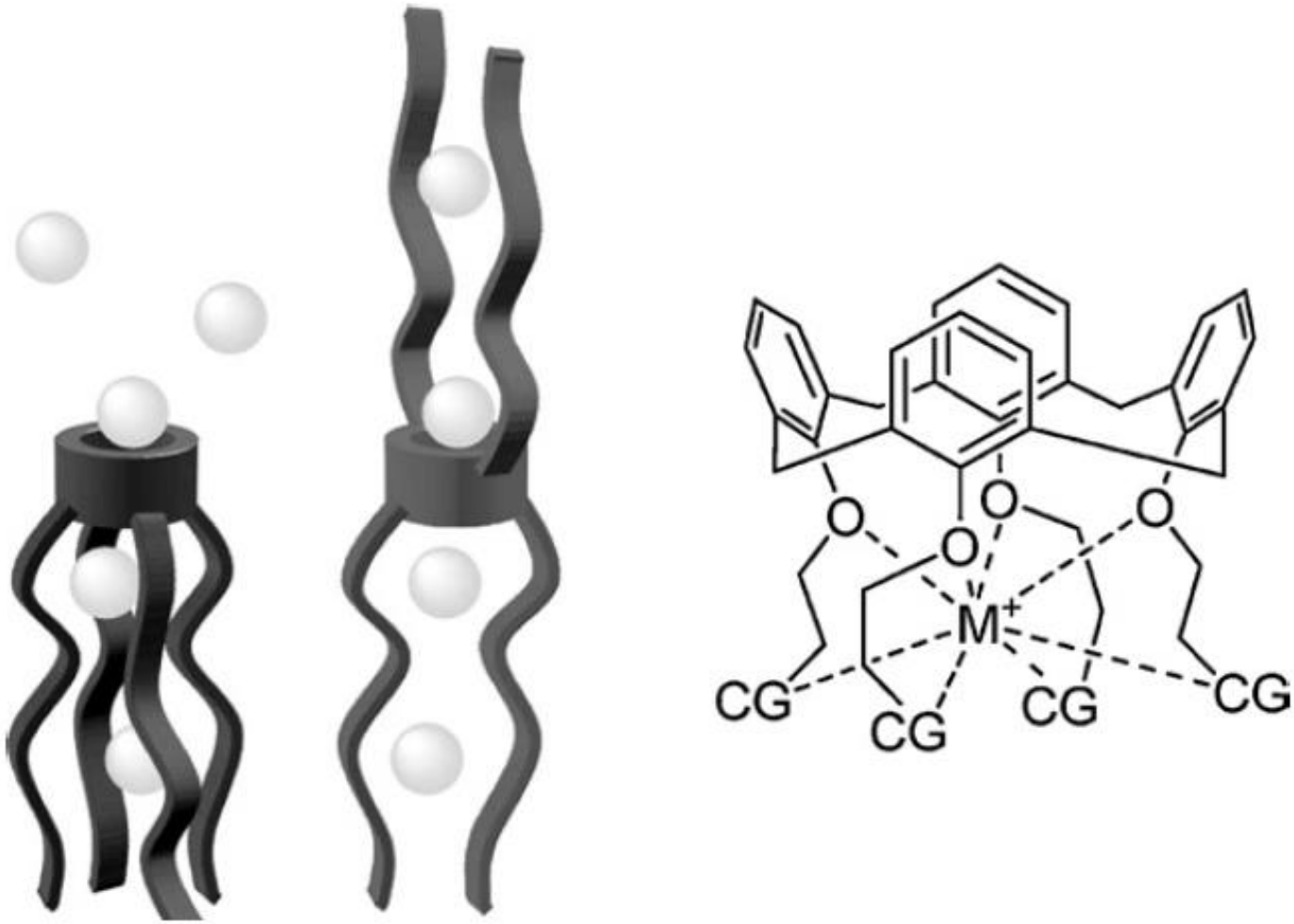
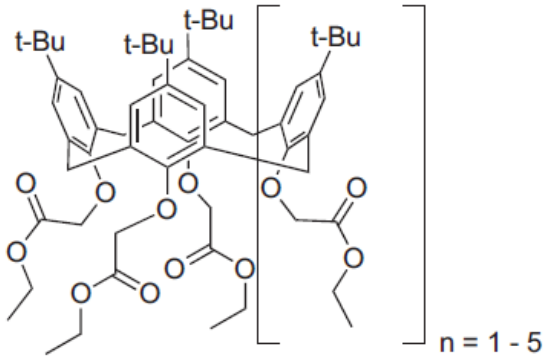


Fig. 3. Two possibilities of how calixarenes can span phospholipid bilayers (left, image reprinted with permission from Ref. [15]) and most common binding motif of calixarene based ion channels (right, CG = coordinating group).

The most promising ion channel mimics should have the potential to be included into lipophilic membranes and at the same time should provide binding sites for metal ions to allow the transport of charged species through the apolar cavity.

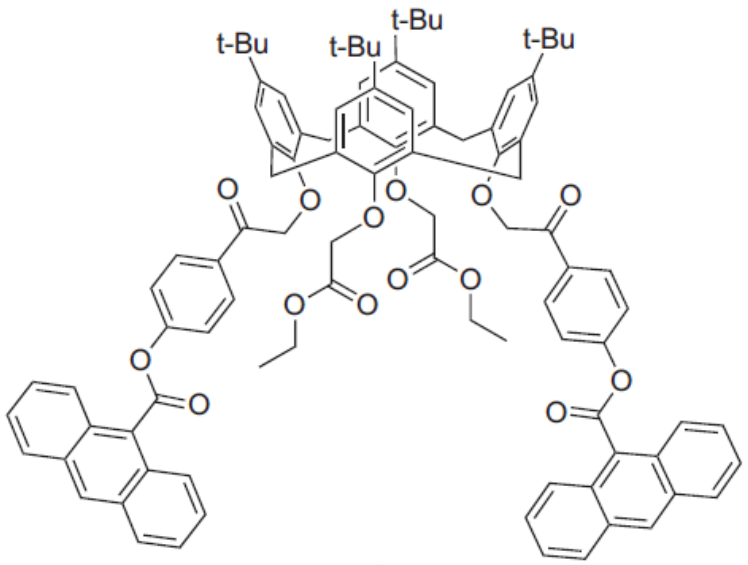
Research on calixarene-based artificial enzyme mimics is limited to Na^+ and K^+ transport so far, but systems that are able to transport other physiologically important cations such as Ca^{2+} or Mg^{2+} can be envisioned as well.

Na^+ channels



1

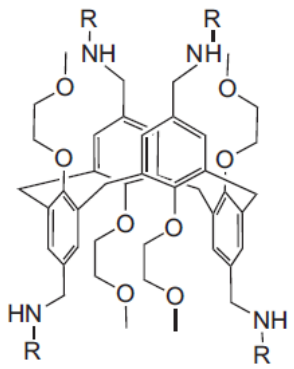
Jin et al. demonstrated that the tetramer (n = 1) leads to a permeability for Na+ ions that is 20 times higher than that for all other metal ions tested [23].
Probably, this calixarene forms a dimer within the bilayer that is able to span the membrane thus allowing efficient Na+ transport or it serves as an ion shuttle. The higher calixarenes of this type have some selectivity for larger alkali ions; compound 1 with n = 3, 4, and 5 show preference for K+, Rb+, and Cs+, respectively



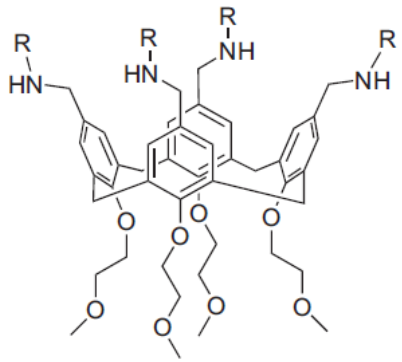
2

In studies of the parameters determining the metal ion flux through artificial cell membranes, it may be useful to have a possibility to block this transport.

For example, 2 [15] formed a photo-switchable artificial Na+ channel which has a Na+-selectivity similar to that of calix[4]arenes 1 (n = 1). Upon irradiation with Uvlight (>310 nm) the anthroyl units dimerize and then block the way
for diffusing metal ions.
Upon switching off the UV-light, the dimer slowly falls apart and ion transport resumes.



3a R'=R"=Ac
3b R'=H; R"=Ac
3c R'=R"=H



3d R'=R"=Ac
3e R'=H; R"=Ac

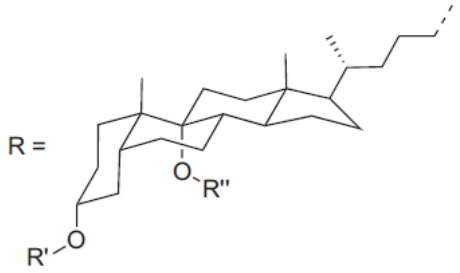
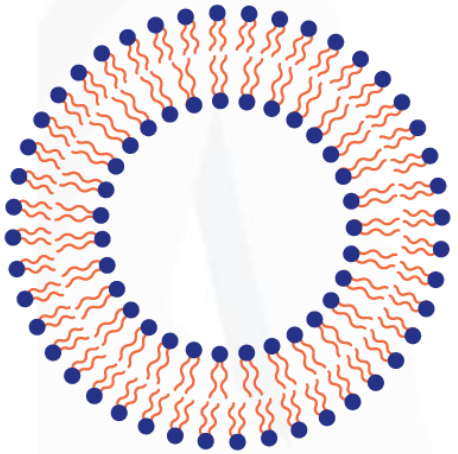


Fig. 5. Cholic acid containing ionophores **3a–e** [24].



liposome

The influence of different conformations of the calixarene backbone on the transport of protons and Na+ ions through liposomes was investigated by inclusion of ionophores 3a–e bearing cholic acid moieties (Fig. 5) into liposomal membranes [24].

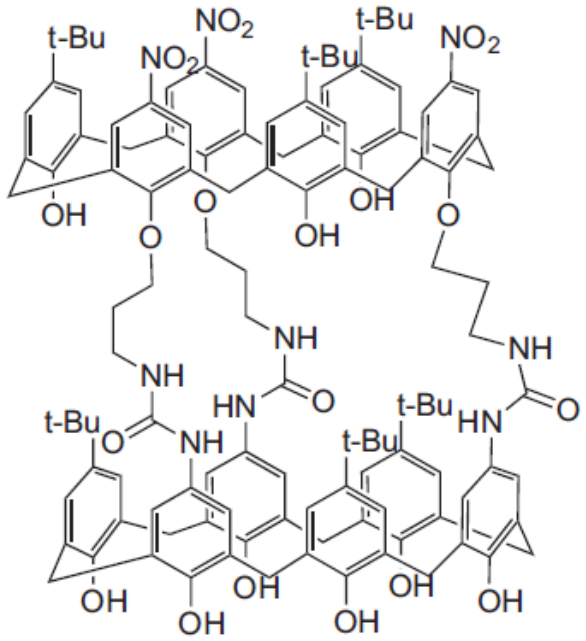
The compounds with 1,3-alternate conformation (3a–c) are much more effective in ion transport than the corresponding compounds in cone conformation.

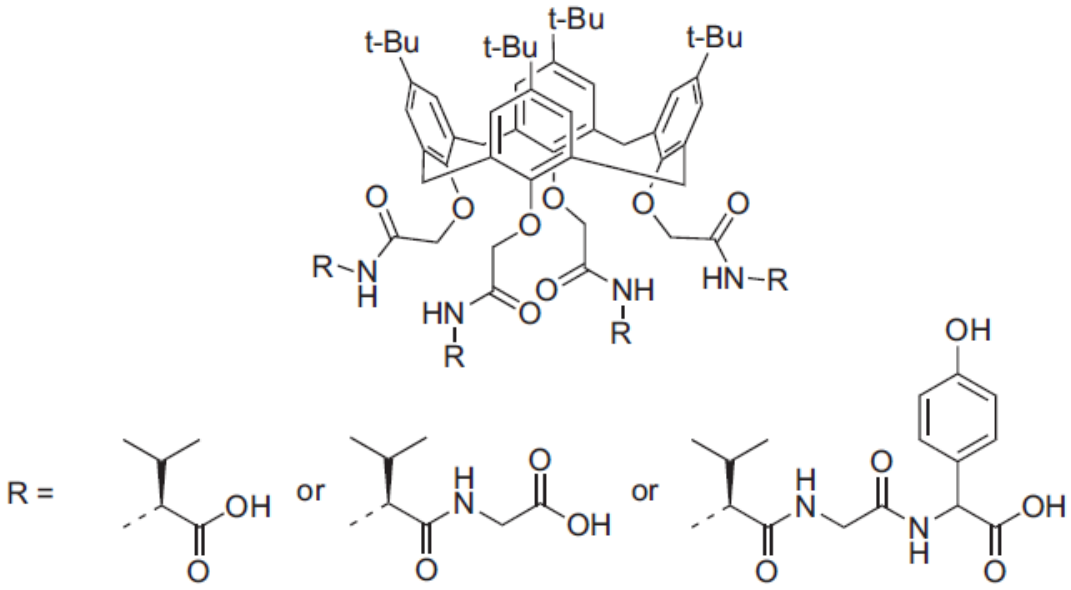
This indicates that different transport mechanisms might be acting in compounds 3.

The authors rationalize that 3d and 3e form dimers in the membrane whereas the 1,3-alternate ionophores are able to span the bilayer providing a much better channel.

K^+ transporters

A promising mimic of K+ transport was reported by Arduini et al., who showed that the tubular 8 forms channels with about the required length to span such a bilayer.





9

A four-fold symmetric arrangement of Thr-Val-Gly-Tyr-Gly polypeptides forms the selectivity filter of the K+ channel in Streptomyces lividans.
Compounds 9 are mimics of this system and were used to learn more about the mechanism of cation transport and about the origin of the selectivity of this system.

Initially, the K+ ion is bounded by the phenolic and the valine carbonyl oxygen atoms of all compounds studied. Hydrogen bonding was crucial in this system: for the tripeptide, the Nval-H···O Cval bonds are much stronger than for the shorter peptides. This effect stabilizes
this species and as a result the metal binding is relatively weak.
This might facilitate the release of the ion and therefore, the ion translocation.

CHEMICAL REVIEWS

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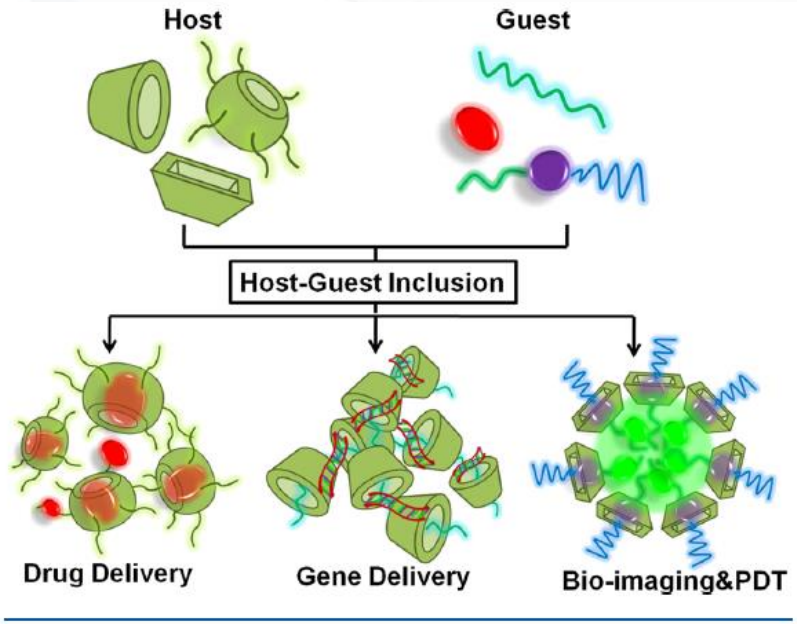
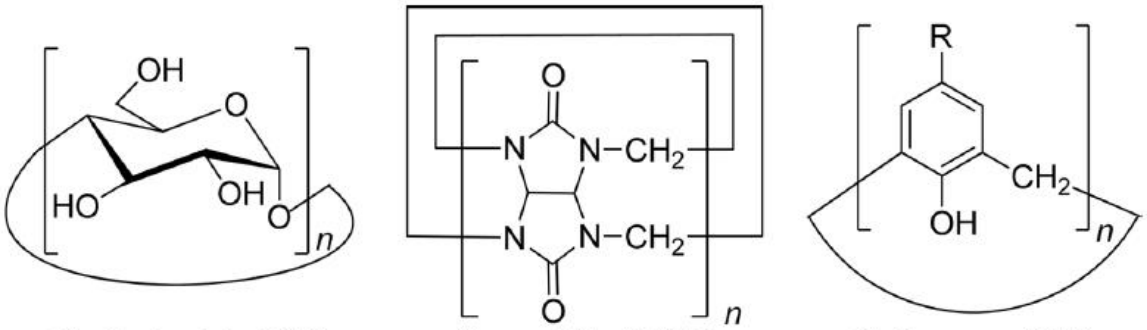
Biomedical Applications of Supramolecular Systems Based on Host–Guest Interactions

Xing Ma[†,‡] and Yanli Zhao*[,†,‡]

[†]

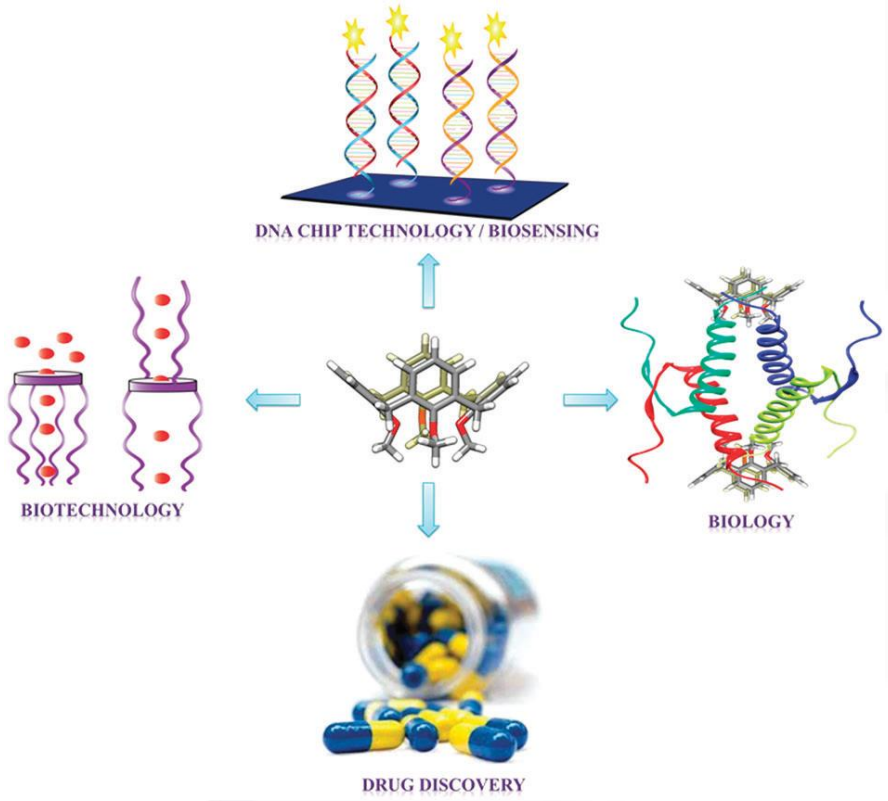




Cyclodextrin (CD) **Cucurbituril (CB)** **Calixarene (CA)**

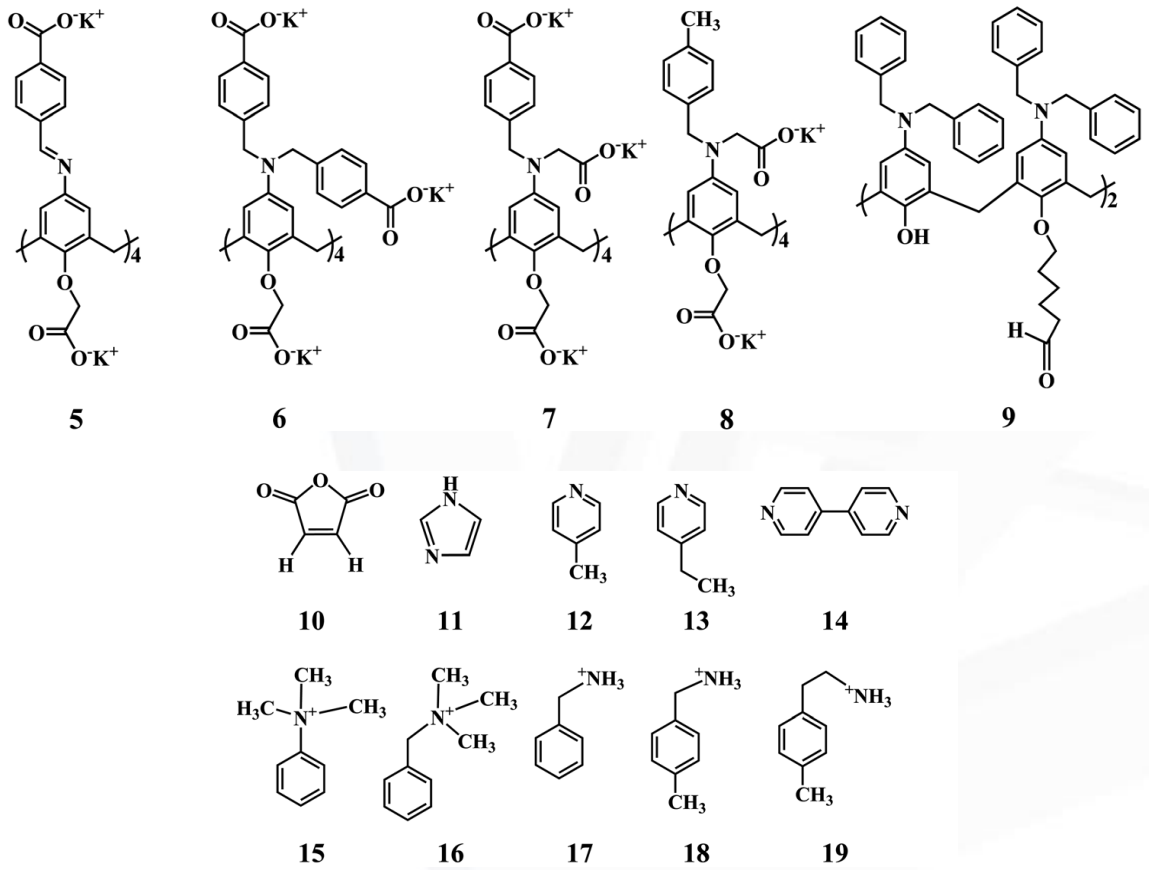
Biological applications of calixarenes

- Water soluble calixarenes
- Amphiphilic calixarenes cross cell membranes
- Calixarenes are non toxic in animals then are suitable for pharmaceutical development



Water-soluble calixarenes

In 2009, and following years, Nimse et al. reported on the molecular recognition properties of the calix[4]arene derivatives 5–9 towards the structurally flat guest molecules 10–19 with binding constant (log K) in the range of 4.4 to 4.9 in the aqueous medium



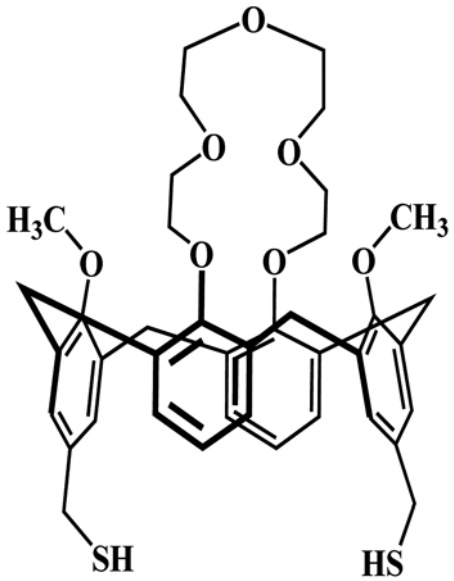
Applications of calixarenes in DNA chip technology/ biosensing technology

It is well known that the lone-pair electrons of the crown ethers in the calix[4]crown-5 derivatives can easily capture the cationic substrates, such as the metal cations and ammonium ions.

The amino acids, as basic structural building blocks of proteins and other biomolecules, sport ammonium ions and hence are attractive targets for calix[4]crown-5 derivatives.

Due to the presence of multiple amino functions in proteins, the possibilities of the complexation of proteins by calixarenes have led to a new class of applications: the protein microarray

One important factor in fabricating protein microarrays is to immobilize proteins, without losing their activity, on a solid. in 2003, Y. Lee and T. Kim developed a highly sensitive microarray protein chip coated with the calixcrown derivatives with a bifunctional coupling property that permits efficient immobilization of capture proteins on solid matrices and makes high-throughput analysis of protein– protein interactions possible.



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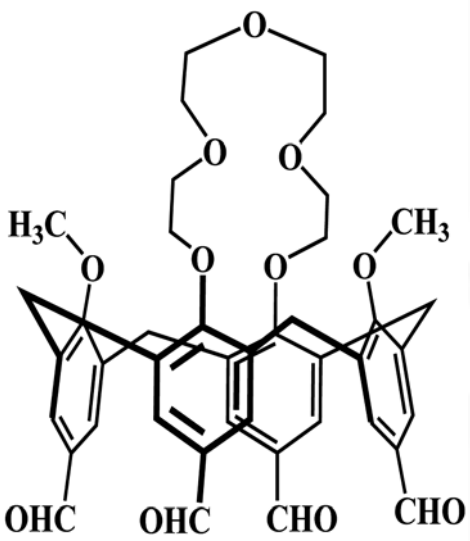

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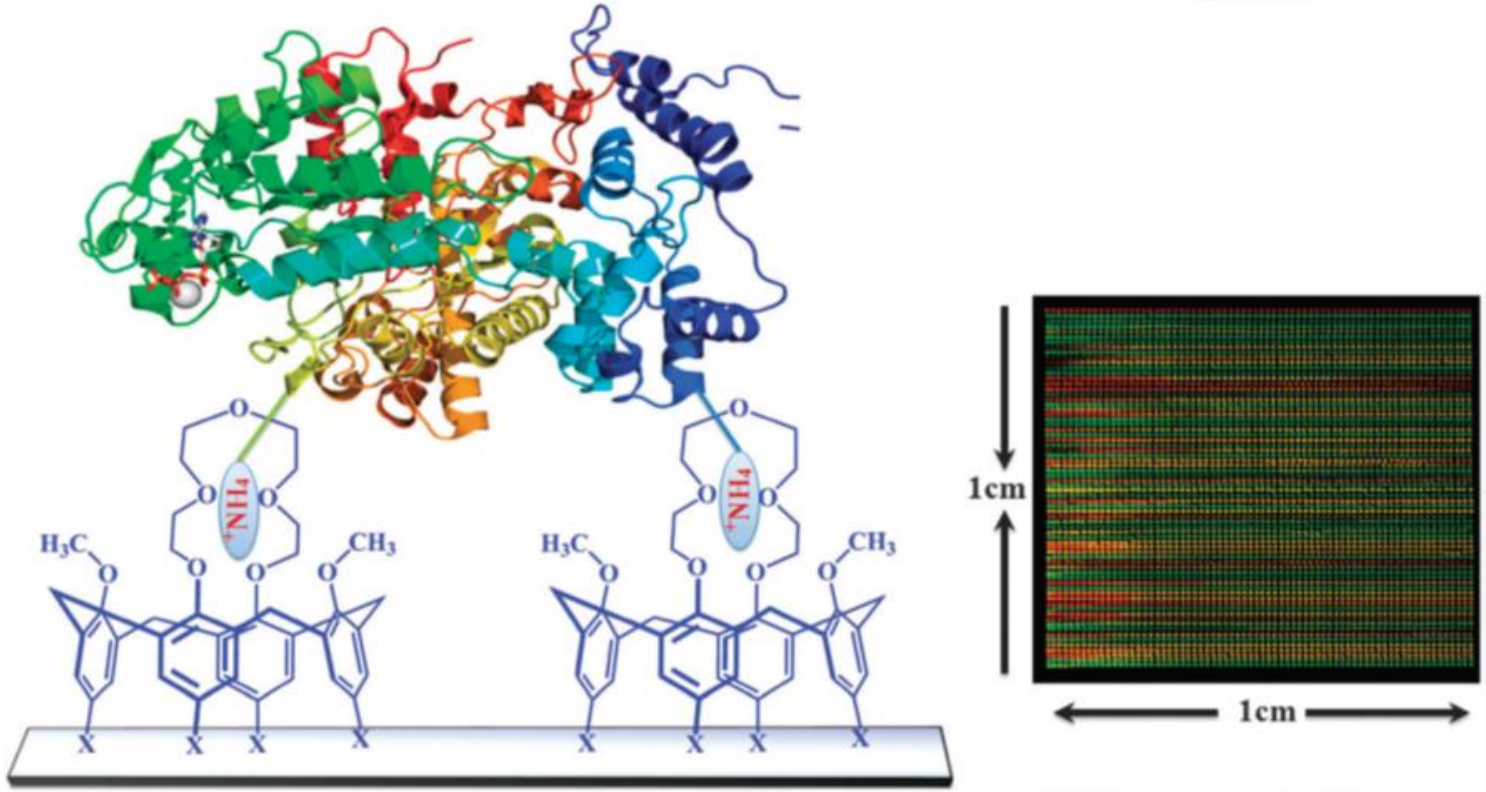
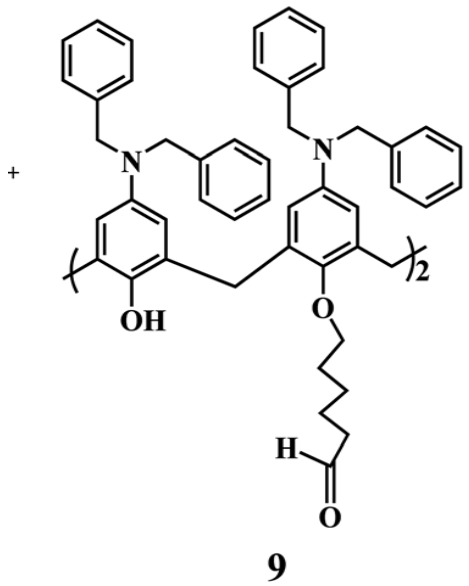


In fabricating the protein microarray, T. Kim et al. created a self-assembled monolayer (SAM) of the calix[4]crown-5 derivatives 3 or 4 on the amine modified the glass slides. In second step, the proteins were immobilized on the surface of a SAM modified glass slide, as shown in Scheme 2.

DNA and RNA microarrays

Based on the knowledge of the mixed-self assembled monolayer and the molecular recognition properties of the imincalix[4]arene 5, and aminocalix[4]arnes 6–9, recently, T. Kim et al. introduced the phenomenon of molecular recognition to immobilize oligonucleotides on aminocalix[4]arene (AMCA) slides for the production of 9G DNAChips.



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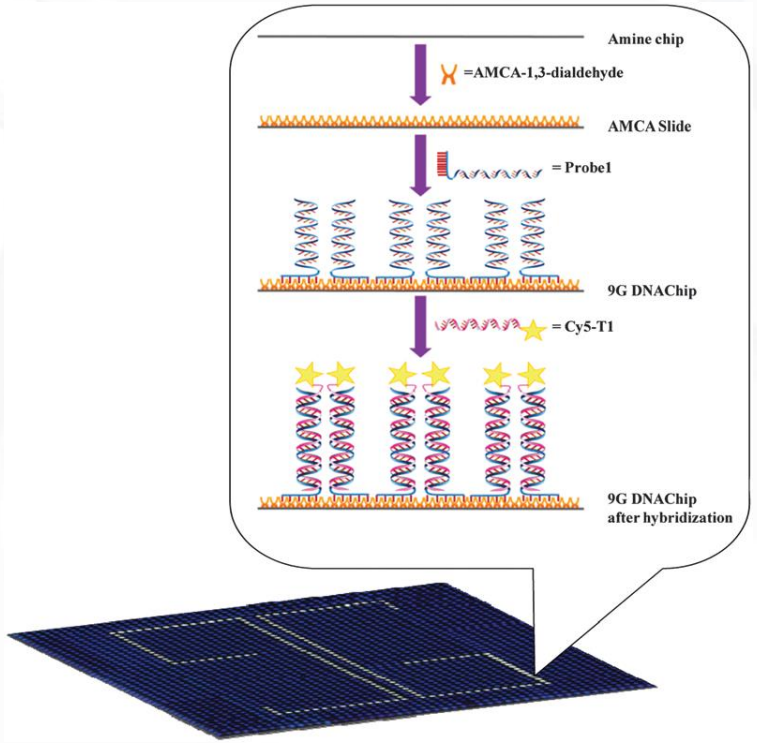


DNA chips

The preparation of the 9G DNAChip and the hybridization thereafter is briefly explained in Scheme 4.

The molecular recognition of the 9 consecutive guanine subunits in the oligonucleotide by the deep hydrophobic cavity of the aminocalix[4]arene was investigated by a competition experiment.

The 9G (guanines) DNAChips shows more than 90% hybridization efficiency at 25°C in 30 min.

Moreover, the 9 consecutive guanines can be easily added to the oligonucleotide probes during their synthesis.

The excellent properties shown by the 9G DNAChip enables it to be a powerful and promising tool for the DNA chip technology.

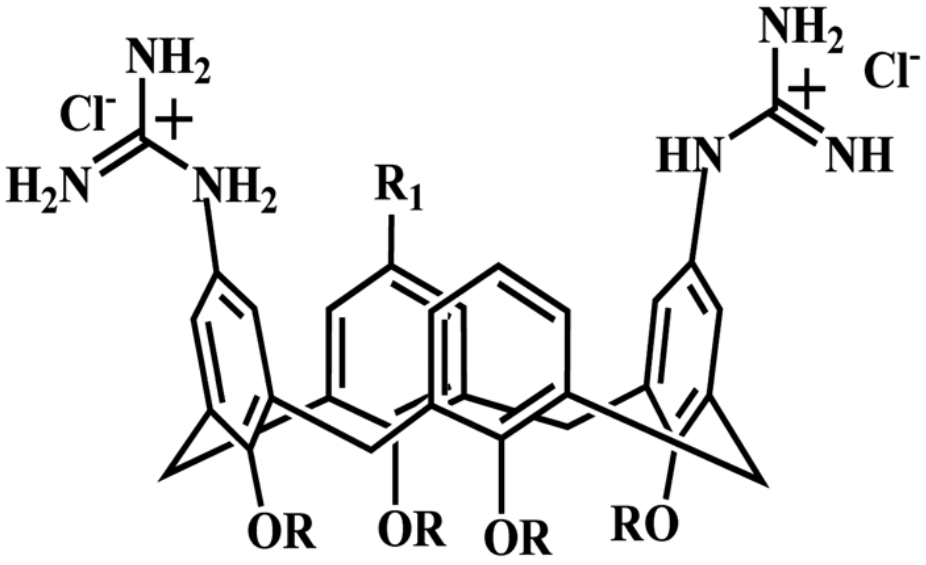
Biotechnology applications: extraction of proteins in organic media

Highly specific recognition of the biological molecules by synthetic compounds is a fast-growing and challenging field both in academic and applied research.

The use of calixarenes has attracted an increasing interest in the last two decades as a powerful tool for biochemical recognition and separation of the bioactive molecules such as amino acids, peptides, proteins, lectins, nucleotides, nucleosides, saccharides and steroids.

In 2005, Oshima et al. reported that a calix[6]arene carboxylic acid derivative exhibited a high affinity for cationic proteins such as cytochrome c (Cyt c) by promoting its extraction in organic media.

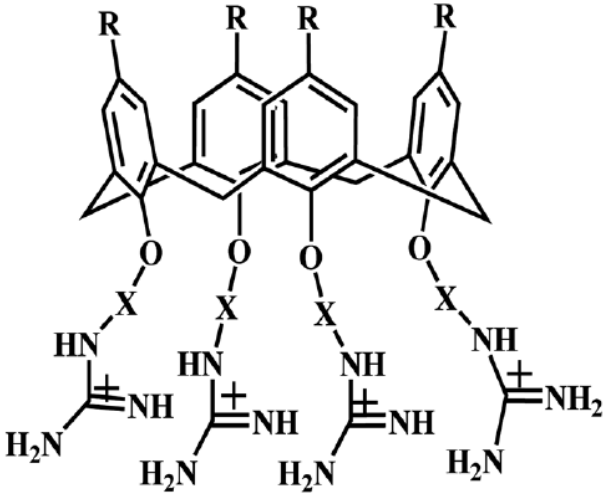
The same group has also investigated several calix[4,6,8]arenes as protein extraction agents, which revealed that the large cavities of the carboxylic acid derivatives of p-tert-octylcalix[6]arene and p-tert-octylcalix[8]arene exhibit high extraction capacities for proteins compared with the p-tertoctylcalix[4]arene.



46 = R = Oct, R_1 = H,

47 = R = Oct, R_1 = —HN(Cl⁻)(NH2)(+)=NH2

In 2006, Ungaro et al. reported on calix[n]arenes 39–47 functionalized with guanidinium groups at the upper rim and alkyl chains at the lower rim could bind to DNA, condense it, and in some cases, promote cell transfection depending on their structure and lipophilicity

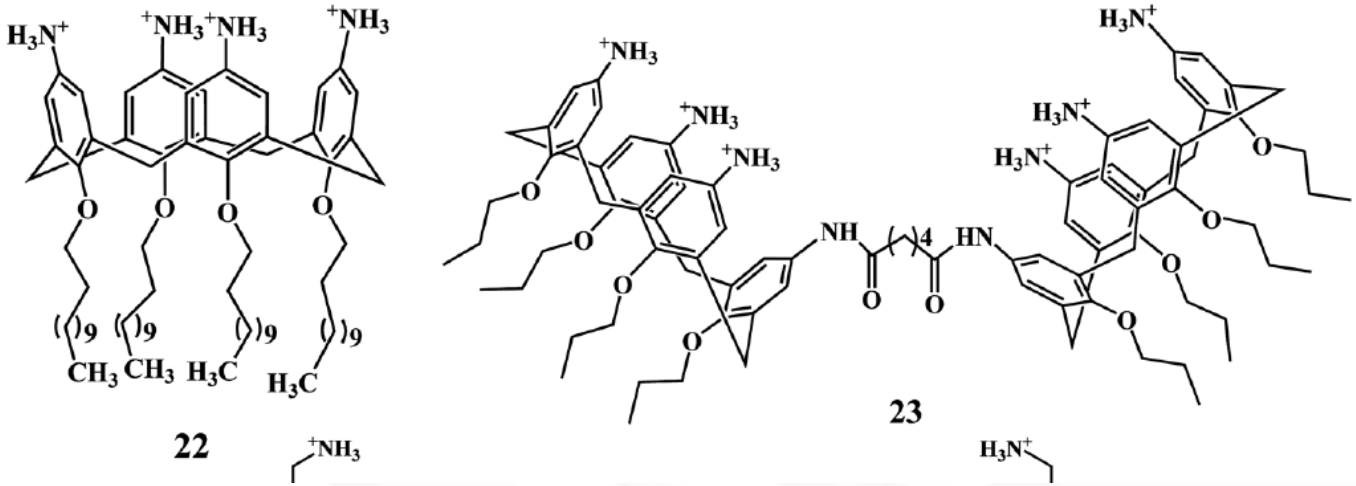


48 = R = *t*Bu, X = $(CH_2)_3$
49 = R = H, X = $(CH_2)_3$
50 = R = Hex, X = $(CH_2)_3$
51 = R = H, X = $(CH_2)_6$

Interestingly, in 2008 Ungaro et al. found that attaching guanidinium moieties at the phenolic OH groups (lower rim) instead of the aromatic moiety (upper rim) of the calix[4]arene through a three carbon atom spacer results in a new class of cytofectins.

Compound 48, when formulated with DOPE, performs cell transfection quite efficiently and with very low toxicity, surpassing a commercial lipofectin widely used for gene delivery

Cationic Calixarenes for plasmid DNA delivery (gener delivery)



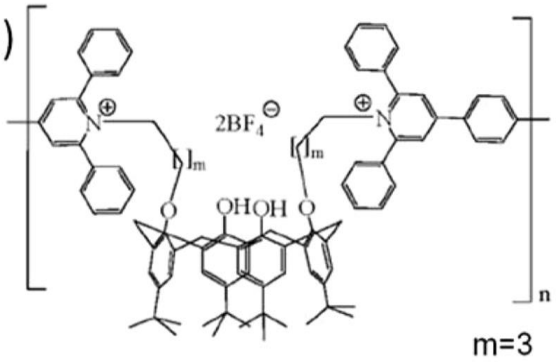
Preorganization of the functional elements onto macromolecular platforms has the potential to allow control of the self-assembling behavior of discrete architectures to produce nanometric objects that can be programmed to be complex, compact, deliver, and release plasmid DNA in a target cell. Compound 22 has been shown to self-assemble, with the absence of a co-surfactant, to form positively charged solid lipid nanoparticles (SLNs) that interact with double stranded DNA.73

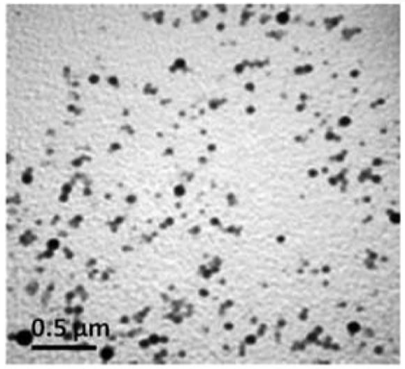
The dimeric derivative 23, with a higher degree of preorganization, had been previously shown to interact with DNA, probably by targeting the major groove, though transfection abilities were not demonstrated.

Water soluble CA and gene delivery

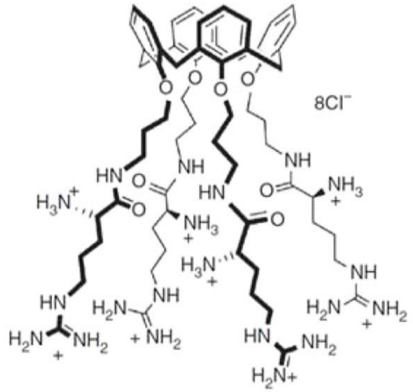
Through the functionalization process, macrocyclic molecules can be imposed with multifunctional capabilities while maintaining their property of encapsulating guest molecules. For instance, poly(pyridinium) salts were functionalized onto CAs, aiming at biosensing application (Figure 3a). Both the lower and the upper rims of CAs could be functionalized with water-soluble groups, including the conjugation with amino containing cationic units for the DNA condensation and gene delivery

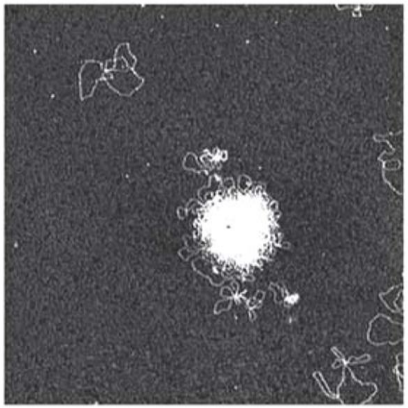
a)



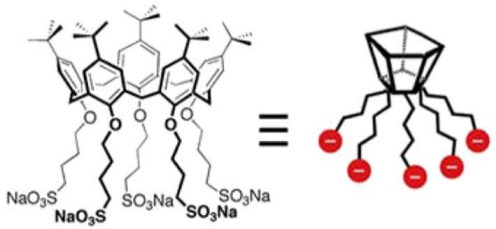


b)

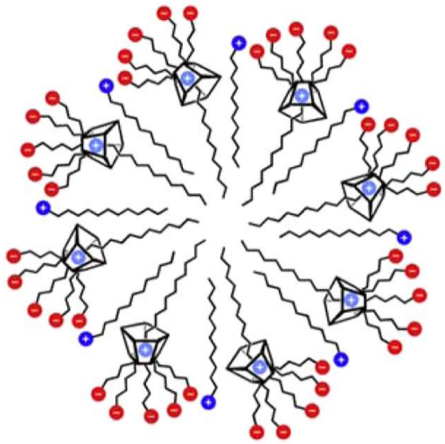




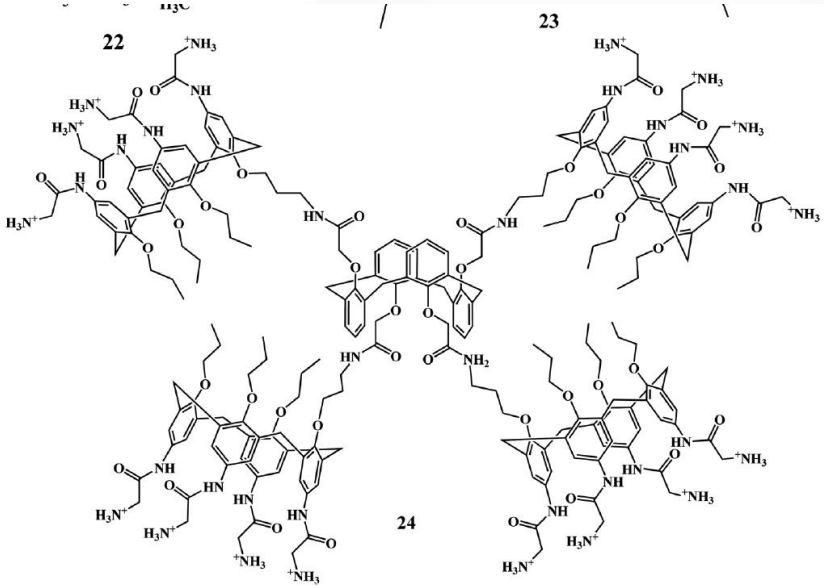
c)



n-dodecylammonium chloride

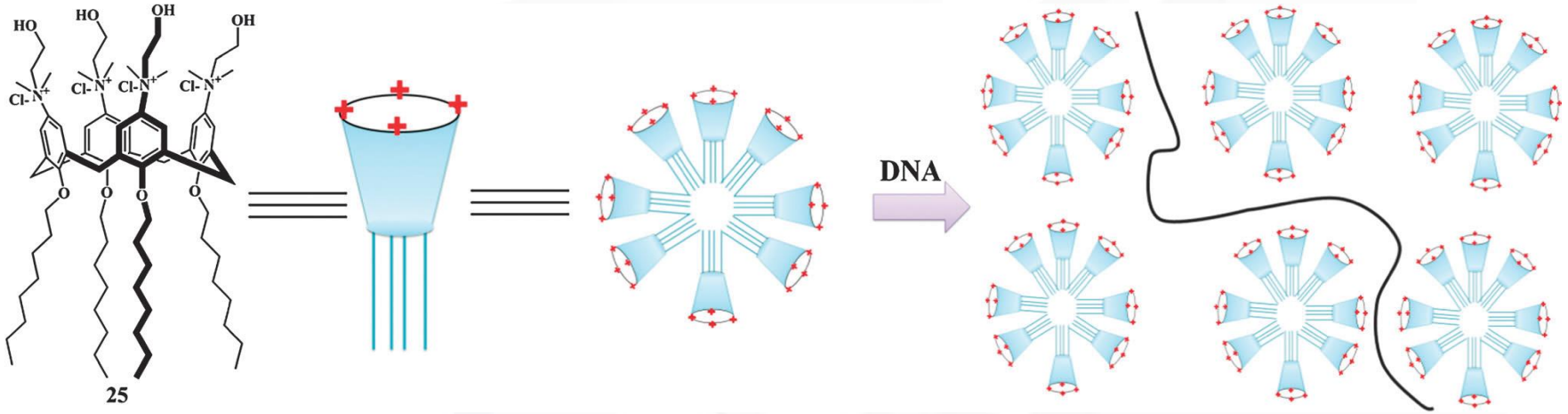


Cationic multi-calix for plasmid DNA delivery into cells



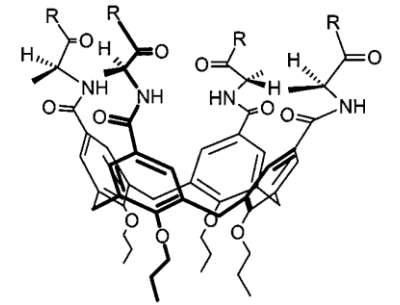
Multicalixarene–pDNA binding turned out to be much stronger than that of their monomeric analogs, indicating the existence of cooperativity effects. Multicalixarenes blocked pDNA electrophoretic mobility, therefore efficiently condensing them, and are virtually nontoxic up to mM concentrations. Transfection experiments carried on Chinese hamster ovary (CHO) cells revealed that the presence of aliphatic amino groups, as in 24, was necessary to efficiently promote the GFP-encoding gene expression. pDNA complexes from multicalixarenes with arylamino groups were ineffective irrespective of the alternating or cone conformation of the central scaffold.

Self-assembled nanoparticles from calixarenes



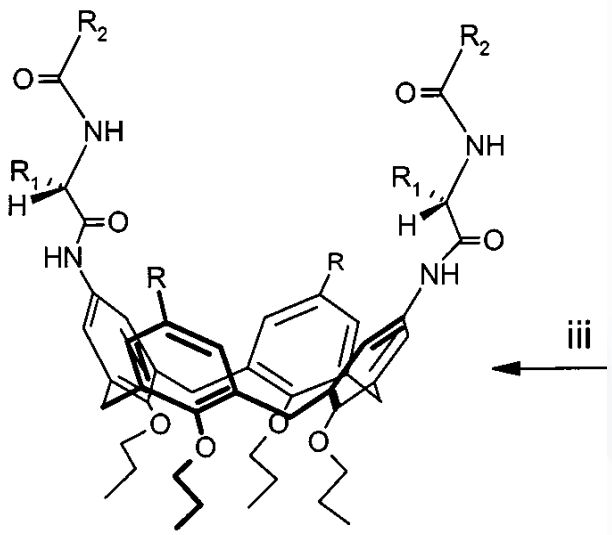
Recently, Rodik et al. reported a two-step hierarchical assembly of small DNA nanoparticles for gene delivery based on amphiphilic cone-shaped cationic calixarenes 25

Peptido-calixarenes



N-linked peptidocalix

C-linked peptidocalix



In 2001, Ungaro et al. synthesized the upper rim modified C-Linked peptidocalix[4]arenes 29–32 of which 29 preferentially interacted with the anionic species. These C-linked peptidocalix[4]arene derivatives were further used to produce the nanotubes.

NMR binding experiments

Table 1. Association constants of selected peptidocalixarenes with various guests at 300 K

Host	Solvent	Guest[a]	K (M^{-1})[b]
4a	$[D_6]$DMSO	acetate	34
		benzoate	12
		N-acetyl-L-alaninate	14
		N-phthaloyl-L-alaninate	17
		Cl^-	18
		Br^-	no complexation
2a	$[D_6]$DMSO	acetate	33
9a	$[D_6]$DMSO	benzoate	19
		benzoic acid	no complexation
9a	$CDCl_3$	benzoate	7
		benzoic acid	49
		N-acetyl-L-alaninate	no complexation
7a	$[D_6]$DMSO	benzoate	21
		acetate	19
		Cl^-	4

[a] Anionic guests as tetra-*n*-butylammonium salts. – [b] The K values are subject to errors of $\leq 10\%$.

The recognition properties of the C-linked peptidocalix-[4]arenes were investigated by 1H NMR titration experiments. The downfield shifts of the amide NH proton signals of the hosts at different host/guest ratios were analysed by nonlinear least-squares fitting procedures, which led to the association constants presented in Table 1.

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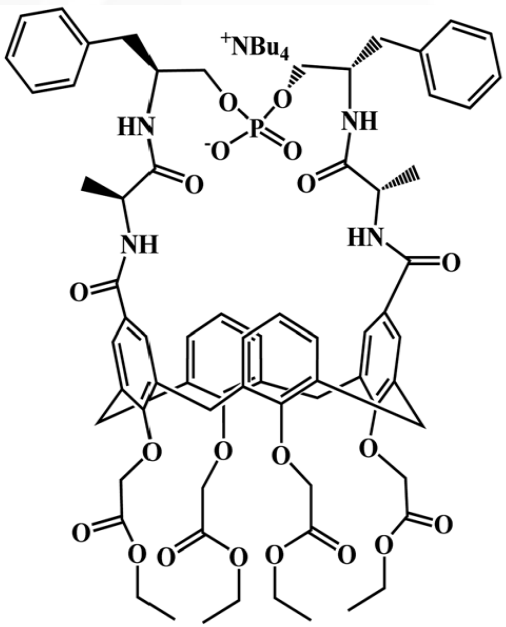
Molecular recognition of sugars (Ungaro, Sansone)

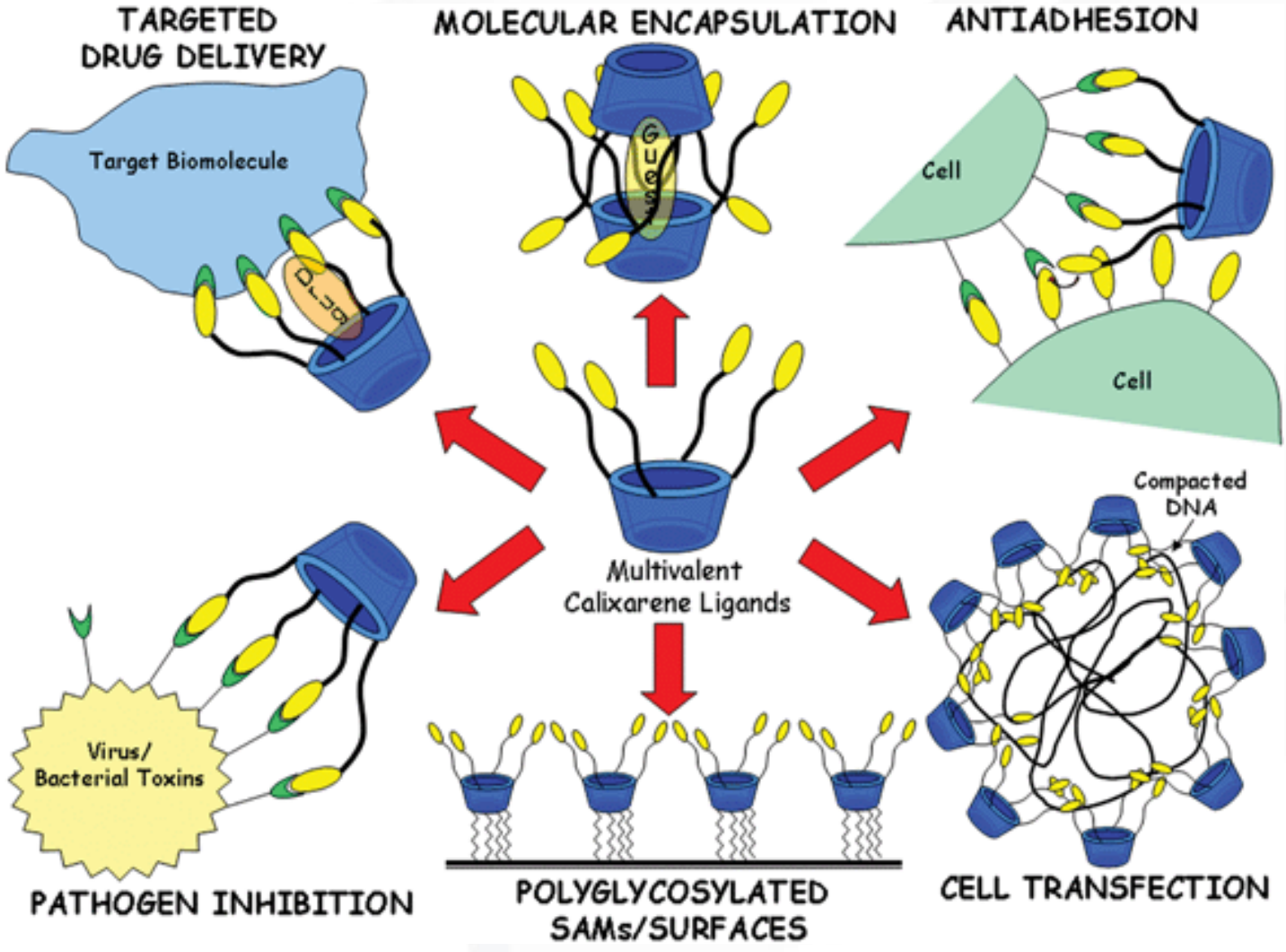
In 2003, Ungaro et al. developed a new macrocyclic receptor 33 for carbohydrate recognition based on upper rim peptide bridged calix[4]arene.

Receptor 33, in which a charged phosphate group cooperates with peptide hydrogen-bonding donor and acceptor groups in the binding process, is the most efficient and selective in the complexation of simple carbohydrate derivatives. The selectivity observed is toward β-glucoside, which is better bound (ΔG1 = 19.6 kJ mol-1) compared to-the corresponding α anomer 35 (ΔG1 = 17.0 kJ mol-1) and β-galactoside 36 (DG1 = 17.7 kJ mol-1) in CDCl3.

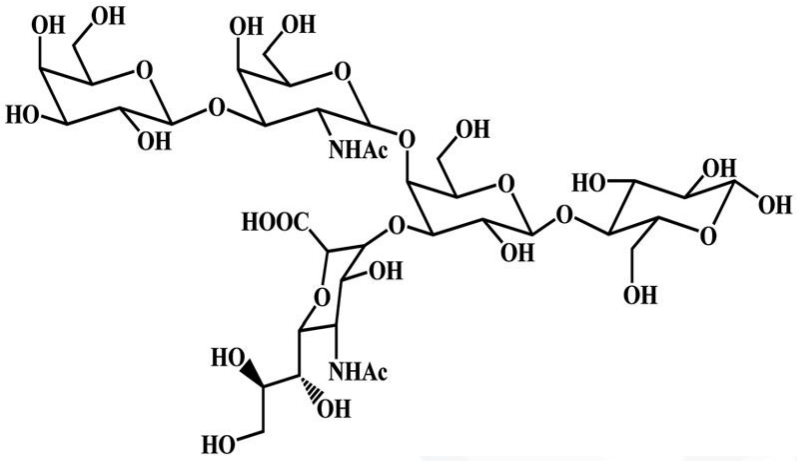


33

Calixarenes as scaffolds for multivalent hosts in biological applications



Cholera toxin



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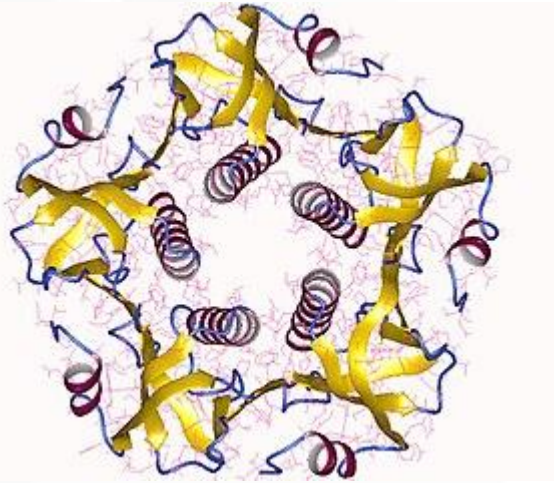
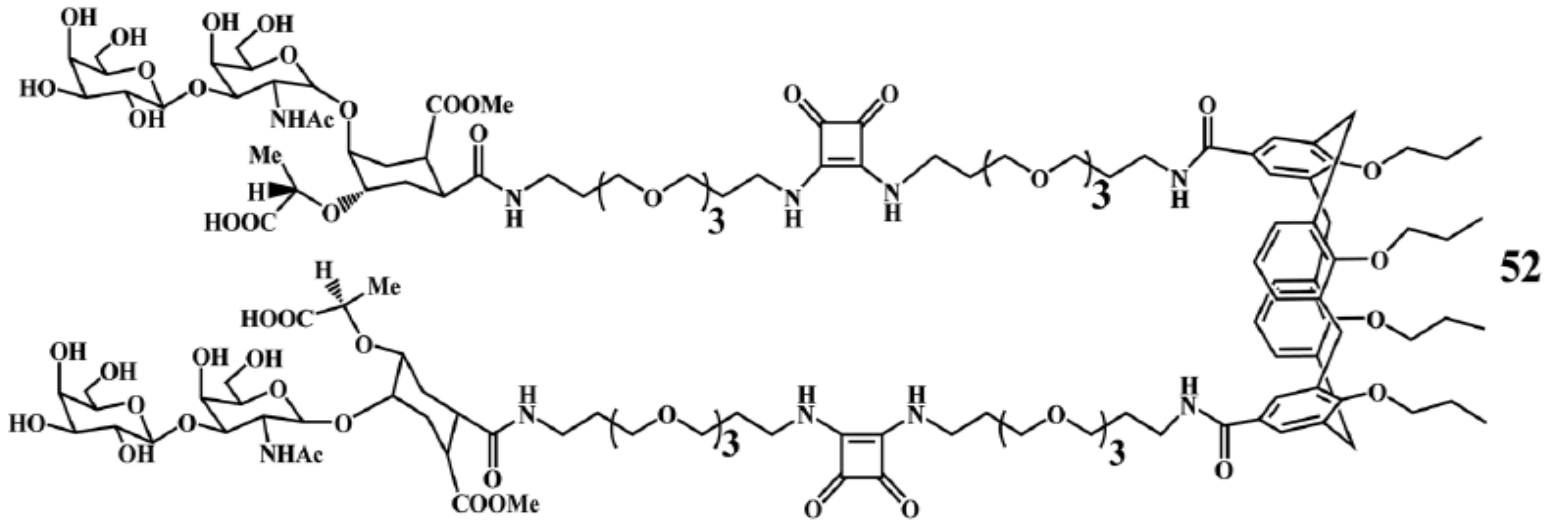


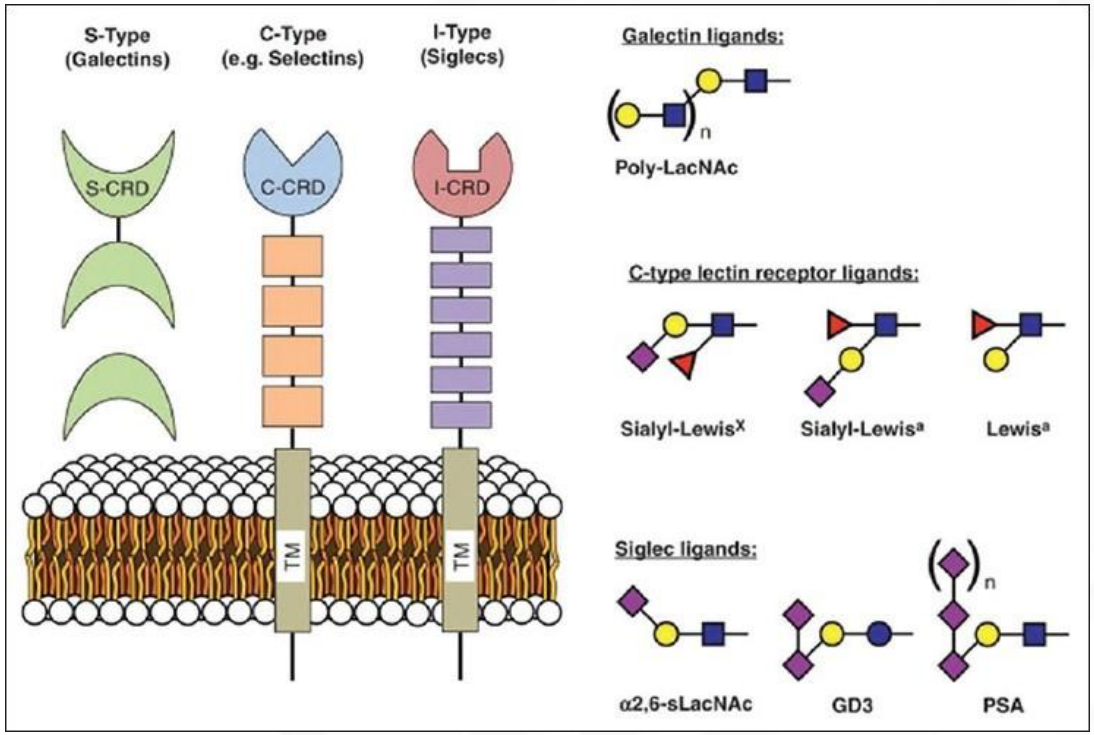
The cholera toxin is a pentavalent sugar-binding protein belonging to the class of AB5 toxins. The doughnut-shaped B pentamer presents five identical sugar-binding sites on a single face and is responsible for cell-surface binding. The cell surface ligand of cholera toxin is ganglioside GM1 [Galb1-3GalNAcb1- 4(NeuAca2-3)Galb1-4Glcb1-1Cer] 53

Cholera toxin ligand GM1 on a calixarene

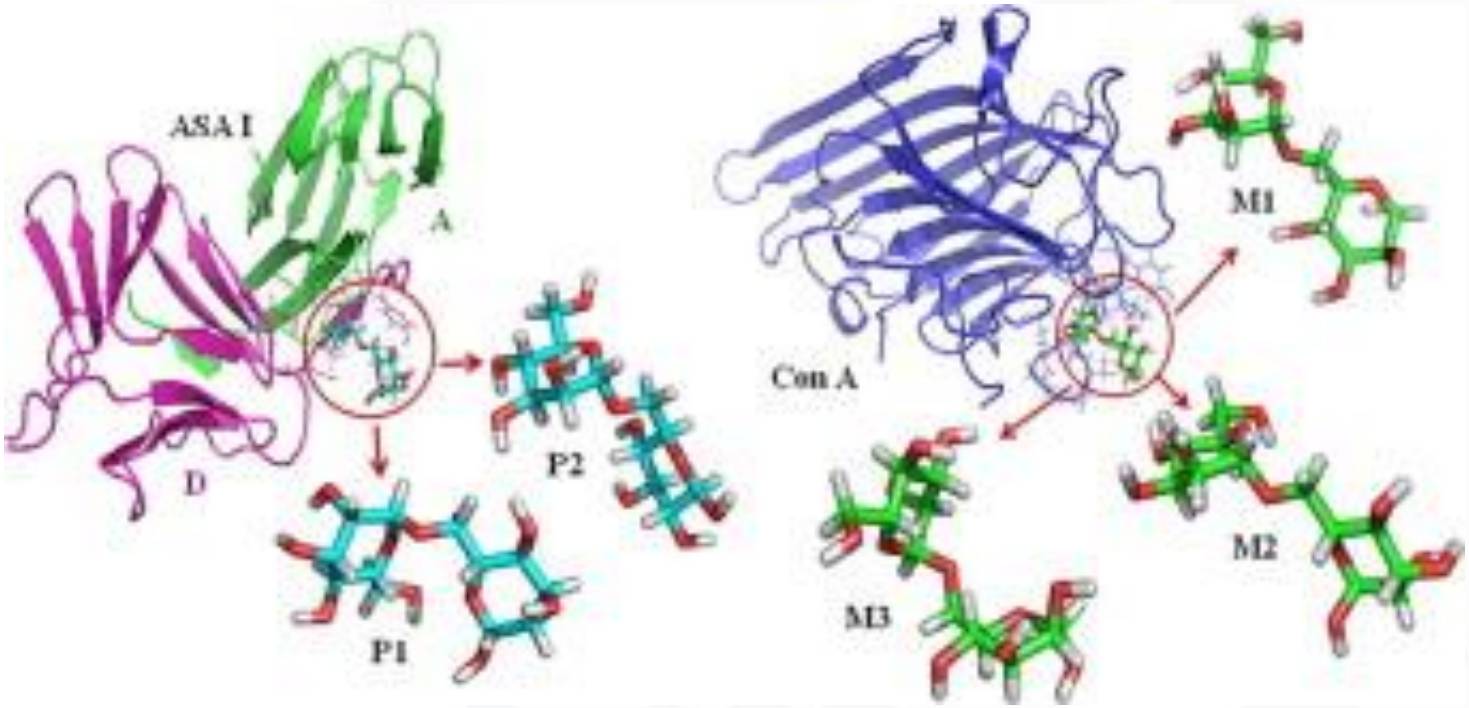


in 2005, Ungaro et al. extended their research on calixarene carbohydrate interactions and developed a novel glycocalix[4]arene 52 which has a higher affinity for the cholera toxin than its natural cell surface ligand ganglioside GM1.

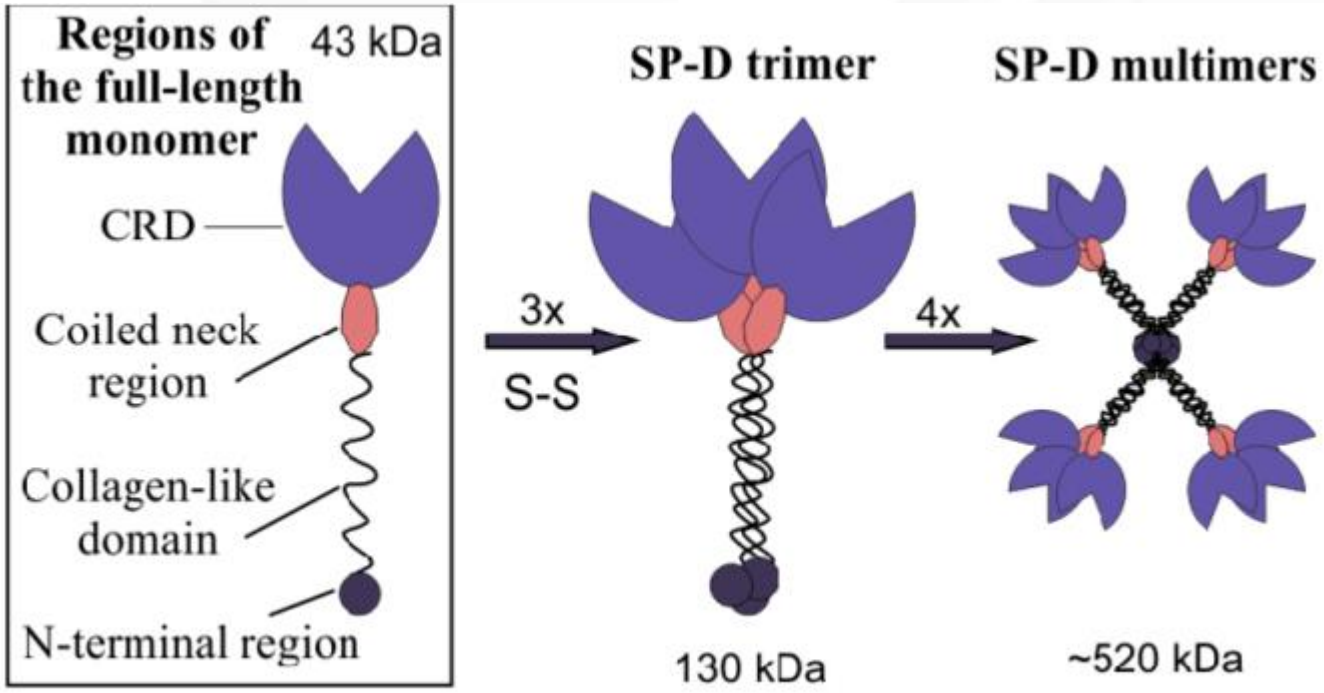
Lectin-carbohydrate interactions



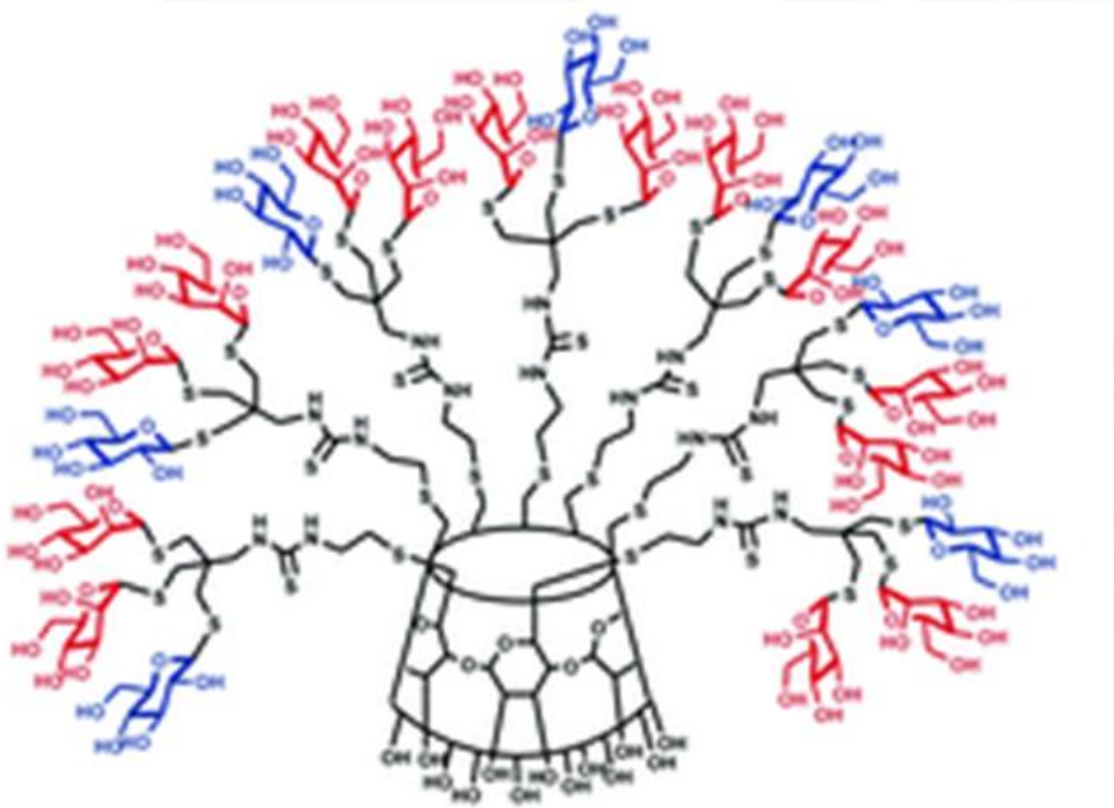
Galectins play an important role in tumor progression: Inhibiting galectin-sugar interactions it is a new strategy to have antitumor drugs



The interaction between lectins and sugars is multivalent



…so ideal lectin ligands display several copies of the sugars that are recognized (glycoclusters)



Representative βCD-centered α-Man−β-Glc heteroglycocluster

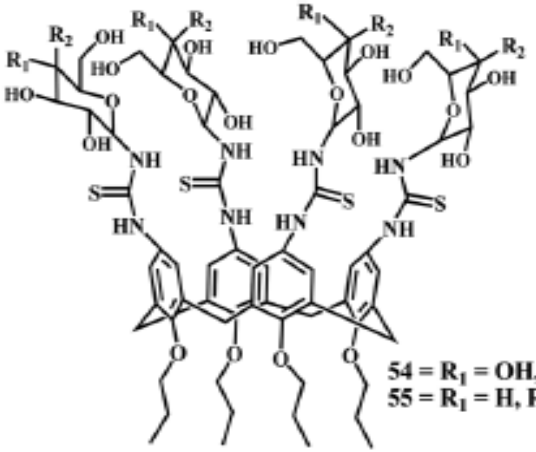
Targeting sugar-lectins interactions

Growing insights into the functionality of lectin–carbohydrate interactions are identifying attractive new targets for drug design.

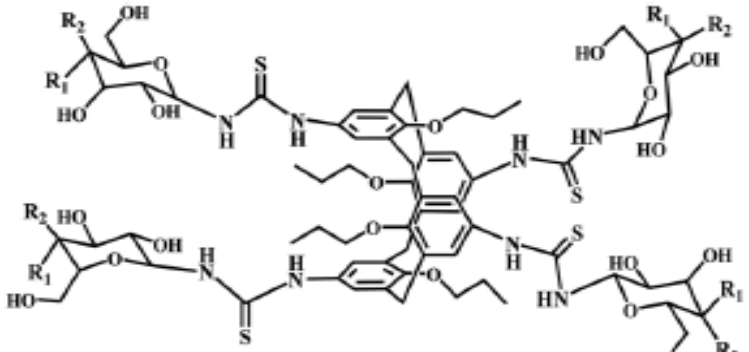
As glycan recognition is regulated by the structure of the sugar epitope and also by topological aspects of its presentation, a suitable arrangement of ligands in synthetic glycoclusters has the potential to enhance their avidity and selectivity.

If adequately realized, such compounds might find medical applications. Ungaro et al. focused on lectins of clinical interest, acting either as a potent biohazard (a toxin from Viscum album L. akin to ricin) or as a factor in tumor progression (human galectins-1, -3, and -4). Using a set of 12 calix[n]arenes 54–65 (n = 4, 6, and 8) with thiourea linked galactose or lactose moieties, Ungaro et al. first ascertained the lectin-binding properties of the derivatized sugar head groups conjugated to the synthetic macrocycles.

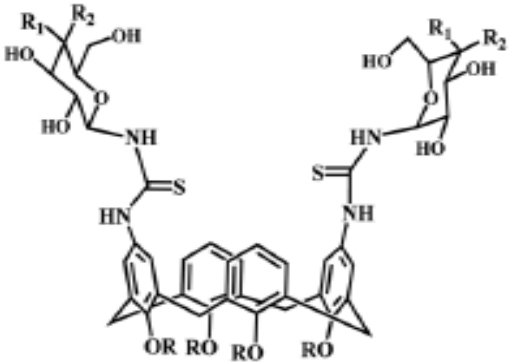
Lectins-binding glycocalixarenes



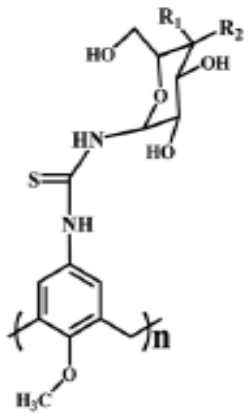
54 = R_1 = OH, R_2 = H
55 = R_1 = H, R_2 = β–galactose



60 = R_1 = OH, R_2 = H
61 = R_1 = H, R_2 = β–galactose

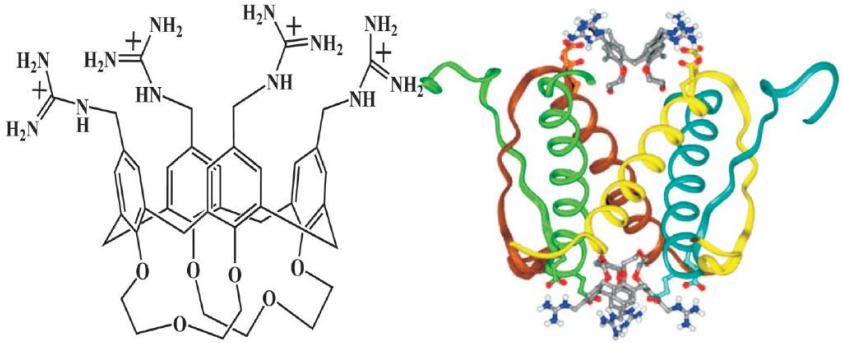


56 = R = -$CH_2CH_2CH_3$, R_1 = OH, R_2 = H
57 = R = -$CH_2CH_2CH_3$, R_1 = H, R_2 = β–galactose
58 = R = CH_3, R_1 = OH, R_2 = H
59 = R = CH_3, R_1 = H, R_2 = β–galactose



62 = n = 6, R_1 = OH, R_2 = H
63 = n = 8, R_1 = OH, R_2 = H
64 = n = 6, R_1 = H, R_2 = β–galactose
65 = n = 8, R_1 = H, R_2 = β–galactose

Calixarenes binding to pharmacological targets



70

Binding model showing two molecules of the rationally designed ligand **70** bound to the two hydrophobic cavities of p53TD.

In 2008, Mendoza et al. explained how an increase in the flexibility of both protein and ligand can result in an increase in the stability of the macromolecular complex. The biophysical study of the interaction between a designed flexible tetraguanidiniumcalix[4]arene 70 and the tetramerization domain of protein p53 (p53TD), and its natural mutant p53TD-R337H shows how the mutant domain interacts more tightly with the ligand than the well-packed wild-type protein.132 Moreover, the flexible calixarene ligand interacts with higher affinity to both wild-type and mutated protein domains than a conformationally rigid calixarene analog previously reported. These findings underscore the crucial role of flexibility in molecular recognition processes, for both small ligands and large biomolecular surfaces.

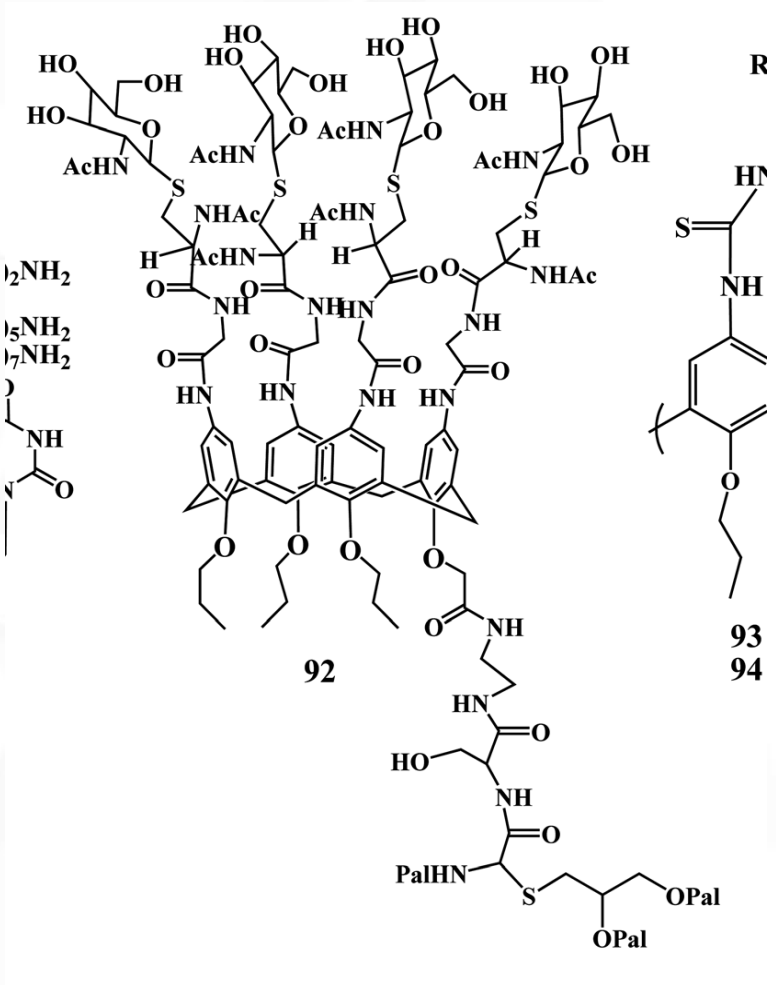
Synthetic vaccines

In 2008, Geraci et al. synthesized a novel fully synthetic cancer vaccine candidate in which a cluster of four Tn antigen glycomimetic units (S-Tn) is conjugated to an immunoadjuvant moiety,

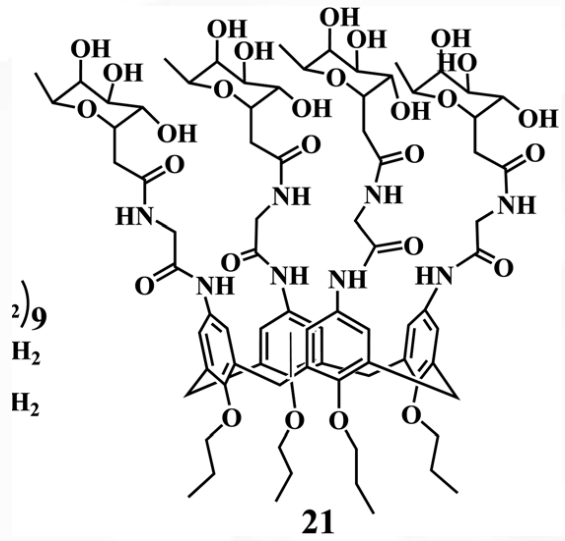
tripalmitoyl-S-glycerylcysteinylserine, through a calix[4]arene scaffold.

The presence of a rigid platform of the calix[4]arene scaffold provided a tetravalent derivative 92 with a definite spatial preorganization of the antigenic moieties. Biological tests, performed in vivo

by the immunization of mice, showed that the target construct possesses higher immunostimulating activity



Calixarenes to block bacterial adhesion to cells



Geraci et al. designed the calix[4]arene functionalized with the α-l-C-fucosyl as a new potential Pseudomonas aeruginosa biofilm inhibitor.

The anti-biofilm activity of the synthesized compound 21 showed dose-dependent activity against the PAO1 strain

Calixarenes for drug delivery

Review

Controlled drug delivery systems based on calixarenes

Yue Zhou, Hui Li, Ying-Wei Yang *

State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, College of Chemistry, International Joint Research Laboratory of Nano-Micro Architecture Chemistry (NMAC), Jilin University, Changchun 130012, China

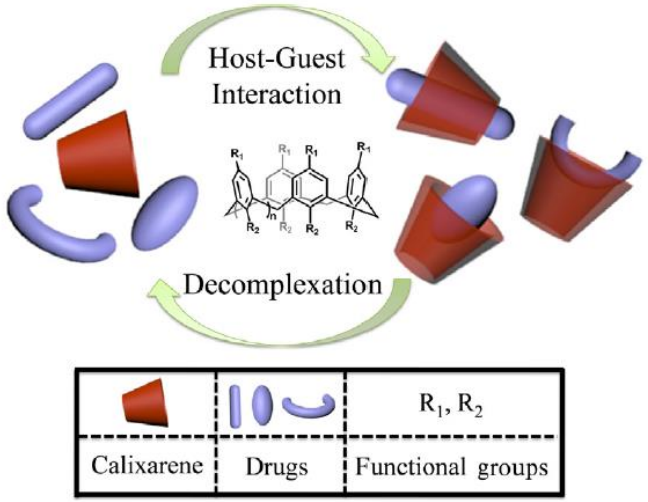
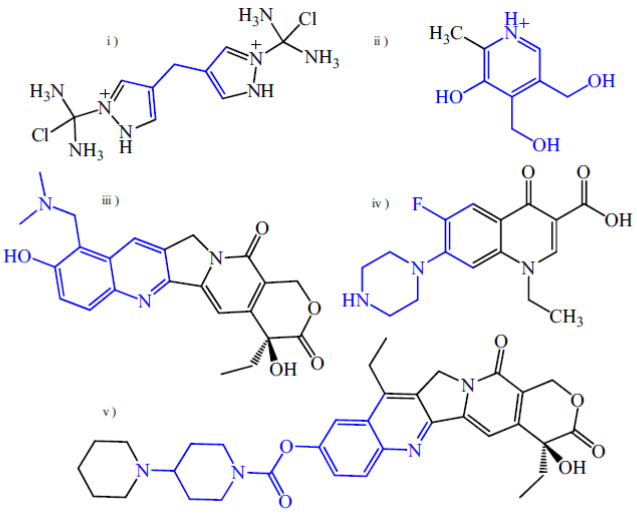


Fig. 1. Schematic representation of the formation of inclusion complexes based on CAs and drugs and their decomplexation.

Drugs as guests



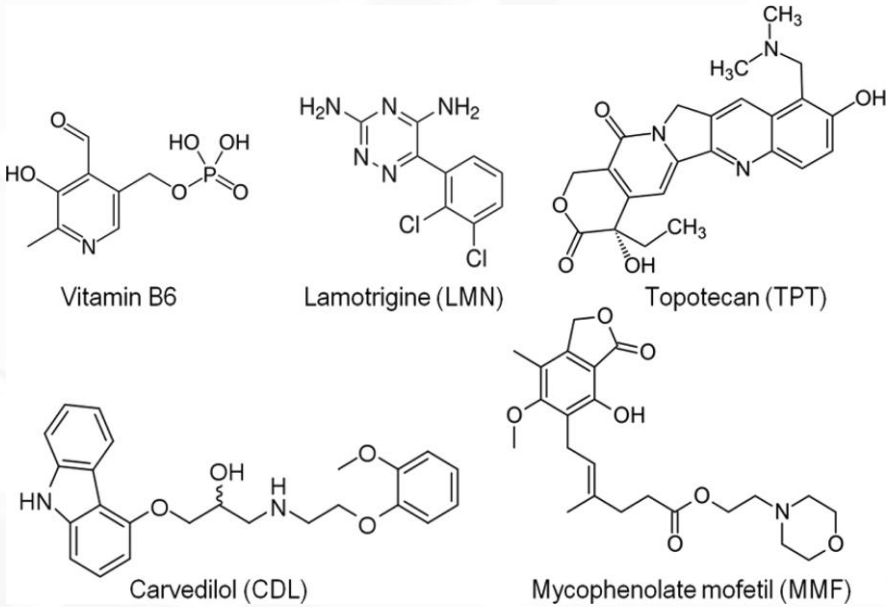


Fig. 2. Chemical structures of the studied drugs. The blue parts are the ones that can inset into the cavity of CAs.

Supramolecular complexes and micelles self-aggregation

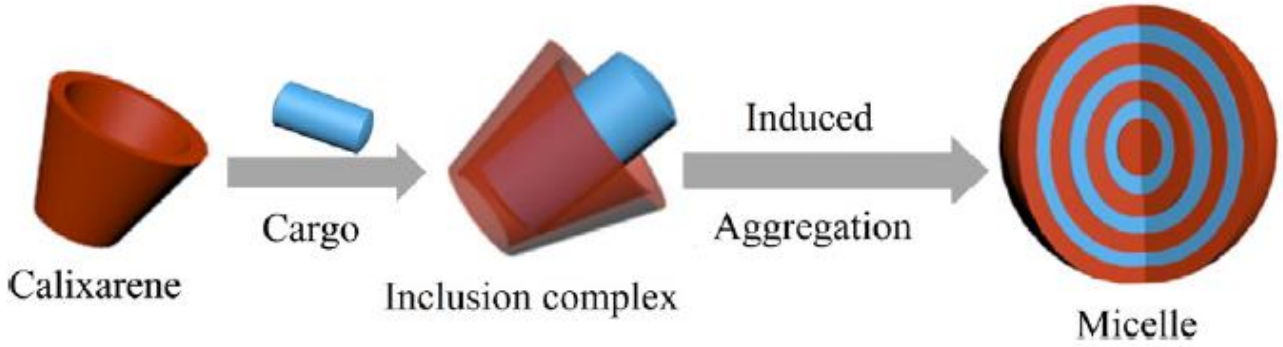
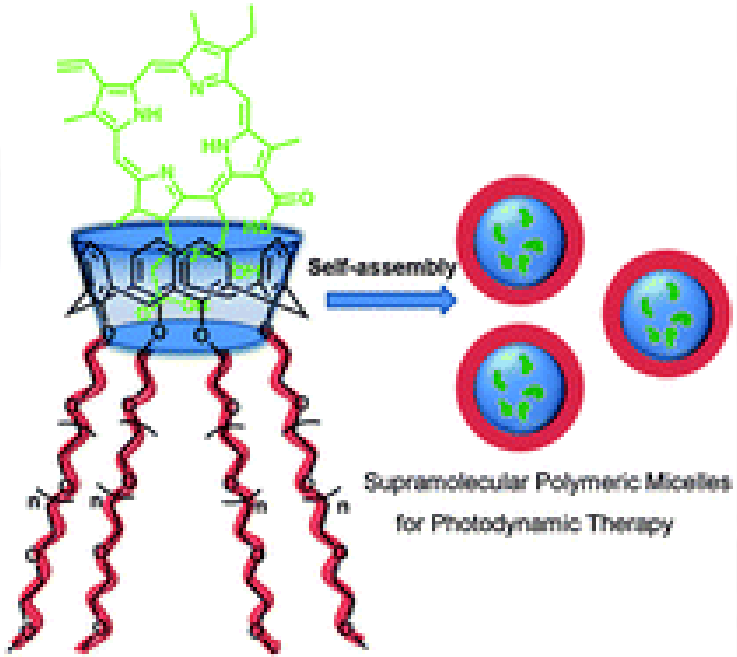


Fig. 3. Schematic illustration of the formation of micelles.

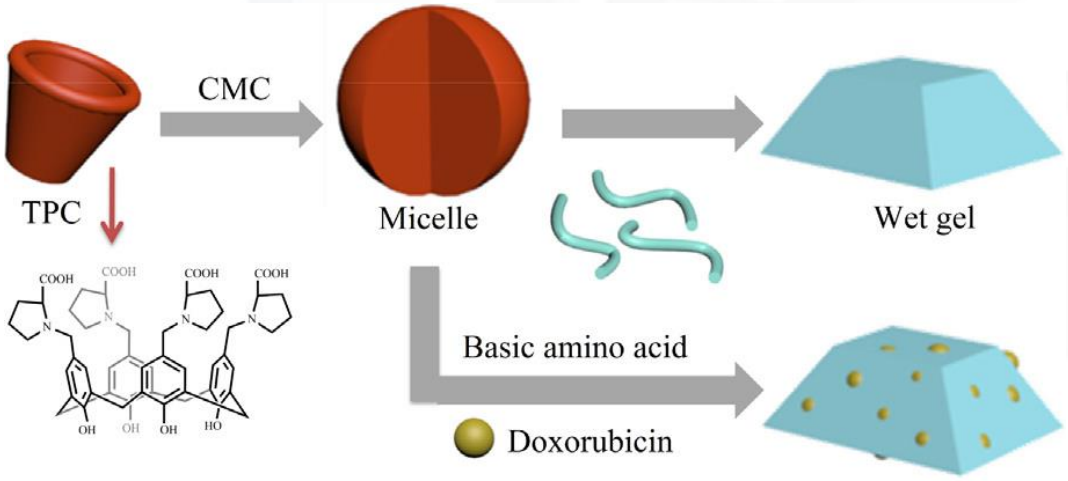
Self-assembly behavior is ubiquitous in living systems. Specific amphiphilic molecules are significant research topics in the fields of materials science and chemical biology because it can be spontaneously developed into micelles (Fig. 3), hydrogels and others.



In 2011, Zhu et al. took advantage of the host-guest interaction of hydrophilic host molecules, that is, PEGylated calix[4]arene, and hydrophobic chlorin e6 to form supramolecular polymeric micelles, which exhibited more efficient photodynamic therapy efficacy than free chlorin e6

Hydrogels

Low molecular weight hydrogels have aroused prodigious attention in many fields. Liu et al. [13] designed a series of supramolecular binary gels by two steps. First, tetra-proline modified calix[4]arenes (TPC) turned to micelles when the concentration of TPC was above the critical micelle concentration (CMC), and then the complexes translated itself into hydrogels with basic amino acids (arginine, histidine and lysine) in acidic conditions. Meanwhile, anticancer drug, doxorubicin hydrochloride (DOX), was entrapped into the gels (Fig. 4) and the systems released DOX upon immersion of the hydrogels into water.



Stimuli-responsive micelles or liposomes

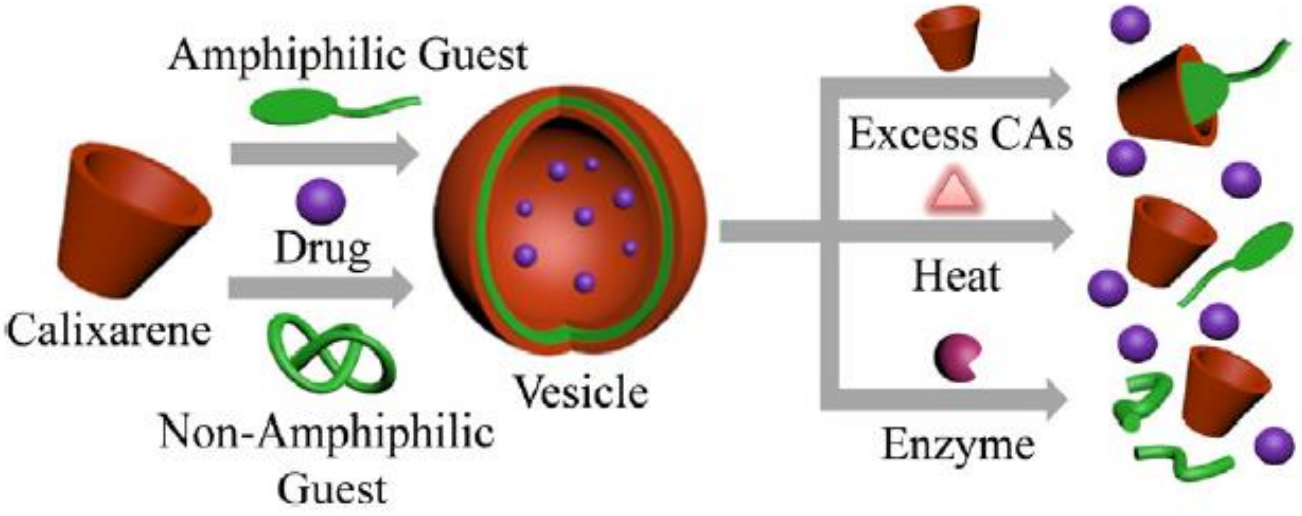
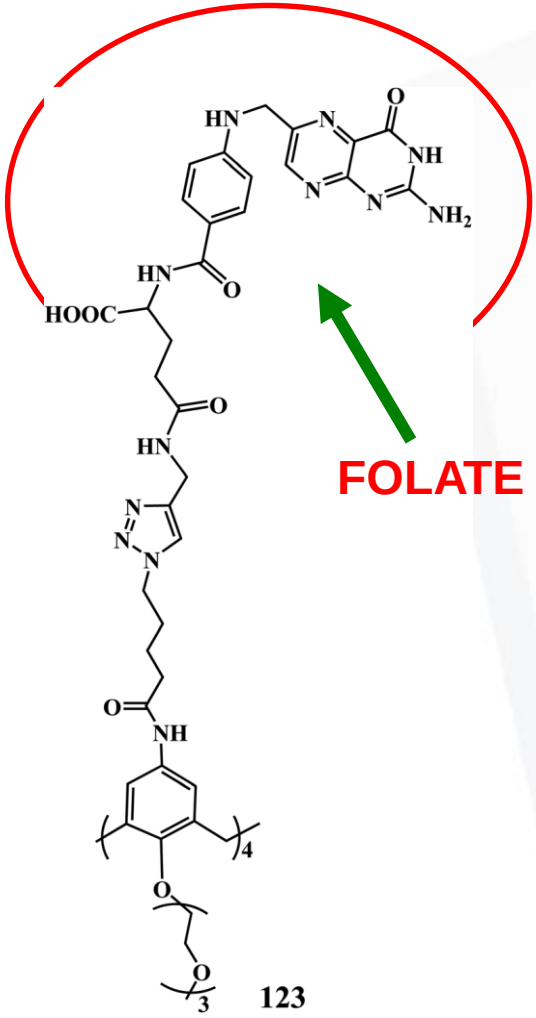


Fig. 5. Illustration of formation of vesicles and their response to multi-stimuli to realize drug release.

Liu et al. constructed a new nanoscale supramolecular vesicle based on p-sulfonatocalix[5]arene (SC[5]A) and 1-pyrenemethylaminium by host-guest interactions, which can form 1:4 molar ratio of complex and respond to temperature to realize the release of DOX. Schematic illustration of formation of vesicles in response to multi-stimuli to realize drug outflow has been represented in Fig. 5.

Targeted tumor drug delivery



A folate receptor (FR) is a highly selective cancer cell and activated macrophage marker, and folic acid vitamin (FA), which binds to FR with high affinity (Kd = 0.1 nM), and behaves as a ‘‘Trojan Horse’’194 that can promote the specific delivery of imaging and therapeutic agents into FR-positive cells.

Recently, Consoli et al. reported the multivalent folate conjugate 123 in which four folate units are clustered by means of a calix[4]arene platform

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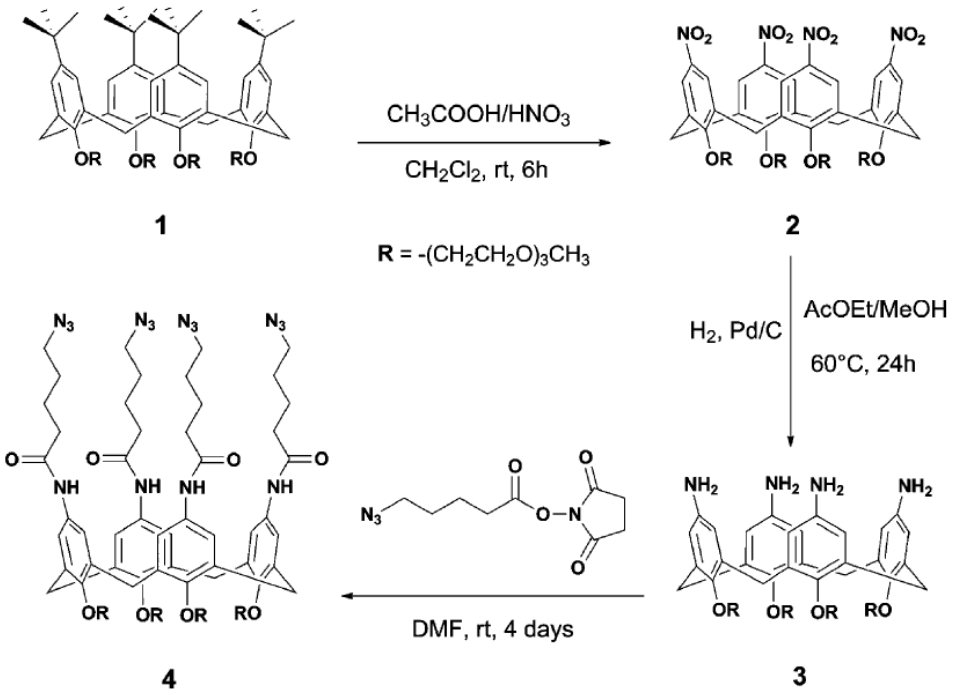
COMMUNICATION

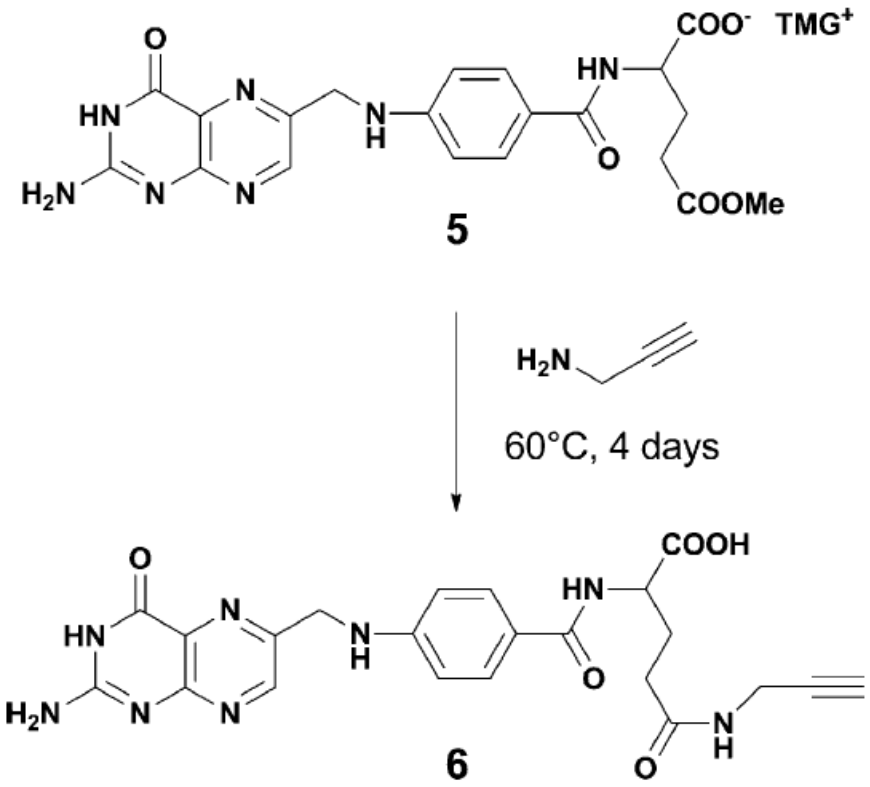
Design, synthesis, and drug solubilising properties of the first folate–calix[4]arene conjugate†

Grazia M. L. Consoli,* Giuseppe Granata and Corrada Geraci*

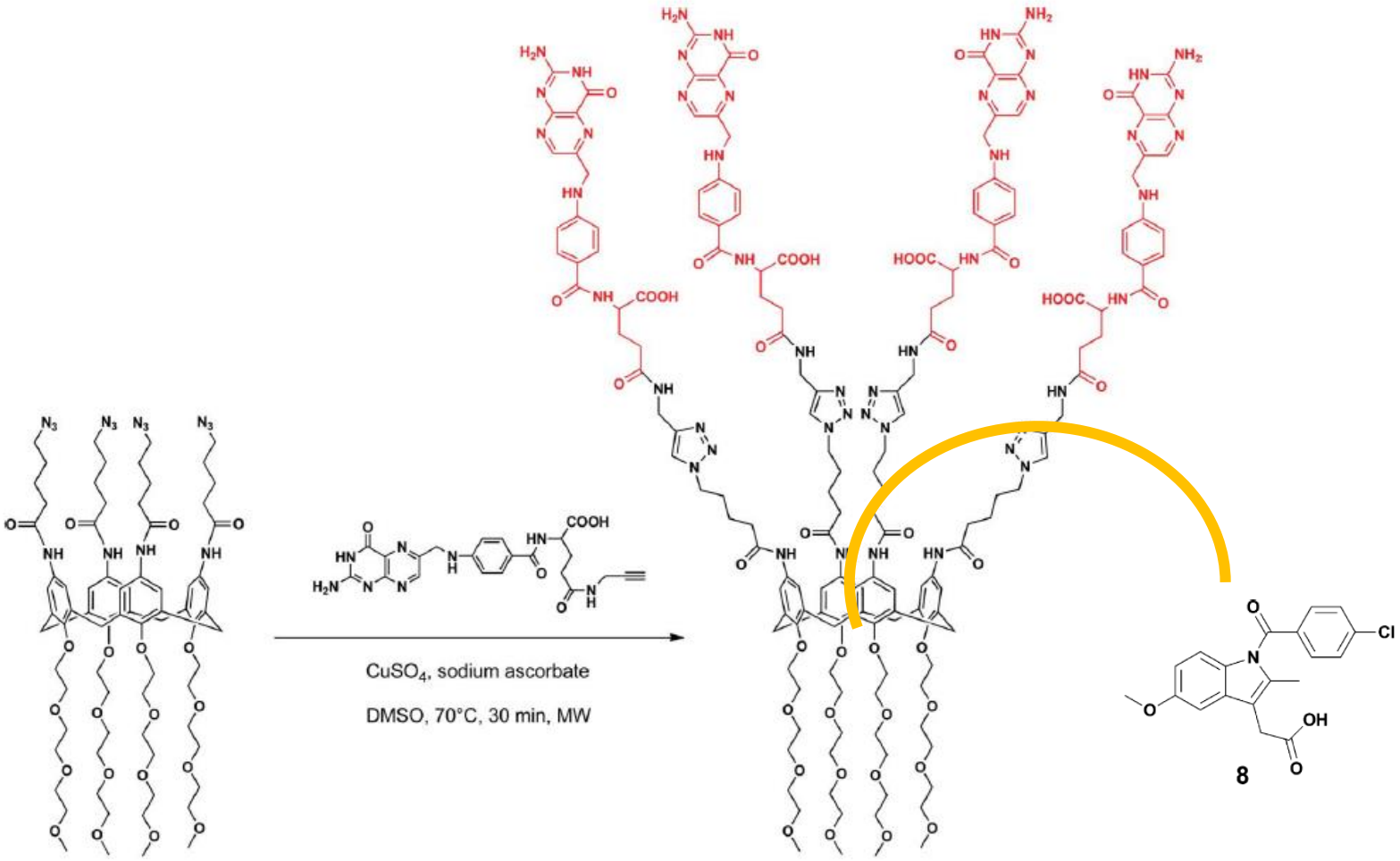
Received 27th June 2011, Accepted 22nd July 2011
DOI: 10.1039/c1ob06032e



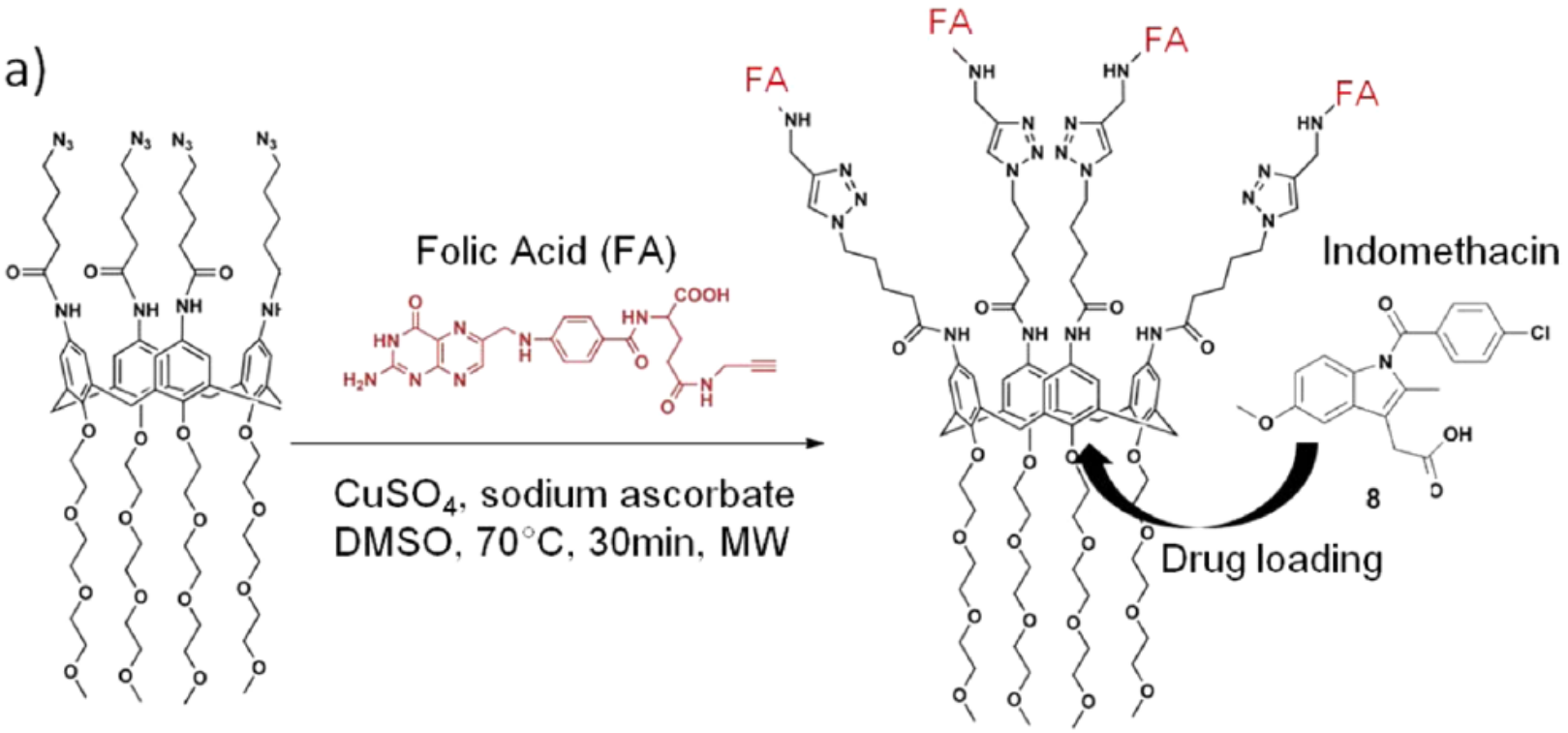
Scheme 1 Synthesis of the azido-derivatized calix[4]arene (**4**).



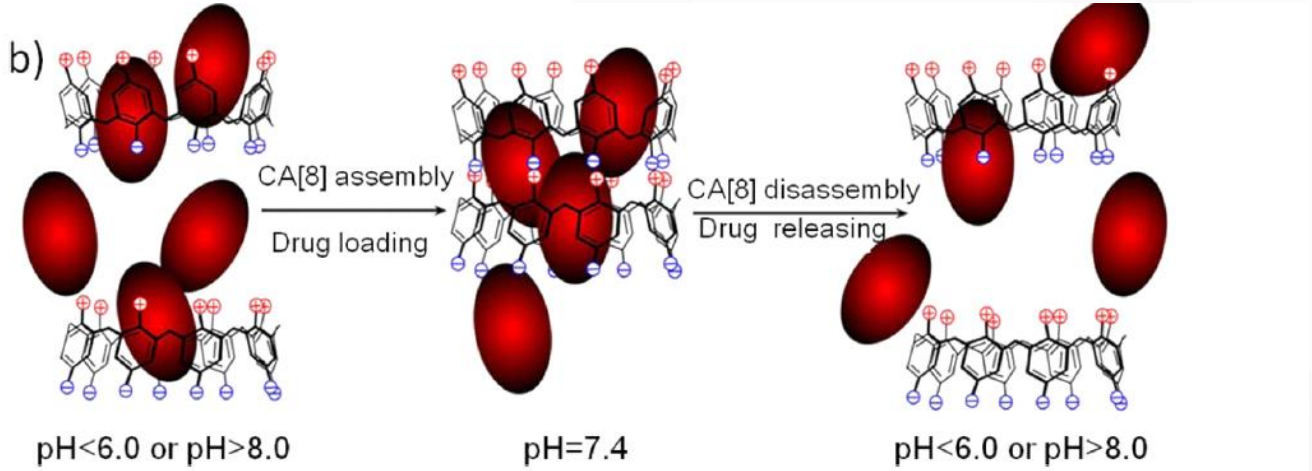
Scheme 2 Synthesis of the propargyl folate (**6**).


CuSO4, sodium ascorbate
DMSO, 70°C, 30 min, MW
4
7
8
INDOMETACIN

Calixarene-based drug delivery



Stimuli-responsive drug delivery from calixarenes



Xiao and co-workers designed and fabricated an amphoteric calix[8]arene (CA[8]) to achieve pH-responsive drug release from CA[8]−drug complex (Figure 10b). The upper rim of the CA[8] was featured with negatively charged sulfonate groups, while the lower rim was grafted with positively charged quaternary ammonium groups.

Hydrophobic model drug, ciprofloxacin (CPF), was loaded into the CA[8] cavity at neutral pH with the loading capacity of 17.8− 24.5%. The CA[8]−CPF complex could self-assemble to form superstructures through the electrostatic interaction between the upper and lower rims.

Under either acidic or basic pH condition, the CA[8]−CPF complex would disassemble and release the CPF drug in a sustained manner. Such pH-responsive drug delivery system shows great potential for future theranostic applications.

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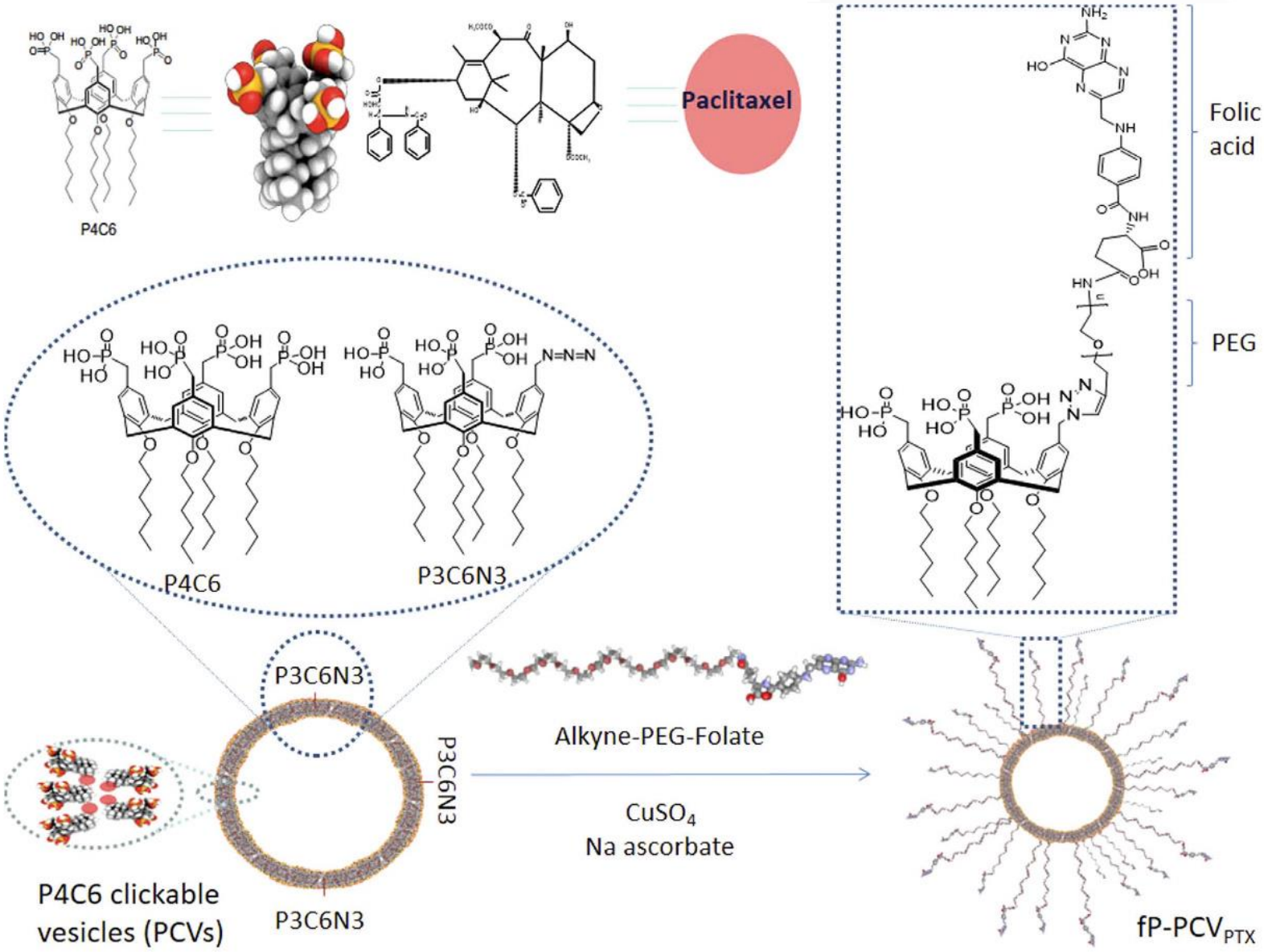
Paclitaxel-loaded phosphonated calixarene nanovesicles as a modular drug delivery platform

The ability to direct a drug payload specifically and solely to a particular cellular type is regarded as a Holy Grail in chemotherapy. Most current chemotherapeutic agents are highly efficacious, but they are also associated with debilitating toxicity, essentially due to their indiscriminate attack on both cancerous and rapidly dividing healthy cells. To improve treatment outcomes, several targeting drug delivery approaches have been devised with various degrees of success.

One approach is to design **pH-sensitive polymeric platforms to trigger the release of a drug payload upon extravasation into cancer tissues, the optimal system being capable of responding with adequate discrimination to the small pH difference between blood and tumour milieu (pH 7.4 vs. pH 5.5)**. Another approach is to target receptors that are overly expressed on cancer cell membranes, in particular the **folate receptor (FR)** which has been the target of choice for a diverse range of delivery platforms.



Metalloenzymes mimics

Enzymes are amongst the most selective catalysts available.
Their active sites provide a variety of non-covalent interactions with substrates that control the recognition process and, therefore, the selectivity of the enzyme. These include hydrogen bonding, charge/charge, CH/π and cation/π interactions. Many enzymes contain metal ions that play a key role in the structural organization of the peptide, in the recognition processes of substrates or as the catalytic centers in the active site.
In the latter case, the enzyme does not only act as a very large ligand with preorganized metal binding sites, but it also provides a cavity and a corridor to control the access of the substrate and, therefore, selectivity and reactivity of the metal ion site.

Metalloenzyme mimics

- Phopshatase-catalyzed reaction 2-hydroxypropyl-p-nitrophenyl phosphate (HPNP) is used as RNA model substrate, the intramolecular cyclization reaction, and the release of p-nitrophenolate being the driving force of the reaction.

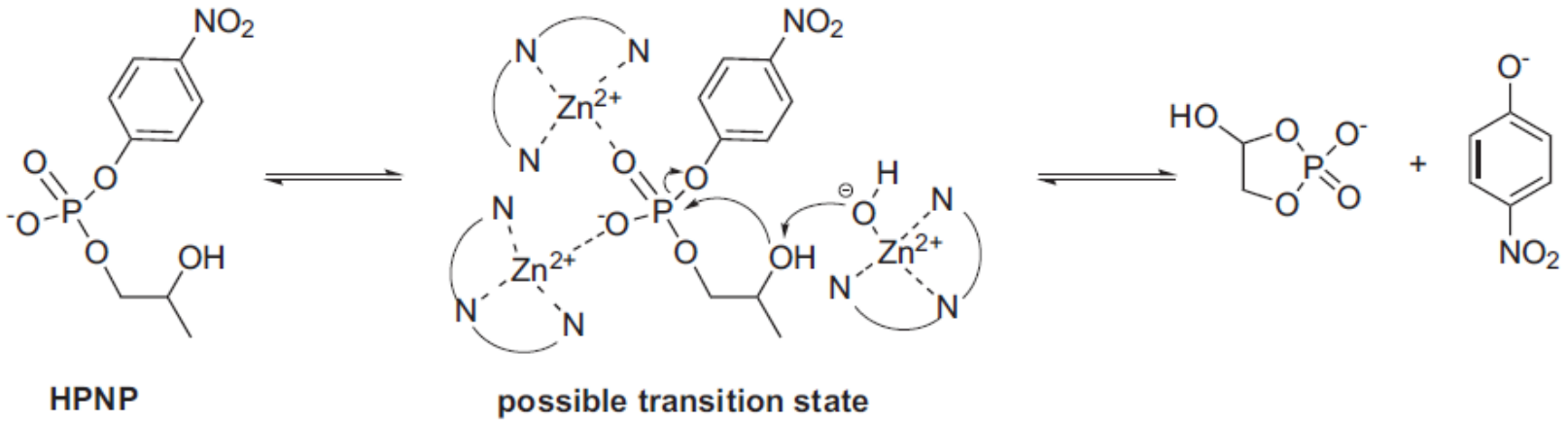


Fig. 10. Possible mechanism for HPNP cleavage by trinuclear phosphatase models [35].

Many metal containing phosphodiesterases use one, two or three divalent metal centers such as Zn^{2+} for the activation of the substrate.
The prearrangement of metal centers using synthetic spacers is crucial in mimics of such enzymes since tetrahedrally coordinated Zn^{2+} centers are assumed to activate the phosphate group and a nucleophilic water molecule, to stabilize the pentacoordinate phosphorus transition state and possibly the leaving group by cooperative action

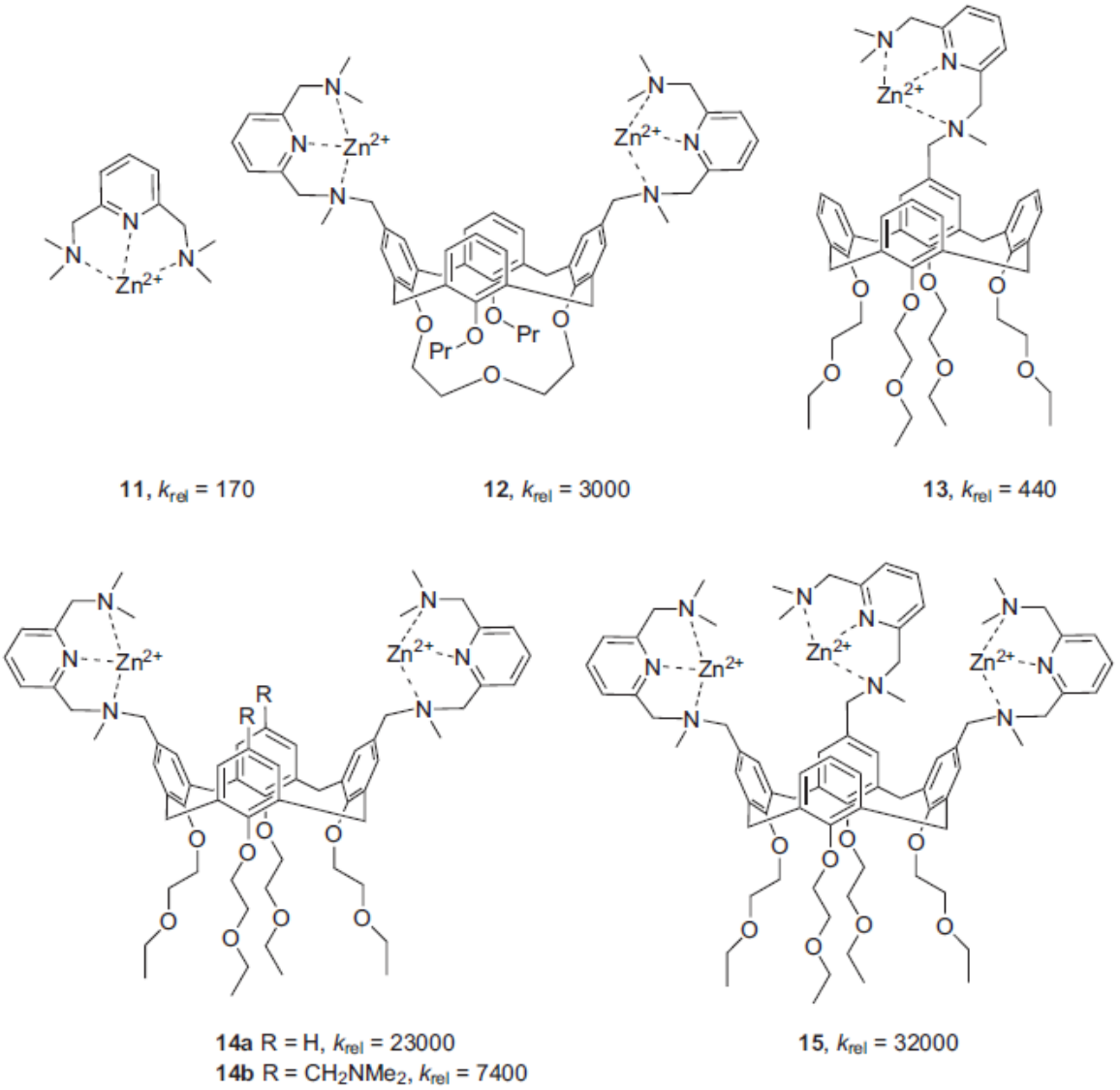


Fig. 11. Mono-, di- and trinuclear Zn(II) phosphatase mimics and relative rate acceleration factors k_{rel} [33–35].

Relative rate acceleration factors k_{rel}, defined as the ratio of the observed rate constant of the reaction in the presence of the catalyst and the rate constant of the non-catalyzed reaction, can be used to directly compare the activity of catalysts.

Reinhoudt and co-workers investigated the performance of calix[4]arenes 12–15 as enzyme mimics.

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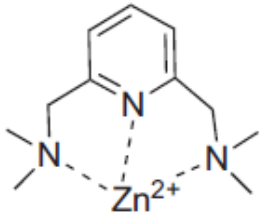
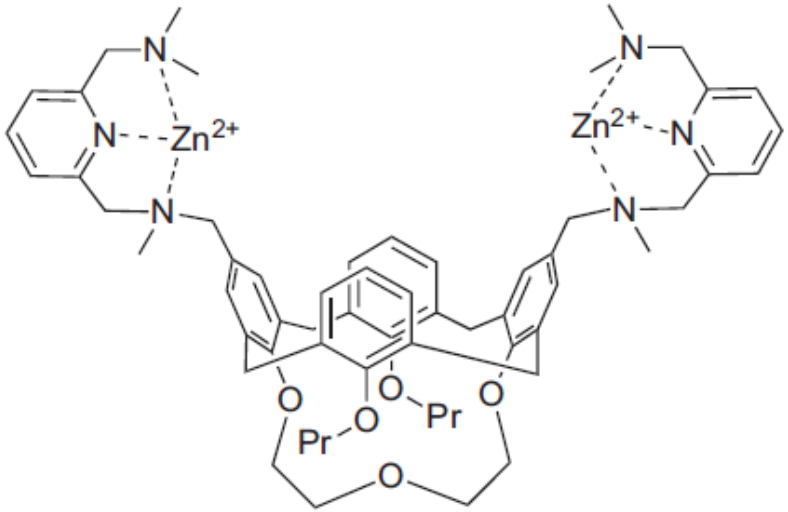
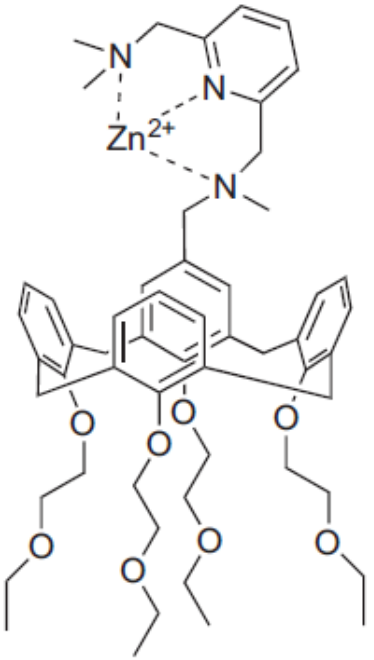


11, k_{rel} = 170

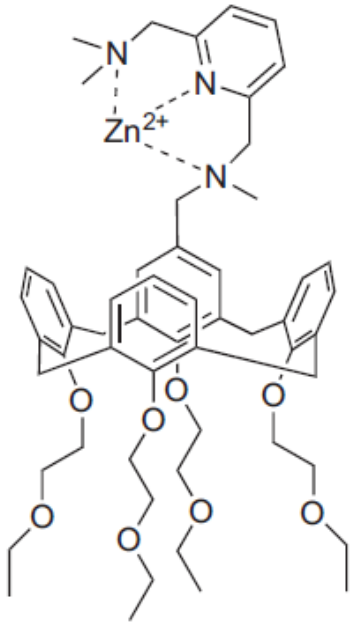


12, k_{rel} = 3000

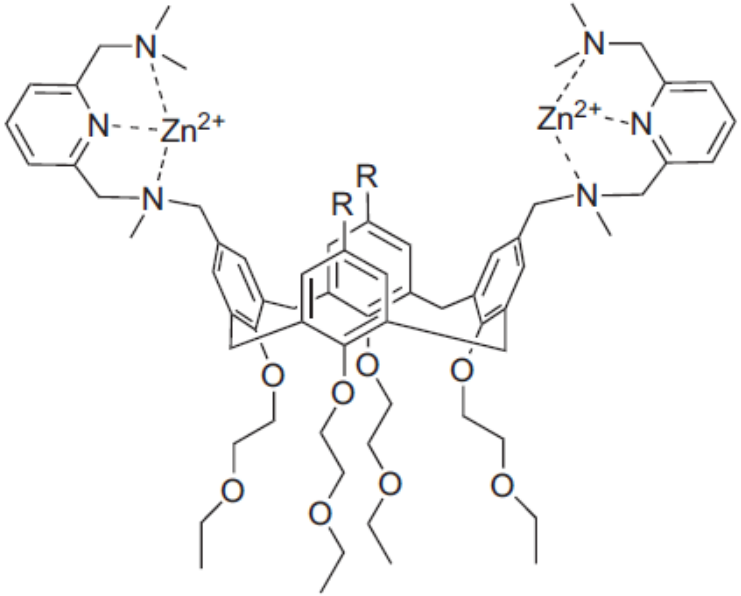


13, k_{rel} = 440

The parent system 11 has a much lower catalytic activity than its mononuclear calixarene based conjugate 13.
This indicates that the calixarene backbone is involved in the catalysis, probably by binding the substrate inside the hydrophobic pocket [35].



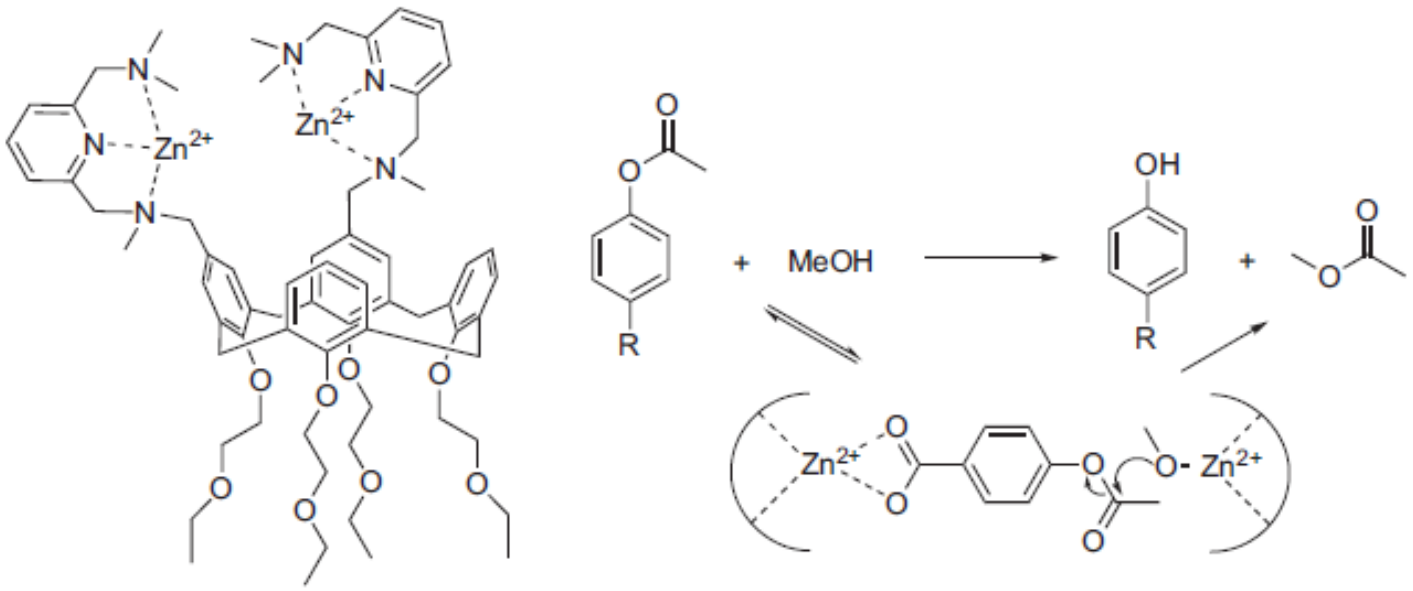
13, k_{rel} = 440



14a R = H, k_{rel} = 23000
14b R = CH_2NMe_2, k_{rel} = 7400

The dinuclear complex 14a shows a rate acceleration of 23,000 in the transesterification of HPNP over the non-catalyzed reaction, whereas the mononuclear 13 analogue is 50 times less active.
This suggests that the metal centers have cooperative action and that the hydrophobic cavity is involved in the binding of the substrate.

The ability of complexes 11, 14a, 15 and 16 to cleave carboxylic acid esters (esterase mimics) was investigated by solvolysis experiments in methanol.



16

17a R = H
17b R = COO^-

Fig. 12. Vicinal Zn^{2+} complex **16** and solvolysis reaction tested [36].

Acylase mimics

Many enzyme catalyzed transacylation reactions proceed via a
double displacement mechanism.
First, an acylated enzyme intermediate is generated, which then acts as acylation agent for the nucleophilic substrate [46]. This principle is well-known in organic chemistry, for example from the pyridine catalyzed esterification of activated carboxylic acids. In such reactions, first pyridine reacts with the acylation agent creating a highly active species that subsequently transfers the acyl group to an attacking nucleophile.

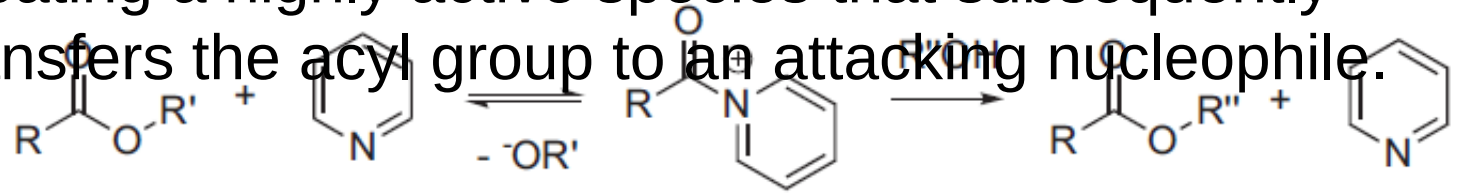


Fig. 15. Double displacement mechanism using pyridine as a catalyst.

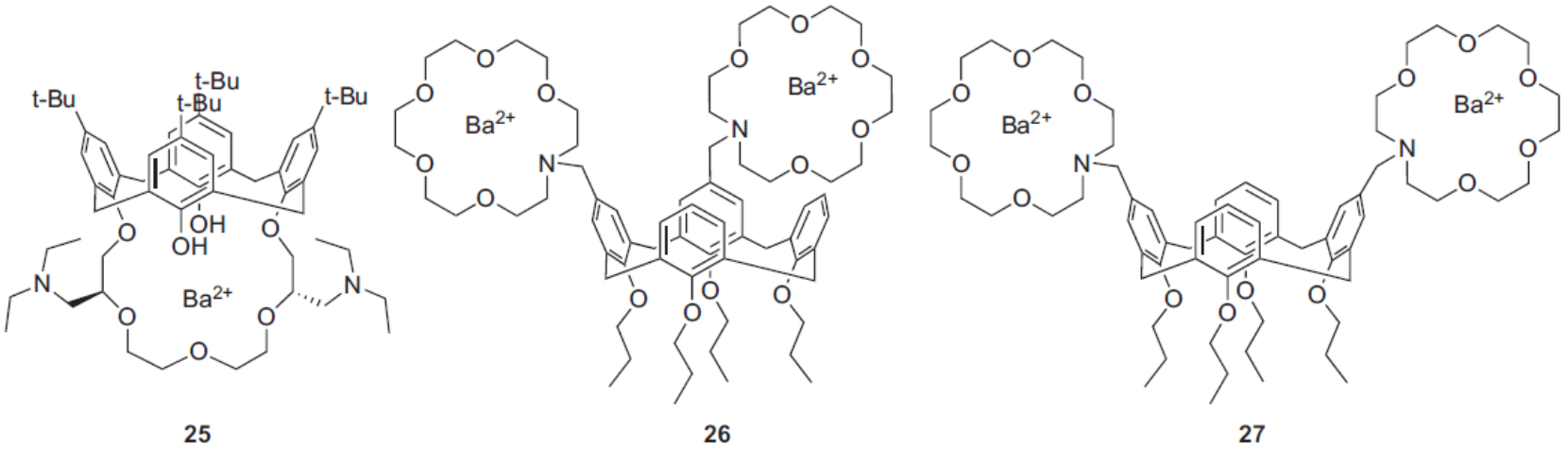
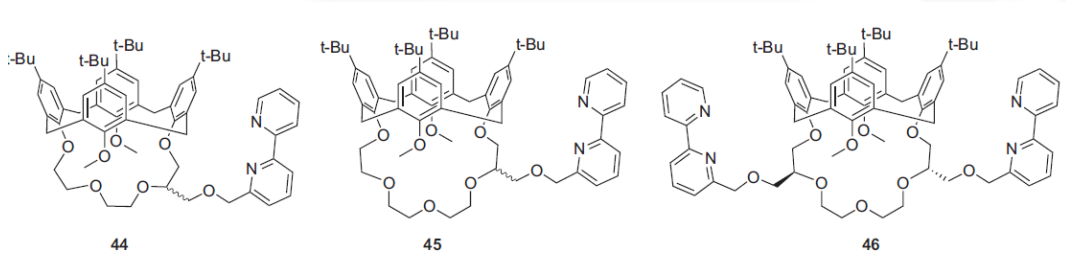


Fig. 16. Ba^{2+} containing artificial acyl-transferase **25** and acylases **26–27** [46,47].

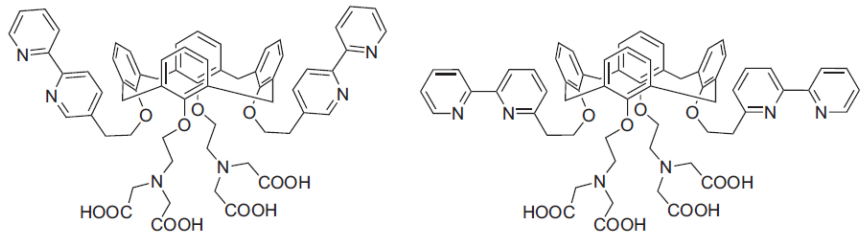
In mimics, the challenge is to achieve efficient transfer of the activated acyl to the nucleophile.
This can be realized by using phenolic alcoholates, which are better leaving groups than alkoxides. In calixarene 25, the phenolic groups can thus act as acyl-acceptors/acyl-donors, whereas the complexed Ba^{2+} acts as activating Lewis acid.

Calixarenes as luminescence agents for in vivo use



44 45 46

Fig. 27. Lanthanide chelators for luminescence studies[93].



47 48

Fig. 28. Strong chelators for luminescent lanthanide ions [94].

The lanthanides Eu3+ and Tb3+ are widely used as luminescent probes. Because of their toxicity, they need to be complexed by strong chelators. The complexation is also necessary to shield the
metal ions from coordination of water, which would quench the luminescence. Still not enough water soluble for in vivo studies

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MRI contrast agents

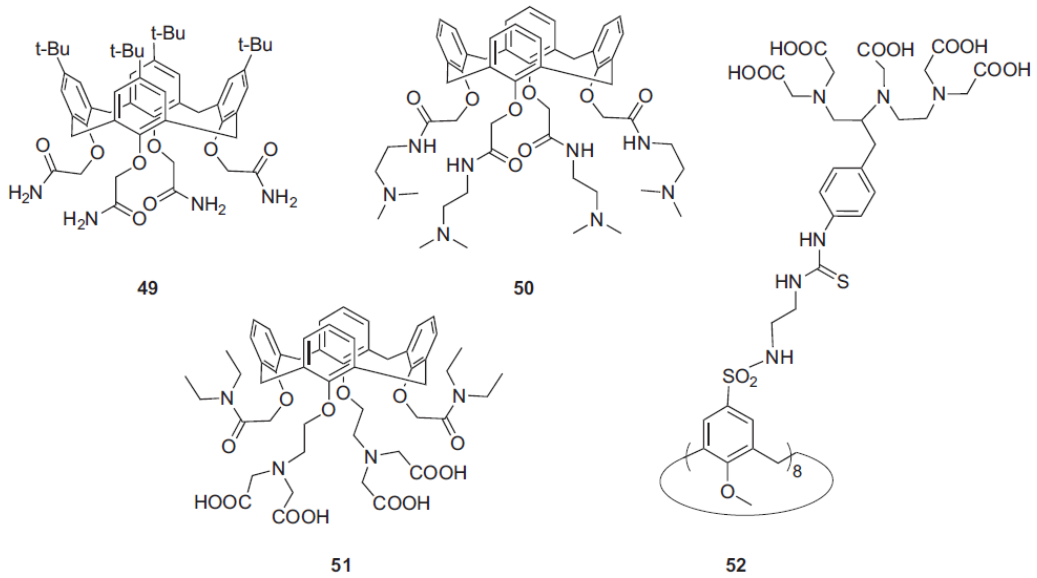

Fig. 29. Calixarene based chelators for MRI contrast agents [96,97].

In most clinical magnetic resonance imaging (MRI) scans, the 1H signal of water protons is observed and the image reflects either the proton density or differences in the longitudinal (T1) or transversal (T2) relaxation time of water protons in certain compartments of the body.

The majority of MRI contrast agents contain Gd3+-ions that are able to shorten the T1 relaxation times of surrounding water molecules very efficiently and lead to a brightening in MR images of areas that contain the agent.

Self-aggregating MRI contrast agents

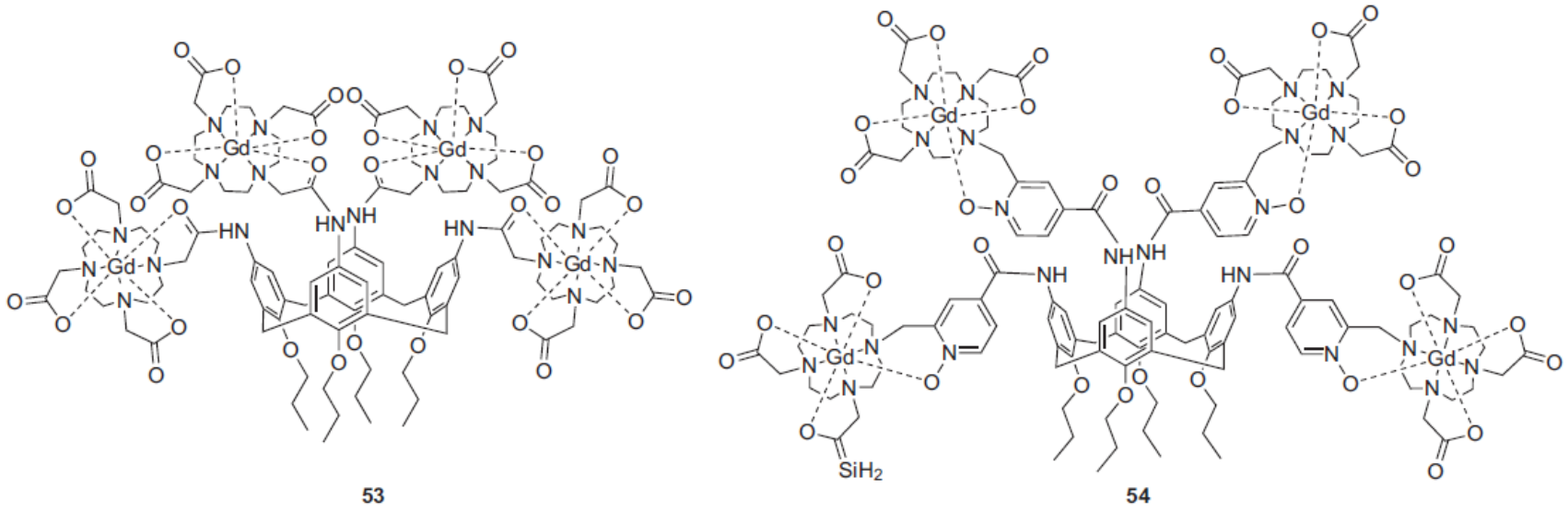

Fig. 30. Self-aggregating MRI contrast agents [100,101].