Peptido- and Glycocalixarenes: Playing with Hydrogen Bonds around Hydrophobic Cavities

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ABSTRACT

This Account reviews the synthesis, conformations, and supramolecular properties of calixarenes endowed with α-amino acids or peptides (Peptidocalixarenes) and carbohydrate units (Glycocalixarenes), with a major emphasis on calix[4]arenes functionalized on the aromatic nuclei (upper or wide rim). Most properties of N-linked peptidocalix[4]arenes are found to be quite different from those of the corresponding C-linked derivatives. An interesting example is the tendency of C-linked peptidocalix[4]arenes to form self-assembled nanotubes in the solid state. In several cases the hydrogen bonding donor and acceptor groups of the amino acid residues and the cavity of cone calix[4]arenes act cooperatively in guest binding in nonpolar solvents but not in water, where hydrophobic interactions dominate. Upper-rim bridged peptidocalix[4]arenes act as vancomycin mimics being able to bind D-alanyl-D-alanine (D-Ala-D-Ala) residues. Glycocalix[4]arenes show the phenomenon of multivalency in their binding to specific lectins, and those bearing thiourea spacers between the calix[4]arene scaffold and the sugar units are able to bind aromatic carboxylates and phosphates, making them attractive as novel site specific drug delivery systems.

Introduction

Calixarenes[1−3] are synthetic macrocycles derived from the condensation of phenols and formaldehyde which, depending on the number of aromatic units in the cyclic array and on the functionalization at the phenolic oxygen atoms (lower or narrow rim), possess a hydrophobic cavity able to encapsulate neutral molecules[4] or charge-diffused quaternary ammonium ions (Quats).[5] The introduction of suitable donor groups at the lower rim of calixarenes produces powerful and selective ionophores, particularly for spherical metal ions.[6] Extensively studied are the calix[4]arene podands (Figure 1, left) and the calix[4]crowns whose efficiency and selectivity are modulated by the conformational features of the calix[4]arene skeleton.[7] In addition to the lower rim, calixarenes have reactive para positions on the aromatic nuclei (upper or wide rim) which have also been used for anchoring binding groups, catalytic centers or other functional units. For example, the attachment of chelating units for Zn(II) and Cu(II)

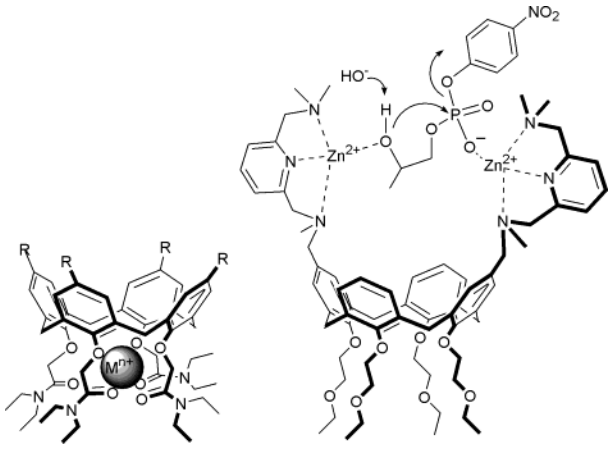

FIGURE 1. Lower rim, calix[4]arene podand for cation complexation (left); and upper rim, functionalized calix[4]arene as phosphodiesterase mimic (right).

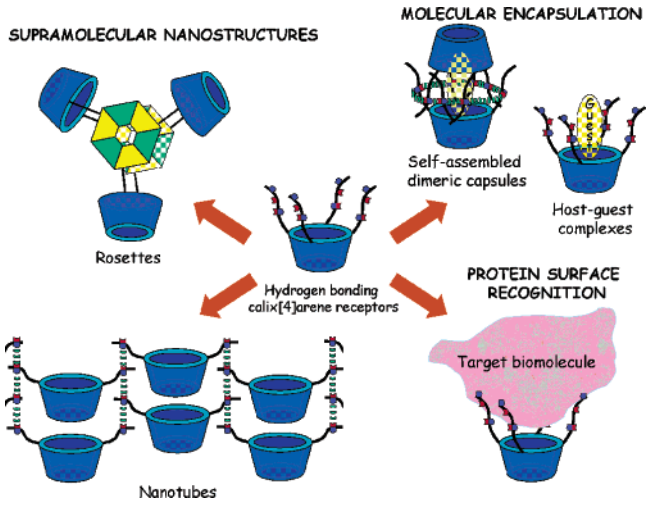

FIGURE 2. Hydrogen bonding calix[4]arene receptors in action.

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Rocco Ungaro was raised in the South of Italy (Potenza province) and studied Chemistry at the University of Parma, where he graduated in 1968 in the laboratory of Prof. Giuseppe Casnati. After a short stay in the pharmaceutical industry, in 1970 he returned to Parma University as lecturer, becoming associate professor in Organic Chemistry in 1982 and full professor in 1986. In 1974−1975 Prof. Ungaro was a postdoctoral fellow at the State University of New York (SUNY) in Syracuse (NY) in the laboratory of Professor Johannes Smid. He has pioneered the chemistry of calixarenes and is currently active in the use of these macrocycles in supramolecular and bioorganic chemistry. In 2002 he received the Research Prize of the Division of Organic Chemistry of the Italian Chemical Society for his studies on novel organic structures and molecular interactions.

Alessandro Casnati was born in 1963 in Milano (Italy). He obtained a Laurea in Chemistry in 1987 and his Ph.D. in 1991 from the University of Parma under the supervision of Prof. Rocco Ungaro. In 1990 he developed part of his Ph.D. program at the Twente University (The Netherlands) with Prof. David N. Reinhoudt. In 1994 he was appointed researcher and in 1998 associate professor of Organic Chemistry at Parma University. In 1997 he was awarded the "G. Ciamician" medal of the Italian Chemical Society. His research interests are in the field of supramolecular chemistry and particularly in the synthesis and study of molecular receptors for ions and neutral molecules.

Francesco Sansone was born in 1968 in Parma (Italy) and graduated in Chemistry in 1993 at Parma University, spending a period of the undergraduate thesis at the Twente University (NL) with prof. David N. Reinhoudt. In 1995 he developed part of his research on carbohydrates in the group of Prof. Alessandro Dondoni at Ferrara University (Italy) and in 1998 he received his Ph.D. in Organic Chemistry working under the supervision of Prof. Rocco Ungaro. Currently he is a researcher at Parma University, and his scientific interests are in the field of supramolecular chemistry, particularly addressed to the synthesis of calixarenes functionalized with amino acids and carbohydrates.

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metal ions at this rim allows the synthesis of polynuclear phosphodiesterase mimics which show high efficiency in the cleavage of P−O bonds (Figure 1, right).[8]

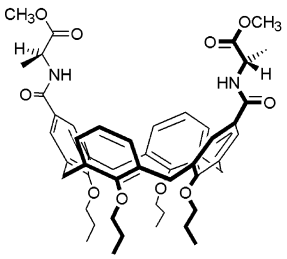
Although the complexation of metal ions continues to occupy a central place in calixarene chemistry,[1−3] more recent attention has also been devoted to the study of calixarenes adorned with hydrogen bonding donor and acceptor groups at the upper rim,[9] which are attractive for a number of reasons (Figure 2). They can function (i) as biomimetic receptors for the encapsulation of guest species of biological interest such as amino acids or carbohydrates,[9] (ii) as building blocks for the noncovalent synthesis of nanostructures[10,11] or self-assembled dimeric capules,[12,13] and (iii) as multivalent ligands capable of affecting biological processes.[14]

In this Account we will discuss a special class of calixarene-based hydrogen bonding receptors obtained by linking α-amino acids and peptide units (peptidocalixarenes) or sugar moieties (glycocalixarenes) to calixarenes. We will mainly concentrate on upper rim calix[4]arene derivatives and only mention, when relevant, lower rim derivatives and hosts obtained from related macrocycles such as resorcinarenes, derived from resorcinol.

Peptidocalix[4]arene Podands

In biological systems the cooperative action of peptide hydrogen bonds plays an important role in organization, assembly, and molecular recognition processes.[15] On the other hand, specially engineered synthetic peptides are able to assemble into ordered nanotubes or other supramolecular architectures[16] or act as hosts for a variety of guest molecules.[17]

The conjugation of α-amino acids or peptides to calixarenes can be performed through the terminal amino or carboxylic groups. We have explored both possibilities synthesizing N-linked[18] and the C-linked[19] peptidocalix-



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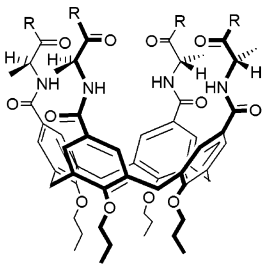
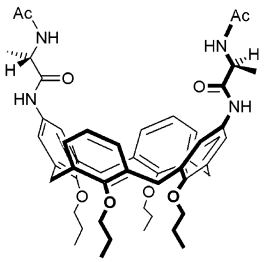


2 R = OCH_3
8 R = O^-



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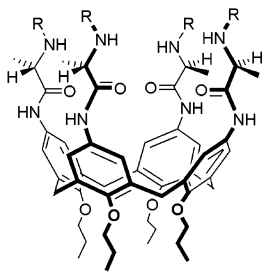


4 R = H
7 R = Ac

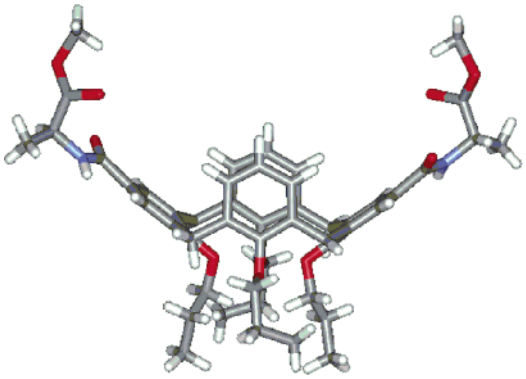


FIGURE 3. X-ray crystal structure of compound **1**, showing the open flattened cone conformation.

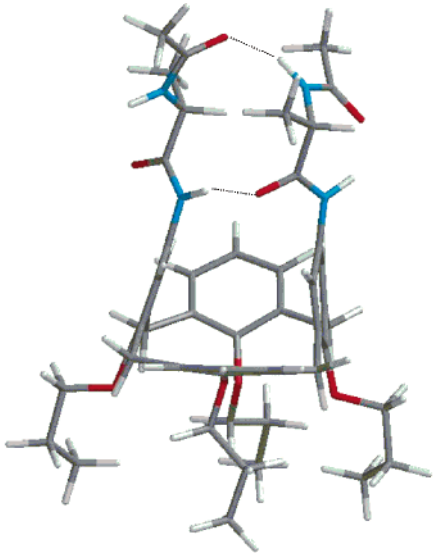


FIGURE 4. Energy-minimized structure obtained by molecular modeling of compound **3** showing the closed flattened cone conformation.[19]

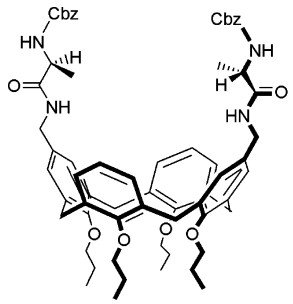
[4]arene podands having two or four amino acid (or dipeptide) units on the upper rim.

In designing these two classes of receptors, we anticipated that they would show different supramolecular properties which was, in fact, the case. The first difference between N- and C-linked peptidocalix[4]arenes appears in the conformational properties of simple difunctionalized L-alanyl derivatives **1** and **3**. Compound **1** (*N*-linked) shows solvent independent ^{1}H NMR spectra with no evidence of intramolecular hydrogen bonding in solution, and this is also in agreement with its X-ray crystal structure (Figure 3).[18]

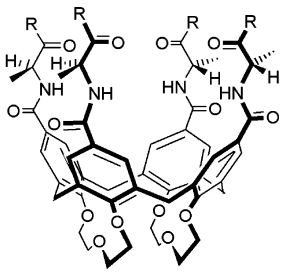
On the contrary, the ^{1}H NMR spectra of the C-linked compound **3** in different solvents very clearly demonstrate the shift from a closed to an open flattened cone conformation on switching from an apolar to a dipolar solvent.[19] This was also supported by molecular modeling studies (Figure 4) which show the closed structure to be stabilized by two strong intramolecular H-bonds between the facing amino acid units.

The two series of receptors also have different self-assembly properties in the solid state, as shown by

comparing the X-ray crystal packing of compound **1** and of the C-linked derivative **5**.[20]



5



6 R = OCH_3
9 R = O^-

In fact, although the C-linked difunctionalized receptor **5** exists in a closed flattened cone conformation in $CDCl_3$, its X-ray crystal structure shows two independent conformers, which are both in an open flattened cone conformation. This rather unexpected result is evidently due to solid-state organization and indicates, once again, that X-ray crystal structures do not always represent the conformational features of organic compounds in solution. Interestingly, the perspective view of the crystal lattice of compound **5** along the *b* axis of the cell reveals the formation of self-assembled nanotubes (Figure 5). They are formed through the cooperative action of several intermolecular hydrogen bonds and not as a consequence of simple van der Waals interactions or packing forces, as observed for the N-linked derivative **1**[20] or other functionalized calix[4]arenes.[21,22]

A closer look to the X-ray crystal structure of compound **5** reveals that in the *a*,*b* plane each calixarene macrocycle is connected by a two-dimensional network of hydrogen bonds (Figure 6, left) involving the H-bonding donor (NH) and acceptor (C=O) groups of each upper rim substituent. This interlocked two-dimensional array of calixarenes, which leads to the formation of the nanotubes, is schematized in Figure 6 (right).

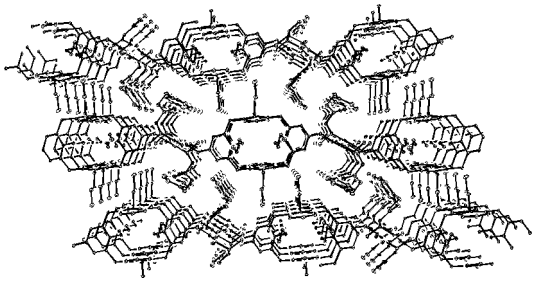


FIGURE 5. Perspective view of the crystal lattice of **5** along the *b* axis of the cell.

N- and C-linked peptidocalix[4]arene podands also behave quite differently in their molecular recognition properties. In fact, both the di- and the tetrafunctionalized receptors **1** and **2** are able to complex carboxylic acids and ammonium cations but not anionic guests. Figure 7[23] reports the association constants in $CDCl_3$ of selected guest species with tetra-L-alanine methyl ester derivatives **2** and **6**, the last one being a conformationally locked analogue of **2**, obtained by functionalization of the rigid cone calix[4]arene platform.[24]

The data indicate that (i) both ligands show shape selectivity in the recognition of primary alkylammonium cations, with linear species showing the strongest binding, (ii) α-branching introduces a steric hindrance to binding, unless H bonding acceptor groups are present in the guest molecules, (iii) the rigidified host **6** is less efficient compared to **2**, which possesses a residual conformational mobility between two C_2 symmetric flattened cone conformations,[25] and (iv) anionic guests are not complexed by either hosts. All available data indicate that the amide carbonyl groups close to the calixarene cavity (and not the terminal ester groups of the hosts) are involved in the complexation of RNH_3^+ cations and that the carbonyl group of the C-protected amino acid guest can interact with an NH group of the host, partially overcoming the steric repulsion introduced through α-branching (Figure 8).

On the contrary, neither the di- (**3**) or the tetrafunctionalized (**7**) C-linked peptidocalix[4]arenes complex primary ammonium cations, but they weakly bind carboxylate anions.[19]

Basic hydrolysis of compounds **2** and **6** produces the corresponding water-soluble N-linked peptidocalix[4]arenes **8** and **9** which were also investigated for their molecular recognition properties.[24] In aqueous solution hydrogen bonding does not play an important role, and guest encapsulation in peptidocalix[4]arenes **8** and **9** is mainly controlled by hydrophobic interactions. Amino

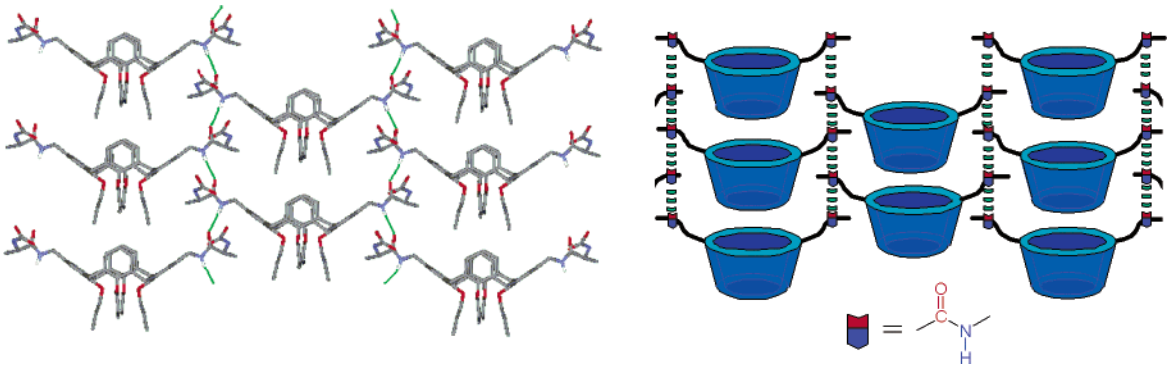


FIGURE 6. H-bonding pattern in the crystal lattice of compound **5** (left), where benzyl groups were omitted for clarity, and its schematic representation (right).

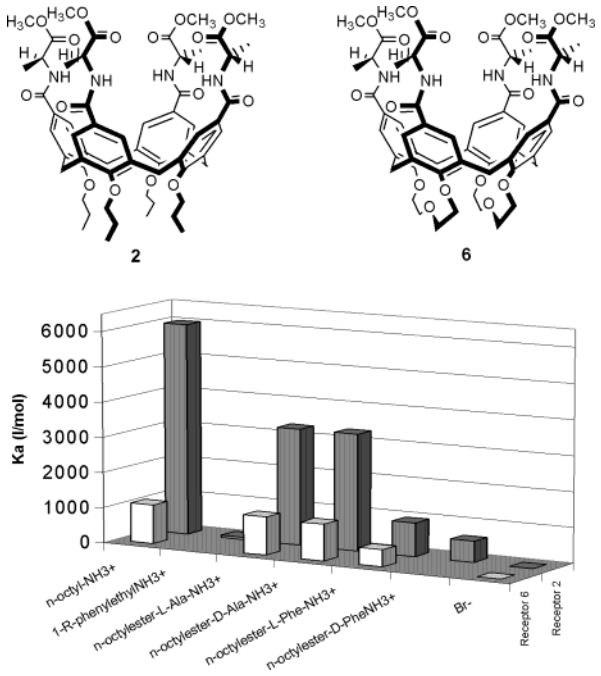


FIGURE 7. Association constants (M^{-1}) with receptors **2** and **6** determined in $CDCl_3$. All ammonium guests were used as bromide salts, except for 1-R-phenylethylammonium, which was used as tetraphenyl borate. Bromide was used as the tetrabutylammonium salt.

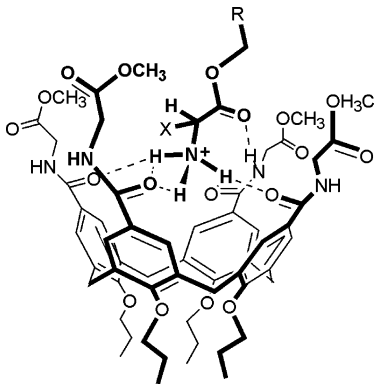


FIGURE 8. Proposed mode of binding of α-amino acid ester derivatives by N-linked tetraalanine methyl ester **2**.

acid methyl esters are better bound than native amino acids, and in both cases, it is the apolar group of the guest which enters the aromatic cavity of the calixarene host. Aromatic α-amino acids are more strongly complexed than aliphatic ones and the selectivity follows the order L-Trp ≈ D-Trp > L-Phe > L-Tyr > L-Ala > Gly. Primary alkylammonium cations are not bound in water, whereas quaternary ammonium cations (Quats) are complexed, with selectivity toward the tetramethylammonium cation. Contrary to the results obtained in $CDCl_3$ for hosts **2** and **6**, the rigid cone receptor **9** is more efficient than the mobile cone **8** in aqueous solution, showing association constants one or two orders of magnitude higher, with all guests examined. From this we learned the important lesson that host rigidity, which has been successfully pursued in designing calixarene-based hosts for spherical metal ions,[6] neutral molecules,[4] and Quats,[5] is detrimental

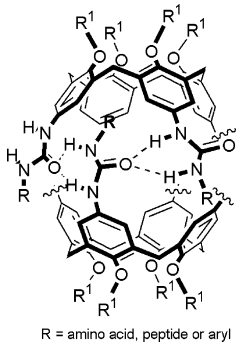


FIGURE 9. Schematic representation of a calix[4]arene dimeric capsule.

when playing with highly directional hydrogen bonding.[26] Protonation of the four terminal NH_2 groups in compound **4** leads to a positively charged, water-soluble receptor[19] which represents the cationic counterpart of the anionic N-linked tetraalanine derivative **8**. The host−guest and aggregation properties of this compound have not been yet investigated. Very recently it has been claimed that compound **4** is able to form hydrogen-bonded self-assembled capsules in polar, protic solvents although the evidence provided is not very strong.[27]

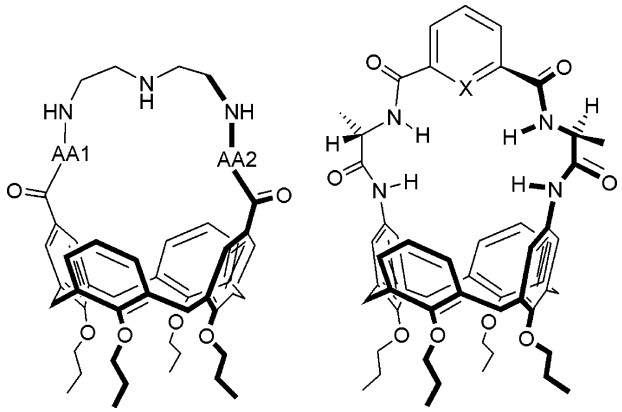
In closing this section, we must briefly mention some related work by other groups regarding calix[4]arenes functionalized with α-amino acid or peptide units at both rims. When four amino acid groups are linked to the calix[4]arene upper rim via the urea spacer, hydrogen bonded, dimeric chiral capsules are formed (Figure 9),[28−30] thanks to the high tendency of the tetraureidocalix[4]arenes to form such supramolecular architectures in apolar solvents.[13] Interestingly, the amino acids on the urea functions have a dramatic effect on the dimerization behavior of the calix[4]arenes since they lead to a preferential hetero-dimerization with calixarenes substituted with aryl ureas, rather than with themselves.[29] Evidence for enantioselective binding of chiral guests inside the chiral capsule was obtained, thus proving that chiral information can be transferred through noncovalent molecular assembly. If a dipeptide containing two α-amino acids of alternating L- and D- configuration (^{L}Leu-^{D}LeuOMe) is used, the calix[4]arene ureidopeptide forms a homodimeric capsule, thanks to the cooperative action of the classical urea-urea interactions and peripheral hydrogen bonds involving the terminal ester groups.[30]

A new family of lower rim cone peptidocalix[4]arene podands, which act as synthetic models for the selectivity filter of the potassium channel from *Streptomices lividans* (KcsA), was recently reported by Lippard and co-workers.[31] The peptide amide functions interact through interchain hydrogen bonds in the free ligands, as previously observed in primary and secondary calix[4]arene amides[32] or in other calix[4]arene amino acid podands.[33] Addition of Na^+ or K^+ breaks the hydrogen bonds leading to metal ion complexation by the amide carbonyl groups and the calixarene oxygen atoms. This H-bonding/M^+-binding switching mechanism could result in a reduction in the strength of metal ion complexation and constitute a

thermodynamic driving force for ion translocation in the model system which could partly explain the same phenomenon occurring in the natural selectivity filter.

Bridged N-Linked Peptidocalix[4]arenes

The vancomycin group of antibiotics is an interesting class of biologically active molecules whose action takes place through a relatively simple molecular recognition process.[34] They are able to bind the terminal L-lysyl-D-alanyl-D-alanine (L-Lys-D-Ala-D-Ala) sequence of the cell wall mucopeptide precursors of Gram-positive bacteria, thus inhibiting the growth of the cell wall and causing cell lysis. The molecular basis of the mode of action of this class of antibiotics has been elucidated by studying the complexation of vancomycin with N-Ac-D-Ala-D-Ala. From all structural data available, it appears that the dipeptide is kept inside the hydrophobic pocket of the natural receptor, by a combination of hydrogen bonds and hydrophobic interactions which act cooperatively. To mimic the vancomycin mode of binding, we designed a series of calix[4]arene macrobicyclic ligands **10** which belong to the class of the upper rim N-linked peptidocalix[4]arenes.[35] They contain a pseudo peptide bridge in 1,3 positions at the upper rim of a cone calix[4]arene, consisting of α-amino acids (AA1 and AA2) of different structure and configuration linked through a 1,3,5-diethylenetriamine spacer. In this way, the hydrophobic cavity of the calix[4]arene could eventually cooperate with the hydrogen bond donor and acceptor groups in the guest binding.



10 **11** X = CH **12** X = N

The minimum inhibitory concentrations (MIC μg/mL) of these macrobicyclic compounds against several Gram-positive bacterial strains indicate (Figure 10) that the biological activity increases as the steric hindrance of the lateral residues of the amino acids decreases. The L-Ala,L-Ala derivative (**10**, AA1 = AA2 = L-alanine) shows activity close to that of vancomycin and quite similar to that of the Gly,Gly derivative.

Increasing the length of the bridge by using the L-Ala-L-Ala dipeptide instead of a simple L-Ala causes a remarkable drop in activity, while protecting the central NH group with a Boc or substituting it with a methylene group completely inhibits the biological activity of the macrobicyclic compounds.[35] Interestingly, the active compounds show a behavior very close to that of the vancomycin: no activity is observed against gram-negative bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*) or cell wall lacking bacteria (*Acholeplasma laidlawii*). This seems to indicate that the biological target of this class of calixarene-based antimicrobials is, as for vancomycin, the terminal D-Ala-D-Ala part of the peptidoglycan. This hypothesis was confirmed by binding studies using NMR and ESI-MS tecniques.[35−37] These show the formation of a 1:1 complex between the L-Ala,L-Ala receptor and N-Ac-D-Ala-D-Ala as well as with simple α-amino acids or carboxylic acids. The stability of the complexes increases in $CDCl_3$ from lauric acid (log K_a = 3.0) to N-lauroyl-D-Ala (log K_a = 4.1) or N-lauroyl L-Ala (log K_a = 4.05) and to N-lauroyl-D-Ala-D-Ala (log K_a > 5). A similar binding order (lauric acid > N-lauroyl-D-Ala > N-lauroyl-D-Ala-D-Ala) was also observed in MS-MS experiments.[37] NMR diffusion studies[36] performed in $CDCl_3$ + 3% DMSO-d_6 show that the N-Ac-L-Ala-L-Ala dipeptide is bound more strongly (log K_a = 3.4) than the simple amino acid derivative N-Ac-L-Ala (log K_a = 2.4), by the host L-Ala,L-Ala. These results indicate that a proton transfer from the carboxylic acids of the guest to the amino group of the host is taking place followed by the formation of a salt bridge, which seems to give the most important contribution to the binding, at least in $CDCl_3$. The presence of additional hydrogen bonding donor and acceptor units in the amino acid or dipeptide guests increases the association constants by one order of magnitude for the former and at least two

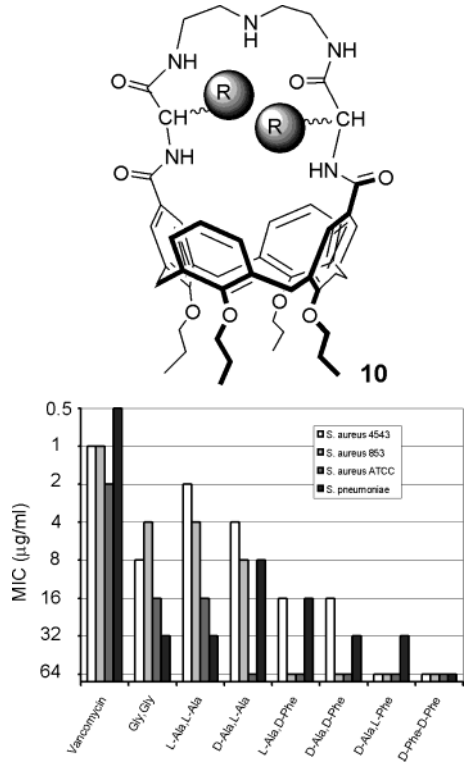


FIGURE 10. Minimum inhibitory concentration (MIC, μg/mL) of macrobicyclic peptidocalix[4]arenes **10** and vancomycin, showing the dependence of antimicrobial activity for **10** on the steric hindrance of R groups (Gly, R = H; Ala, R = CH_3; Phe, R = $CH_2C_6H_5$).

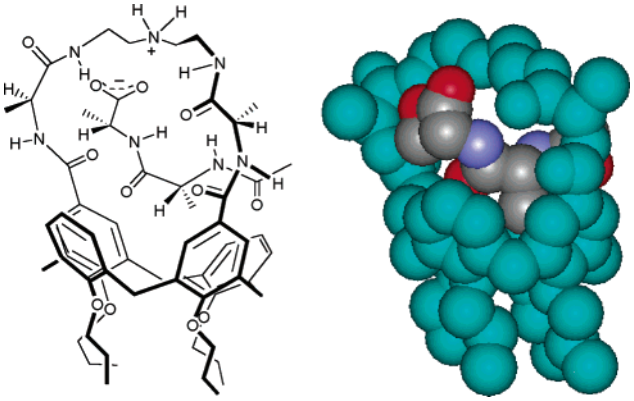


FIGURE 11. Proposed mode of binding of *N*-acetyl-D-Ala-D-Ala by receptor **10** (AA1 = AA2 = L-alanine).

for the latter. On the basis of the experimental data, we proposed the structure reported in Figure 11 for the complex between N-Ac-D-Ala-D-Ala and the calixarene-based vancomycin mimics **10**. The peptide guest is threaded under the pseudopeptide bridge where the electrostatic interaction between the ammonium ion and the carboxylate anion stabilizes the complex together with additional H-bonds between the NH donor and CO acceptor units of host and guest. We cannot rule out that additional stabilization of the complex (especially in aqueous solution) could derive from the insertion of one of the CH_3 groups of the D-Ala-D-Ala guest into the hydrophobic cavity of the peptidocalix[4]arene host **10**.

Bridged C-Linked Peptidocalix[4]arenes

Cleft-like C-linked peptidocalix[4]arenes **3**, **5**, and **7** were shown to be poor receptors for anions mainly because of their tendency to form extensive inter- and intramolecular hydrogen bonds (vide supra). To reduce the conformational flexibility of the difunctionalized hosts, we introduced rigid aromatic spacers between the two amino acid units thus obtaining the isophthalic (**11**) and pyrido (**12**) derivatives.[38] The X-ray crystal structure of **12** (Figure 12) shows that two molecules of acetone are coordinated to the host through three hydrogen bonds, one with a calixarene NH and two with the alanine NH groups. We reasoned that, possibly, the acetone molecules could be replaced by suitable guest species.

Exploratory binding studies performed by ESI-MS showed a preference of ligands **11** and **12** for anionic guests. In all cases a 1:1 stoichiometry was established for the complexes. Competitive experiments carried out with **12** and a mixture of inorganic and carboxylate anions (as tetrabutylammonium salts) indicated the selectivity order benzoate > p-MeO-benzoate > N-Ac-Phe-COO^- > N-Ac-Ala-COO^- > Cl^- ≫ NO_3^- > $H_2PO_4^-$. Solution studies performed in acetone-d_6 by ^1H NMR confirm the high selectivity for Y-shaped carboxylate anions and show no interaction with the corresponding carboxylic acids. The significant downfield shifts experienced in all cases by the amide NH protons indicate that complexation is mainly due to H bonding of the anions with the amide NH groups of the receptors. The data obtained with ligand **12** (Figure

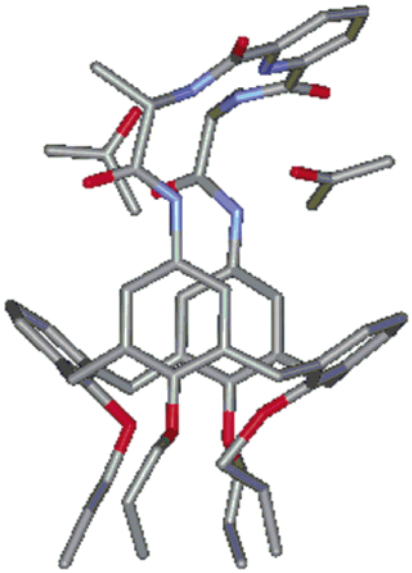
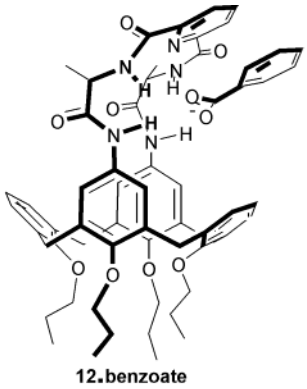


FIGURE 12. X-ray crystal structure of peptidocalix[4]arene **12**.



12.benzoate

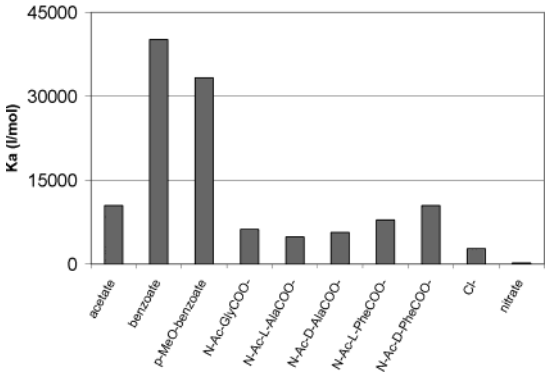


FIGURE 13. Binding constants (Ka, M^{-1}) for the 1:1 complexes of ligand **12** and anionic substrates (as tetrabutylammonium salts) in acetone-d_6.

13) show a selectivity for carboxylate anions having aromatic nuclei in their structure (benzoate > acetate, N-Ac-Phe-COO^- > N−Ac−Ala-COO^-) and only a modest enantioselectivity for the D series of amino acids.

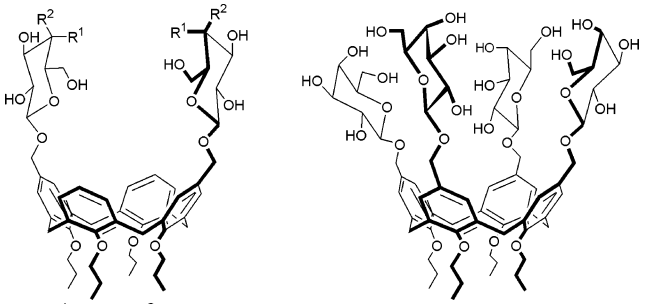
Compared to the cleft-like C-linked peptidocalix[4]-arene **3**, which presents a $K_a = 375\ M^{-1}$ for the benzoate anion in acetone-d_6, the more preorganized ligand **12** shows an increase in the association constant by 2 orders of magnitude. This is remarkable, considering that the H-bonding donor groups in neutral receptor **12** are simple amides of amino acids. Of particular interest is the

preference shown by ligand **12** for benzoate over acetate despite the higher basicity of the latter. This could indicate that, besides the most important $COO^{-}\cdots HN$ hydrogen bonding, additional stabilization of the complexes with benzoate and N-Ac-Phe-COO^{-} may result from $\pi-\pi$ stacking interaction between the aromatic nuclei of the guest and the pyridine or phenolic units of the host.

Glycocalix[4]arenes

For a long time, it was considered that carbohydrates played only two major roles in biological systems. First as a storage medium for chemical energy, and second as structural elements of cellular membranes and nucleic acids. This view has drastically altered in the last two decades however, as it has been discovered that carbohydrates act as substrates for specific receptors in a wide range of biological processes.[39,40] In fact, intercellular communication, cell trafficking, immune response, pathogenesis of infections by bacteria and viruses, and growth and metastasis of tumor cells all occur thanks to the binding of sugar residues at cell surfaces by saccharide receptors.[41,42] Since the affinity of a single carbohydrate unit for its receptor is usually low, the strong binding observed in these recognition events is determined by the simultaneous complexation of several identical glycoside residues, exposed at the substrate surface, by receptors (often proteins) bearing several equivalent binding sites. This phenomenon has been named multivalency,[40] or glycoside cluster effect,[43] and has inspired the synthesis of a variety of polyglycosylated multivalent ligands able to mimic the biological ligands and their functions.[44−46] Calixarenes, because of their oligomeric nature and shape, which can be controlled by the size of the macrocycle and by the substituents on the lower rim, are attractive in this context since they can act as cores for linking identical carbohydrate units and obtaining multivalent systems. Furthermore, the calix[4]arenes blocked in the cone conformation can, to some extent, mimic a small portion of the cell surface presenting a series of glycosylated residues on the exterior of a lipophilic cavity. Moreover, one could, in principle, use the cavity and the spacer between the calixarene upper rim and the sugar units to cooperatively bind suitable guest molecules. In this way complexes could be directed toward selected biological targets which recognize the carbohydrate units, with the glycocalix[4]arenes acting as a novel type of site directed molecular delivery system.

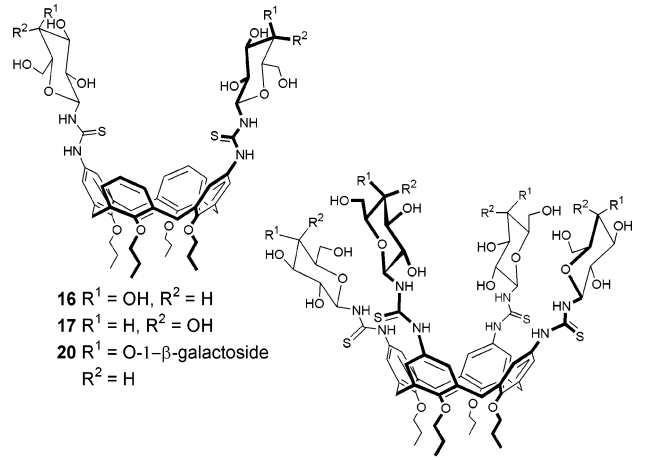
In 1994, in collaboration with Dondoni's group, we reported the first examples of lower- and upper-rim glycocalixarenes (called calixsugars)[47] and the early work in this field has recently been reviewed by Fulton and Stoddart.[48] For upper-rim cone glycocalix[4]arenes, the major problem has been the development of efficient synthetic methodologies for linking the sugar units to the aromatic rings of the macrocycles. We originally started from the bis- and tetrahydroxymethyl derivatives of the tetrapropoxycalix[4]arene, and using benzoyl protected thioethyl-β-galactoside and thioethyl-β-lactoside as glycosyl donors, we synthesized the bis- and tetraglycocalixarenes **13**−**15**.[47,49] The presence of the benzoyl protecting group at C-2 of carbohydrates favored the total β selectivity in their coupling to the calix[4]arene scaffold.



13 R^1 = OH, R^2 = H
15 R^1 = H, R^2 = O-1–β-galactoside

14

Although compounds **13** and **14** were obtained in good yields (60−65%), this procedure did not give good results with the more hindered lactoside as the glycosyl donor, whose conjugates were obtained in ca. 25% yields. Moreover, the host−guest properties of these glycocalix[4]arene prototypes were quite poor, discouraging further studies. Better results were obtained with the thiourea linked glycocalix[4]arenes **16**−**19**.[50] These were synthesized through a condensation reaction between the di- and tetraamino calix[4]arenes and the β-isothiocyanate of the tetraacetyl-gluco- and galactoside, followed by deprotection (75−80% overall yield). Thiourea represents an interesting spacer group because it is usually obtained in high yield and the acidity of its protons makes it a potential binding group for anions and for neutral molecules with hydrogen bonding acceptor groups. Using the heptaacetyl-β-aminolactoside and the diisothiocyanate-calix[4]arene, it was also possible to synthesize the bisthioureido-lactosyl calix[4]arene **20** in 45% yield.



16 R^1 = OH, R^2 = H
17 R^1 = H, R^2 = OH
20 R^1 = O-1–β-galactoside
R^2 = H

18 R_1 = OH, R_2 = H
19 R_1 = H, R_2 = OH

ESI-MS and ^{1}H NMR experiments reveal that both di- and tetrathiourea-linked glucocalixarenes **16** and **18** preferentially bind to guests having an anionic headgroup (carboxylates, phosphates) and an aromatic tail. For example, in a very competing solvent such as DMSO-d_6,

the binding order for receptor **16** is benzyl phosphonate ($Ka = 170\ M^{-1}$) > benzoate ($103\ M^{-1}$) > $H_2PO_4^-$ ($90\ M^{-1}$) > Cl^- ($31\ M^{-1}$) > acetate ($17\ M^{-1}$). The observed selectivity order suggests that, in addition to hydrogen bonding, sugar−aromatic interactions[51] could stabilize the complexes. The tetrafunctionalized receptors **18** and **19** also show the phenomenon of selective multivalency in their binding with two lectins, concanavalin A (Con A) and peanut lectin (PNA, *Arachis hypogaea*). Through a simple, qualitative turbidimetric analysis, we were able to show that the tetraglucosyl derivative **18** causes microprecipitation of Con A, which is a glucose and mannose binding protein. The multivalent interaction of glycocalix[4]arene **18** with several Con A molecules causes cross-linking and Con A agglutination. No microprecipitation was observed with the galactose binding lectin PNA, while a large excess of glucose partially inhibited the agglutination of Con A by **18** demonstrating the binding specificity. Similar behavior was shown by the tetragalactosyl derivative **19** toward PNA.

Interesting examples of upper rim polyglycosylated resorcinarenes were reported in recent years by Aoyama and co-workers.[52,53] The ability of these compounds to show the cluster glycoside effect was demonstrated by adhesion experiments to glass surfaces,[52] surface plasmon resonance[54] and turbidimetric measurements in the presence of proteins.[53] Evidence of saccharide directed molecular delivery performed by these macrocyclic sugar clusters was reported toward lectins[53] and, more significantly, toward hepatocytes.[55]

Conclusions and Outlook

The introduction of hydrogen bonding donor and acceptor groups at the lower and upper rims of calixarenes has profoundly affected their chemico-physical, self-assembly, and host−guest properties and opened new horizons for a more extensive use of calixarenes in supramolecular and bioorganic chemistry. This also applies to the peptido- and glycocalixarenes which are chiral, soluble in polar solvents and even in water, and therefore quite attractive for biomolecular recognition studies. The results so far obtained for peptidocalix[4]arenes have shown that the α-amino acid or peptide chains at the upper rim can serve the purpose of organizing the macrocyclic cavities into well-defined supramolecular structures which, in some cases, resemble self-assembled nanotubes, or can be used as binding units for the biomimetic recognition of polar guests. Although the amide NHs are not very strong hydrogen bonding donor groups, once they are cooperatively assisted by other weak noncovalent interactions such as CH−π or $\pi-\pi$ stacking, the binding of (e.g.) aromatic carboxylate anions or dipeptides can be quite strong. The ability of a few selected peptidocalix[4]arenes to bind N-acetyl-D-alanyl-D-alanine, used as a model for the terminal part of the peptidoglycan of the Gram positive bacterial cell wall, renders these compounds biologically active in vitro and attractive as vancomycin mimics. Since the cone calix[4]arenes can be viewed as easily available U-turn inducers if incorporated into selected peptide chains, their use in the synthesis of novel peptidomimetics can be easily envisaged. The study of glycocalixarenes is still in its infancy and certainly less advanced than that of peptidocalixarenes and of cyclodextrin-based neoglycoconjugates.[45] However, considering the large variety of carbohydrate structures available, the many calixarene scaffolds described in the literature and the numerous biological recognition phenomena in which sugars are involved, the interest in calixarene-based neoglycoconjugates should increase in years to come. So far the studies have mainly concerned calix[4]arenes and resorcin[4]arenes with the major aim of exploring and tuning synthetic methodologies for the efficient linking of carbohydrates to calixarene scaffolds. An attractive perspective is to also use calix[6]- and calix[8]arene derivatives, which have larger cavities and a higher number of reactive groups for the attachment of sugar units. Apart from the opportunity of offering new molecular architectures for studying protein-carbohydrate and carbohydrate-carbohydrate interactions, the calixarene-based neoglycoconjugates, together with the cyclodextrins, are also attractive novel site-directed drug delivery systems.

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