CHEMISTRY
A EUROPEAN JOURNAL

Water-Soluble Molecular Capsules: Self-Assembly and Binding Properties

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Abstract: The self-assembly and characterization of water-soluble calix[4]arene-based molecular capsules (**1·2**) is reported. The assemblies are the result of ionic interactions between negatively charged calix[4]arenes **1a** and **1b**, functionalized at the upper rim with amino acid moieties, and a positively charged tetraamidiniumcalix[4]arene **2**. The formation of the molecular capsules is studied by ^{1}H NMR spectroscopy, ESI mass spectrometry (ESI-MS), and isothermal titration calorimetry (ITC). A molecular docking protocol was used to identify potential guest molecules for the self-assembled capsule **1a·2**. Experimental guest encapsulation studies indicate that capsule **1a·2** is an effective host for both charged (*N*-methylquinuclidinium cation) and neutral molecules (6-amino-2-methylquinoline) in water.

Keywords: docking · host–guest systems · ionic interactions · molecular capsules · self-assembly

Introduction

Nature is the main source of inspiration when looking for novel synthetic systems with functional properties. Particularly interesting architectures are the molecular containers obtained from the self-assembly of preorganized scaffolds,[1] resembling the shape of natural shell-like containers such as viruses and ferritin. Beside their fascinating design, the possibility to isolate molecules or ions of different size and shape in the interior of their cavities makes noncovalent molecular capsules very attractive. Thus far supramolecular capsules have found applications in binding, separation, and sensing of small molecules and ions,[2] stabilization of reactive intermediates, and catalysis.[3–6] However, one of the most challenging objectives of supramolecular chemistry is the synthesis of supramolecular capsules for medical purposes (like storage of molecules and drug delivery)[7–12] or biological model studies (for example, the mimicry of the catalytic efficiency of enzymes).[13] Needless to say that to achieve these aims water solubility represents an essential requirement. Water soluble noncovalent molecular cages have been obtained mainly by metal–ligand interactions,[1c,14] although few examples of molecular capsules based on hydrogen bonds[15] soluble in wet solvents have been reported. Ionic interactions,[16] employed as an important attractive noncovalent force both in biological and artificial molecular recognition, have been only marginally used for building molecular capsules.[17] Previously, we have reported the formation of stable molecular capsules based on ionic interactions between a tetrasulfonate and several tetraamidiniumcalix[4]arenes.[18] Although the 1:1 complexes were found to be soluble in methanol and in mixtures of methanol/water (up to 40% of water) precipitation was observed upon increasing the percentage of water. Only recently a water-soluble molecular capsule based on ionic interactions has been obtained when one of the calix[4]arene building blocks was functionalized with four L-alanine moieties.[19]

Here we describe a study on the synthesis and characterization of water-soluble calix[4]arene-based molecular capsules **1·2**. The formation of the assemblies **1a·2** and **1b·2** is the result of the ionic interactions between the amino acidic residues and the amidinium groups of the calix[4]arene building blocks **1a**,**b** and **2**, respectively. The capsules were stud-

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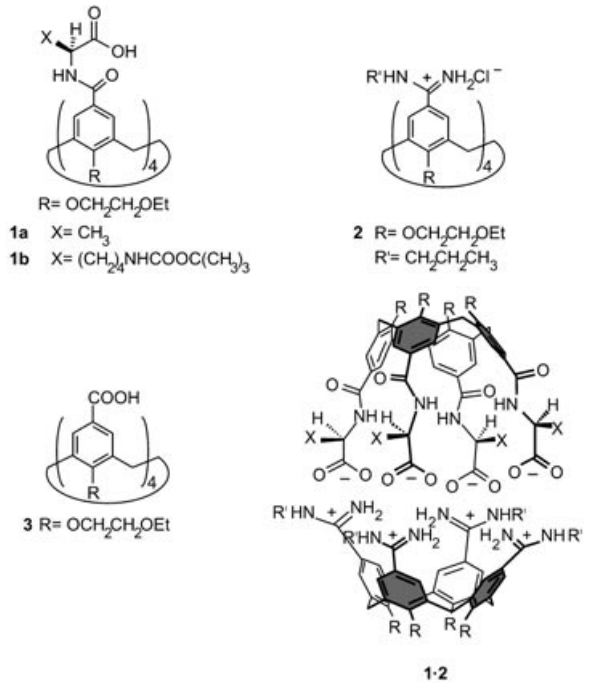
Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

DOI: 10.1002/chem.200400849



ied by ^{1}H NMR spectroscopy, ESI mass spectrometry (ESI-MS), and isothermal titration calorimetry (ITC). For comparative purposes calix[4]arene **3**, derivatized with carboxylic acid groups, was also synthesized and the formation of the molecular capsule **3·2** investigated. The analysis of the data obtained by ITC allows an estimation of the influence of the size of the amino acid side chains in **1a** and **1b**, and of the distance of the carboxylic acid groups from the calix[4]arene scaffold, on the self-assembly process.

Additionally, we describe the encapsulation properties of the molecular capsule **1a·2**. Evidence for the inclusion of the *N*-methylquinuclidinium (NMQ) cation was provided by ^{1}H NMR spectroscopy and mass spectrometry. Moreover, a computational method (docking)[20,21] has been successfully applied to identify possible guest molecules for the capsule **1a·2**. Molecular docking is a virtual screening method that allows for the rapid evaluation of the steric and electrostatic complementarity of potential guest molecules with a host. The docking study was performed with **1a·2** as the host and a selection of commercially available small molecules. The actual binding properties of the selected guest molecules were subsequently experimentally tested by ^{1}H NMR spectroscopy.

Results and Discussion

Synthesis: The formation of assemblies **1a**,**b·2** and **3·2** is the result of the electrostatic interactions between the positively charged calix[4]arene **2** and the negatively charged calix[4]-arenes **1a**, **1b**, or **3**.

Tetracarboxycalix[4]arene **3** was obtained in a Br–Li exchange reaction on the precursor tetrabromo derivative[22] with *t*BuLi in THF at −78 °C followed by quenching with CO_2. Compounds **1a** and **1b** were synthesized from calix[4]-arene **3** (Scheme 1). After the formation of its tetraacyl chloride derivative **4**, L-alanine methyl ester hydrocloride or N^{ε}-(*t*Boc)-L-lysine methyl ester hydrocloride were added to a solution of **4** in CH_2Cl_2 affording calix[4]arene tetraesters **5a** and **5b**, respectively. Hydrolysis with LiOH in MeOH/H_2O provided **1a** and **1b** (Scheme 1), which were fully characterized by NMR spectroscopy, ESI-MS, and elemental analysis. The ^{1}H NMR spectra of both compounds in D_2O ([**1**] = 2 mM) display well-resolved patterns, indicating that under these conditions aggregation is not likely. Moreover, no changes in the ^{1}H NMR spectrum were discernible upon dilution of concentrated solutions of **1a** and **1b** in D_2O, thus confirming their monomeric structure.

Characterization of the molecular capsules 1a,b·2: Building blocks **1a** and **1b** are readily soluble in H_2O, while a cloudy solution is obtained by mixing the two building blocks in H_2O. However, the 1:1 mixture of **1** and **2** is completely soluble in H_2O buffered at pH 9.2 (borate buffer). In the case of assembly **1a·2** the ^{1}H NMR spectrum of the 1:1 mixture of **1a** and **2** in buffered D_2O shows upfield shifts ($\Delta\delta_{Ha} = 0.22$, $\Delta\delta_{Hb} = 0.20$, $\Delta\delta_{Hc} = 0.25$ ppm; for assignment of these protons see Figure 1) for the protons of the propyl

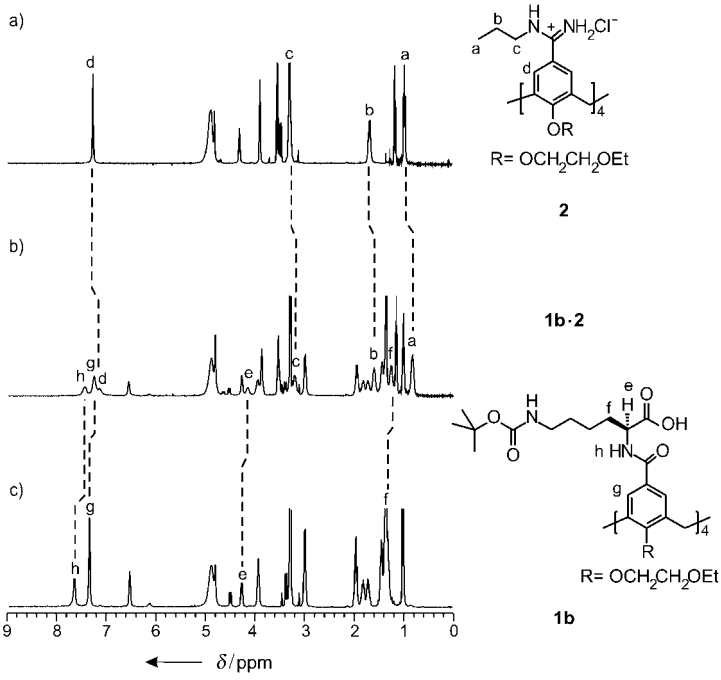
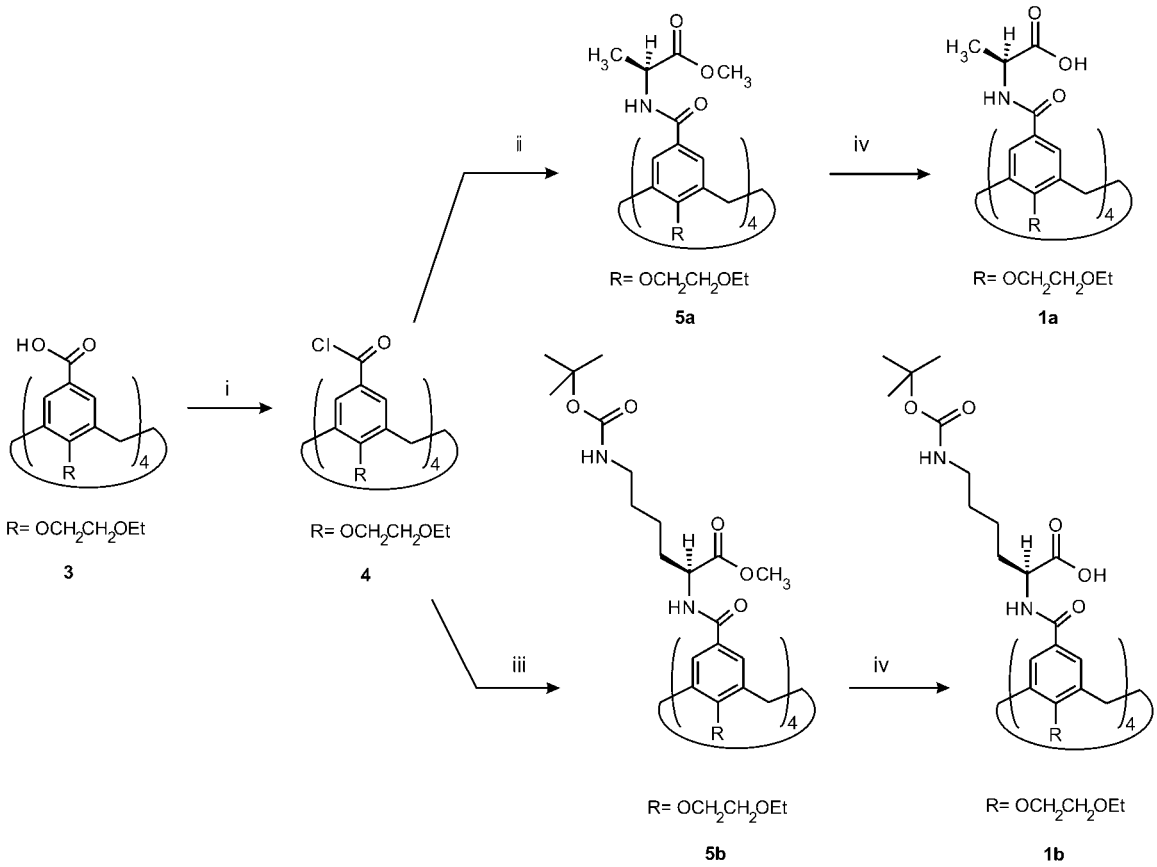


Figure 1. ^{1}H NMR spectra (borate buffer, CD_3OH/H_2O ($x_{water} = 0.4$), 298 K) for a) **2**, b) **1b·2**, and c) **1b**.

amidinium chains of **2**. In analogy with what was observed for a previously described tetrasulfonate–tetraamidinium capsule,[18] upfield shifts are attributed to the shielding provided by the aromatic walls of the calix[4]arene upon inclu-



Scheme 1. i) $SOCl_2$, CCl_4/DMF, 50 °C; ii) L-alanine methyl ester, DMAP, Et_3N, dry CH_2Cl_2, RT; iii) N^{ε}-(*t*Boc)-L-lysine methyl ester, DMAP, Et_3N, dry CH_2Cl_2, RT; iv) LiOH/H_2O/MeOH, THF, RT.

sion of the propyl side chain in the cavity of the capsule **1a·2**.

An equimolar solution of **1b** and **2** in buffered D_2O showed a complex ^{1}H NMR spectrum with broad signals, but analogously to the assembly **1a·2**, the upfield shifts of the protons of the propyl chains are evident. A more resolved spectrum was obtained in CD_3OH/H_2O ($x_{water}=0.4$) in the presence of borate buffer (Figure 1). Upfield shifts accompanied by broadening were observed for the protons of the propyl chains of **2** ($\Delta\delta_{Ha}=0.09$, $\Delta\delta_{Hb}=0.08$, $\Delta\delta_{Hc}=0.15$ ppm). An upfield shift ($\Delta\delta_{Hd}=0.11$ ppm) of the signal of the aromatic protons of **2** was also observed, while small downfield shift changes are detectable for the protons of the methylene bridge of **2**. The rest of the signals of **2** did not undergo any significant changes ($\Delta\delta_{H\alpha}\leq 0.02$ ppm).

The signal of the aromatic protons H_g of **1b** (Figure 1) also experiences a small upfield shift ($\Delta\delta_{Hg}=0.09$ ppm). Interestingly, the protons NH_h, H_e, and H_f (Figure 1) are all upfield shifted ($\Delta\delta_{Hh}=0.21$, $\Delta\delta_{He}=0.12$, $\Delta\delta_{Hf}=0.06$ ppm), probably as a consequence of their proximity to the carboxylic groups, which interact with the oppositely charged amidinium groups of **2**. No changes were observed for the rest of the protons of the side chains. This finding indicates that the side chains of **1b** are most probably outside the capsule's cavity.

Addition of an excess of either calix[4]arenes **2** or **1a**,**b** to the 1:1 mixture of the components resulted in averaged signals for the free and complexed building blocks indicating that the assembly formation is fast on the NMR timescale.

Electrospray mass spectrometry (ESI-MS) provided additional evidence for the formation of self-assembled molecular capsules. The spectrum of an equimolar mixture of **1a** and **2** ($c=0.1$ mM) in buffered H_2O shows a peak at *m/z* 1134 corresponding to the capsule $[(\mathbf{1a\cdot 2})+2Na]^{2+}$ together with two other major peaks, one at *m/z* 1049 and the other at *m/z* 620 corresponding to $[(\mathbf{2}-4HCl)+H]^+$ and $[(\mathbf{1a}-H+Na)+2Na]^{2+}$, respectively (Figure 2).

Analogously, the ESI-MS spectrum of a 0.1 mM solution of the assembly **1b·2** in buffered H_2O shows a doubly charged peak at *m/z* 1681 corresponding to the capsule $[(\mathbf{1b\cdot 2})+2Na]^{2+}$ together with other two relatively intense signals at *m/z* 1049 and at *m/z* 526 corresponding to $[(\mathbf{2}-4HCl)+H]^+$ and $[(\mathbf{2}-4HCl)+2H]^{2+}$, respectively.

The thermodynamic parameters for the self-assembly of the molecular capsules **1a·2** and **1b·2** were studied by means of isothermal titration calorimetry (ITC). To evaluate the effect that the side chains of **1a** and **1b** and the distance of the carboxylate groups from the calix[4]arene scaffold play in the assembly formation, self-assembly of **3·2** was also investigated. Titrations were carried out in MeOH/H_2O

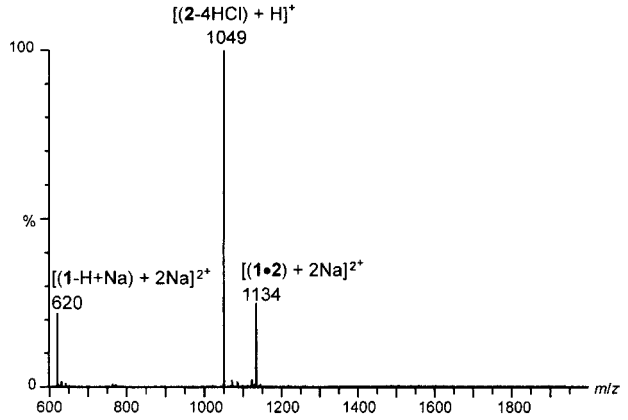


Figure 2. ESI-MS spectrum (borate buffer, H_2O, 298 K) for **1a·2**.

($x_{water}=0.4$) and in pure H_2O containing in both cases borate buffer (pH 9.2, $I=0.03$ M).

In an MeOH/H_2O solvent mixture the formation of a 1:1 complex was observed for all the assemblies (Figure 3) with association constants (K_a) in the order of $10^5\,M^{-1}$ (Table 1).

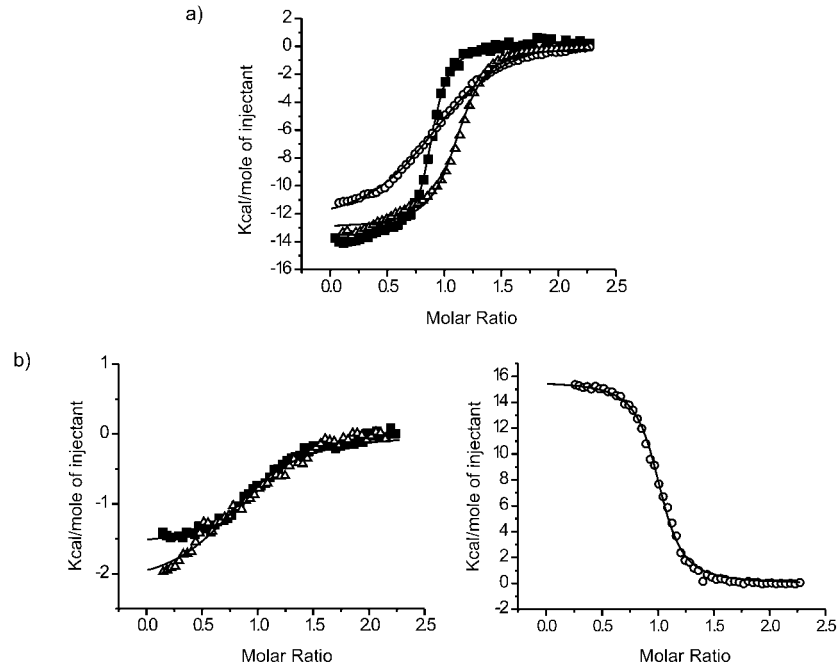


Figure 3. ITC binding curves for capsule formation △: **1a·2**, ○: **1b·2**, ■: **3·2** a) in MeOH/H_2O ($x_{water}=0.4$) at 298 K, borate buffer, $I=0.03$ M, pH 9.2; b) in H_2O at 298 K, borate buffer, $I=0.03$ M, pH 9.2.

Analysis of the thermodynamic parameters shows negative values for both $\Delta H°$ and $\Delta S°$ in this solvent mixture that account for an exothermic and enthalpy driven association process. The favorable change in enthalpy is the result of the formation of four ionic interactions upon self-assembly of the molecular capsules. This contribution overrides the cost in energy needed for the desolvation of the charged groups prior to the self-assembly process. The large unfavorable entropy change reflects instead a loss of degrees of freedom associated with conformational restrictions and/or reorganization upon capsule formation.[13c]

Table 1. Association constants and thermodynamic parameters for the formation of assemblies **1·2** and **3·2** as determined by ITC at 298 K. [**1a**], [**1b**], [**3**] = 1 mM, [**2**] = 0.1 mM.

Assembly	K_a [M^{-1}]	$\Delta H°$ [kJ mol^{-1}]	$\Delta S°$ [J K^{-1} mol^{-1}]
1a·2[a]	$(1.4\pm0.2)\times10^5$	-58.5 ± 0.4	-98 ± 1
1a·2[b]	$(2.3\pm0.7)\times10^4$	-12.3 ± 0.2	43 ± 2
1b·2[a]	$(0.9\pm0.1)\times10^5$	-69.9 ± 0.3	-140 ± 1
1b·2[b]	$(1.7\pm0.1)\times10^6$	74.7 ± 0.4	370 ± 2
3·2[a]	$(1.8\pm0.1)\times10^5$	-49.7 ± 0.2	-66 ± 2
3·2[b]	$(1.5\pm0.7)\times10^5$	-8.9 ± 0.2	69 ± 1

[a] MeOH/H_2O ($x_{water}=0.4$), borate buffer, $I=0.03$ M, pH 9.2. [b] H_2O, borate buffer, $I=0.03$ M, pH 9.2.

Analysis of the thermodynamic data indicates that the assembly **1b·2** shows the largest negative value for $\Delta S°$ ($-140\,J\,K^{-1}\,mol^{-1}$) in MeOH/$H_2O$, indicative of a higher loss of entropy relative to assemblies **1a·2** and **3·2** upon complex formation. Calix[4]arene **1b** is indeed less preorganized than **1a** and **3**, due to the long side chain of the lysine moieties, thus explaining the higher penalty in entropy paid for the formation of the molecular capsule **1b·2**. The formation of the complex **3·2** has the lowest loss of entropy ($\Delta S° = -66\,J\,K^{-1}\,mol^{-1}$). In compound **3**, the carboxylic acid moieties are directly attached to the calix[4]arene scaffold allowing more preorganization than in **1a** and **1b**.

On the other hand, the small differences between the association constants determined for **1a·2** and **1b·2** ($\Delta\log K_a=0.10$) suggest that under these conditions the binding strength is independent of the nature of the amino acidic moieties at the upper rim of the calix[4]arene scaffold. Moreover, comparison with assembly **3·2** indicates that the strength of the binding is not affected by the structural differences of the anionic building components of the capsule. Only a small decrease in association constant in the order $K_{a3\cdot2} > K_{a1a\cdot2} > K_{a1b\cdot2}$ is observed ($\Delta\log K_{a(3\cdot2-1b\cdot2)}=0.30$).

In pure H_2O changes in the thermodynamics of binding were determined for the formation of complexes **1a·2**, **1b·2**, and **3·2**. In general, when compared to the self-assembly in MeOH/H_2O, much less favorable values for $\Delta H°$ but more favorable (positive) values for $\Delta S°$ are observed. As depicted in Figure 3, the resulting titration curves for the self-as-

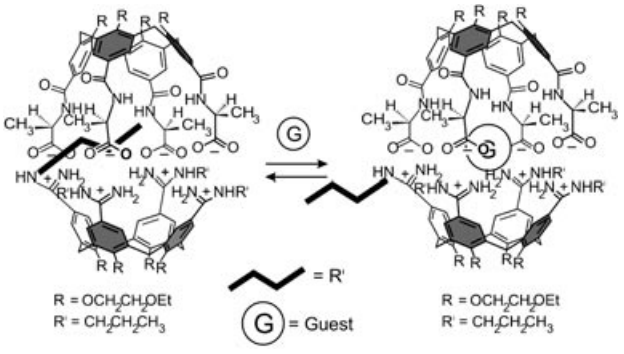
sembly of **1a·2** and **3·2** in H_2O account for an exothermic process; while the binding curve for **1b·2** is indicative of an endothermic binding event (vide infra).

A slight decrease in the association constants for both **1a·2** and **3·2** was found in H_2O versus MeOH/H_2O. This can be qualitatively explained by considering the higher dielectric constant (ε) of H_2O relative to an MeOH/H_2O solvent mixture. The shielding of the electrostatic interactions depends on the ε of the surrounding medium. The coulombic interaction energy is proportional to ε^{-1}, that is, higher ε values result in a weaker binding.

The thermodynamic parameters indicate that the formation of **1a·2** and **3·2** in water is enthalpically favored. The formation of capsule **1b·2** is instead strongly entropically driven. The nature of the carboxylate–amidinium interactions should be similar for assemblies **1a·2**, **1b·2**, and **3·2**, thus the different behavior was rationalized considering the differences in the side chain of the amino acidic residues.[23,24]

In particular the microcalorimetric data suggest a contribution coming from hydrophobic effect in the formation of capsule **1b·2**. The complex formation involves rearrangements of the more hydrophobic and N^{ε}-protected lysine chains. However, the entropy loss for this process is overcompensated by the release of ordered water molecules, thus leading to a large entropy gain ($\Delta S° = 370\ J\,K^{-1}\,mol^{-1}$). The large positive $\Delta H°$ value ($74.7\ kJ\,mol^{-1}$) is most probably the reflection of the energy needed to set these solvent molecules free upon assembly formation. Similar changes in thermodynamics of binding due to side-chain lengths have been observed by Hamilton[25] in the molecular recognition of tetraanionic peptides.

Guest encapsulation in 1a·2: Priority has been given to the study of the encapsulation of quaternary ammonium cations which, according to previous results, act as suitable guests for a molecular capsule held together by ionic interactions.[18] Molecular mechanics calculations suggested that the *N*-methylquinuclidinium cation (NMQ), exhibits a good fit for encapsulation in the assembly **1a·2**. ^{1}H NMR studies were performed in $D_2O/Na_2B_4O_7{\cdot}10H_2O$. The propyl side chain of **2** was used as a probe to detect guest encapsulation (Scheme 2, Figure 4). According to the model (Scheme 2)



Scheme 2. Model for guest encapsulation.

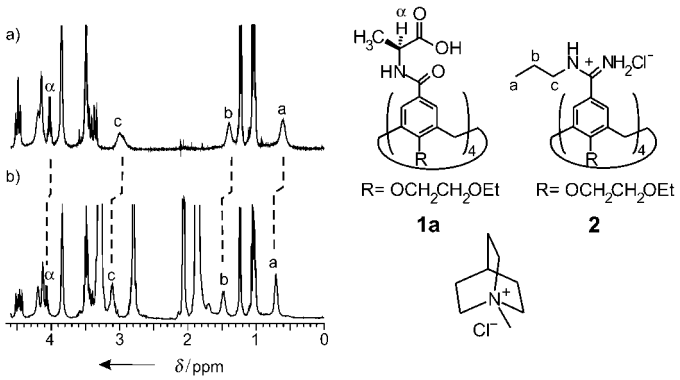


Figure 4. Part of the ^{1}H NMR spectra ($Na_2B_4O_7{\cdot}10H_2O$, D_2O, 298 K) for a) **1a·2** and b) **1a·2** + 30 equiv of NMQ chloride.

the inclusion of a suitable guest molecule should result in the extrusion of the alkyl chain from the capsule's cavity.[18,19]

Changes in the chemical shifts of the signals of the protons of the amidinium propyl chains ($\Delta\delta_{Ha} = 0.17$, $\Delta\delta_{Hb} = 0.15$, $\Delta\delta_{Hc} = 0.17$ ppm) were observed upon addition of 30 equivalents of *N*-methylquinuclidinum chloride to a 1.2 mM solution of **1a·2** (Figure 4) in $D_2O/Na_2B_4O_7{\cdot}10H_2O$. A slight downfield shift was also observed for the α-proton of compound **1a**. The chemical shifts of the other protons, including the ones of the guest, did not show significant changes. The fact that there is only one set of resonances of the guest indicates that the encapsulation is a fast process on the NMR timescale. Therefore, the chemical shifts are averaged signals between free and complexed NMQ. A ^{1}H NMR titration was performed by adding increasing amounts of NMQ chloride to a solution of **1a·2** in $D_2O/Na_2B_4O_7{\cdot}10H_2O$, giving an association constant $K_a = 36 \pm 12\ M^{-1}$.

An experiment to detect chemical shift changes in the guest by adding increasing amounts of the capsule **1a·2** to a 1×10^{-4} M solution of the guest in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ was also performed. Due to solubility reasons only an excess of ten equivalents of the capsule could be reached; this did not result in significant changes in the guest's resonances ($\Delta\delta = 0.005$ ppm). Interestingly, addition of an excess (up to 100 equiv) of acethylcholine (Ach) and tetramethylammonium (TMA) chloride to a solution of **1a·2** in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ did not show any indication for guest encapsulation. As reported by Rebek, binding of molecules in the cavity of a molecular capsule in solution can be expected when the packing coefficient (PC), that is, the ratio of the guest volume to the host volume, is around 0.55.[26] Presumably, even if the ACh and TMA cations could fit into the capsule's cavity their PCs are too small for the encapsulation to occur.

A calorimetric titration of **2** with **1a** in presence of a large excess (60 equiv) of NMQ chloride was performed. The experimental data were consistent with a 1:1 binding mode with an association constant K_a of the same order of magnitude as that found in absence of the NMQ; this fact rules

out the possibility that the addition of NMQ, being a charged guest, dissociates the capsule.

The encapsulation of the NMQ cation was also supported by mass spectrometry. The ESI-MS spectrum of an aqueous solution of **1a·2** containing 30 equivalents of NMQ chloride shows signals at *m/z* of 2222.6 and 2347.7 corresponding to $[(\mathbf{1a{\cdot}2})+H]^+$ and $[(\mathbf{1a{\cdot}2})+NMQ]^+$, respectively (Figure 5).

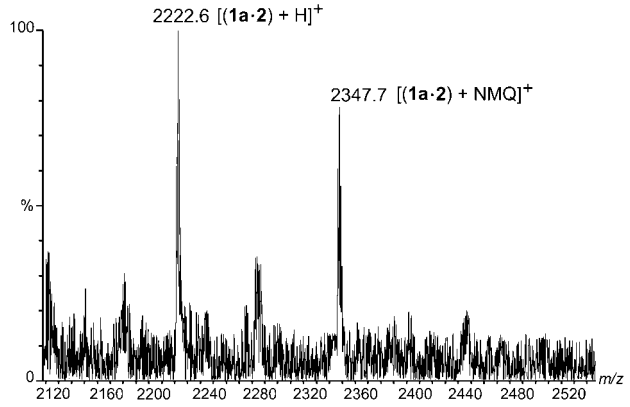


Figure 5. Portion of the ESI-MS spectrum ($D_2O/Na_2B_4O_7{\cdot}10H_2O$) of **1a·2** + 30 equiv of NMQ chloride.

Analogous results were obtained by using FAB mass spectrometry (FAB-MS). Signals at *m/z* 2222.6 and 2244.4 corresponding to $[(\mathbf{1a{\cdot}2})+H]^+$ and $[(\mathbf{1a{\cdot}2})+Na]^+$, respectively, and *m/z* 2347.7 and 2369.7 corresponding to $[(\mathbf{1a{\cdot}2})+NMQ]^+$ and $[(\mathbf{1a{\cdot}2}-H+Na)+NMQ]^+$, respectively, were observed. An additional experiment with FAB-MS was performed: the addition of a small amount of K_2CO_3 resulted in a shift of the peaks of 16 mu, indicating the exchange between the Na^+ for the K^+ ion, which confirms the molecular weight of the complexes.

To identify suitable guest molecules for the molecular capsule **1a·2**, a computational method (docking) was utilized, which allows for rapid screening of molecular databases for potential guest molecules on the basis of their steric and electrostatic complementarity to a receptor. Docking techniques are often used in medicinal chemistry to identify new leads or to suggest possible binding modes of known ligands.[27–29] Applications involving the screening of molecular databases requires a simplified representation of the binding pocket of a protein or receptor from a crystal structure by, for instance, a number of spheres or interaction site points. Subsequently, a large number of potential substrates are fitted into this binding site model and the docking algorithm decides whether the interaction energy of each guest in the binding site is favorable or unfavorable. Suitable orientations within the cavity for molecules from the database are obtained by the DOCK 4.0 (see Experimental Section) by superimposing atoms of the ligands onto the spheres describing the cavity of the host. A maximum of 75 orientations was generated per ligand, with those matches that have the smallest differences in distances between the atoms in the ligand and the spheres describing the cavity being generated first.

In the absence of an X-ray crystal structure, a molecular modeling study provided the conformation of the molecular capsule **1a·2** that was used for docking of the available chemicals database (ACD; Figure 6).

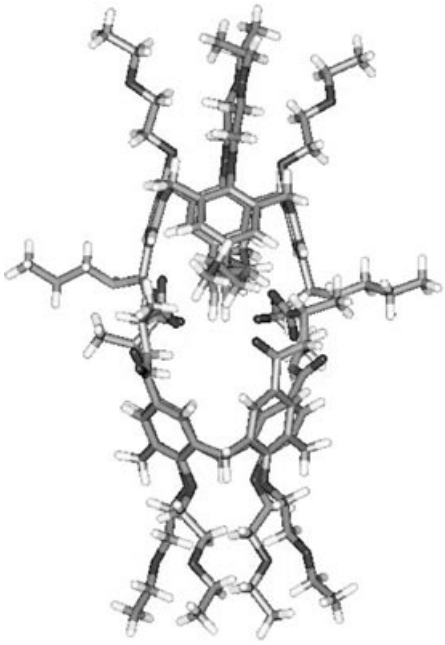


Figure 6. Conformation of the molecular capsule **1a·2** used for docking study. The propyl chains of **2** are pointing outside the capsule's cavity.

Docking into the molecular capsule **1a·2** was a challenging exercise due to the small size/volume ratio and the extremely charged nature of the assembly. Potential guest molecules with molecular weights below 250 were selected from the ACD database of commercially available chemicals (MDL, San Leandro, USA). After filtering out molecules with reactive functionalities, a database of 27999 compounds was obtained. A further selection was made retaining only those molecules possessing a 0 or 1 charge. This yielded a database containing 22818 molecules for docking. Two different scoring functions were used for docking of the selected ACD molecules to **1a·2**. The "energy scoring", which applies a grid-based representation of the AMBER force field, provided a large number of neutral molecules with hydrogen-bond donor groups (−OH and/or −NH) for the interaction with the oxygen of the ethylene glycol chains located at the lower rim of the components of the capsule **1a·2**. Most of the molecules also have polar groups that could interact with the belt of carboxylate–amidinium moieties. The "chemical scoring" function, however, provided quite hydrophobic molecules with some polar functionalities that could interact with the charges that hold the capsule together.

Based on cost and commercial availability, a restricted number of guest molecules were selected for experimental screening. To obtain a rapid indication as to whether a guest

binds or not, ^{1}H NMR experiments were performed. This method takes advantage of the downfield shifts of the protons of the propyl chains of **2** upon addition of the guest molecules; this shift is considered an indication of guest encapsulation (Scheme 2). The study was performed by adding the pure compounds to a solution of the preformed molecular capsule **1a·2** in $D_2O/Na_2B_4O_7{\cdot}10H_2O$. Unfortunately, most of the guest molecules provided by the docking studies are very insoluble in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ and their addition to the host solution resulted in precipitation. A further limitation was due to the fact that **2** undergoes hydrolysis over time or with heat. Therefore, no heating was allowed for dissolution of the guest molecules and their encapsulation, limiting the amount of studies that could be performed.

However, among the selected molecules, compounds **6**–**10** showed good indication of guest encapsulation as their addition to a solution of **1a·2** shifted the signals for the protons of the propyl side chains of **2** downfield (Table 2).[30] More-

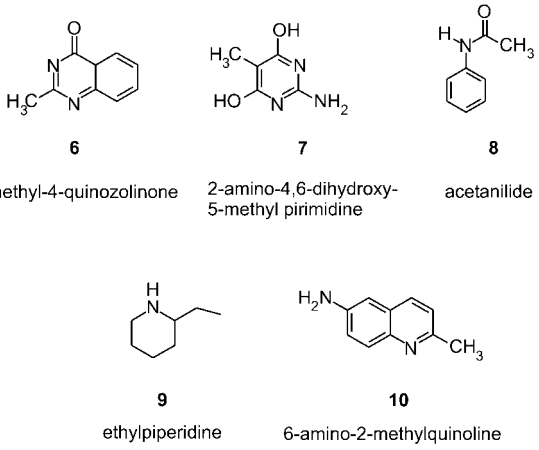


Table 2. ^{1}H NMR chemical shift changes (downfield) for the protons of the propyl side chains of **2** upon addition of an excess (25–30 equiv) of guest molecules to the capsule **1a·2**. ($D_2O/Na_2B_4O_7{\cdot}10H_2O$, 298 K). For assignment of H_a, H_b and H_c see Figure 4.

Guest	$\Delta\delta_{Ha}$	$\Delta\delta_{Hb}$	$\Delta\delta_{Hc}$
6	0.13	0.06	0.1
7	0.09	0.08	0.07
8	0.13	0.05	0.07
9	–	–	0.04
10	0.03	0.03	0.02

over, in all the cases, small but reproducible changes were also observed for the methylene, α, and CH_3 protons of the alanine moieties of **1a** ($\Delta\delta \leq 0.05$ ppm).[31]

Among the guest molecules studied for encapsulation, 6-amino-2-methylquinoline (**10**) is the one that shows better solubility under the experimental conditions reported above. For this reason we decided to further investigate its binding to the capsule **1a·2** by ^{1}H NMR spectroscopy. According to the docking studies, compound **10** is expected to be included into the capsule with its $-NH_2$ group pointing towards the first oxygen atom of one of the ethylene glycol chains located at the lower rim of the calix[4]arene. In this way the protons of the aniline ring of the guest would reside in the core of the calix[4]arene and, therefore, experience the shielding provided by the aromatic rings of the aromatic scaffold.

Interestingly, upon addition of an excess of **10** to a 0.5 mM solution of **1a·2** in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ significant upfield shifts of the resonances of the guest were observed, accompanied by small downfield shifts for the protons of the propyl chains of **2** (Table 2).

Additionally, ^{1}H NMR experiments were carried out to assess whether the upfield shifts observed for the resonances of the guest **10** were generated from the binding to one of the calix[4]arene components or indeed to inclusion in the capsule's cavity. Addition of one equivalent of **1a** to a 1 mM solution of **10** in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ caused an upfield shift for the protons of the guest (Figure 7 and Table 3), ac-

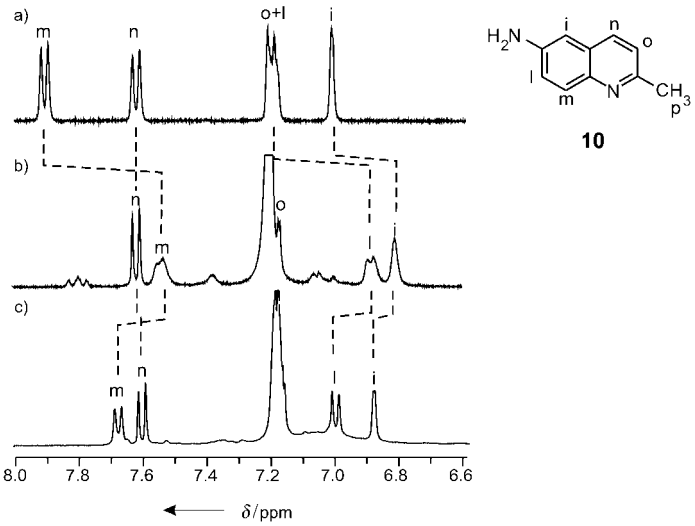


Figure 7. Part of the ^{1}H NMR spectra ($Na_2B_4O_7{\cdot}10H_2O$, D_2O, 298 K) of a) 6-amino-2-methylquinoline **10**, b) **10** + 1 equiv **1a**, and c) **10** + 1 equiv **1a** + 1 equiv **2**.

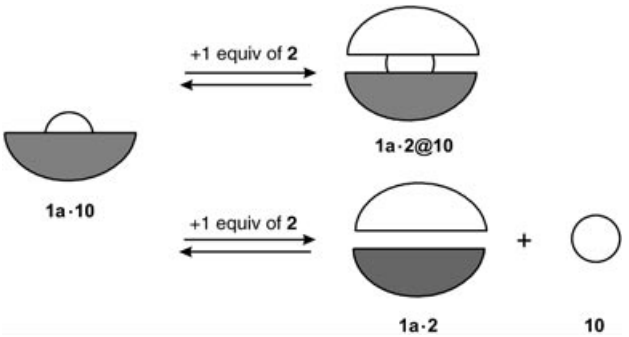
Table 3. ^{1}H NMR chemical shift changes [ppm] for the protons of 6-amino-2-methylquinoline (**10**) for the binding to the tetraalanine calix[4]-arene **1a** and to the capsule **1a·2** ($Na_2B_4O_7{\cdot}10H_2O$, D_2O, 298 K). For assignment of the protons see Figure 7.

	10 δ_{free}	**10** + 1 equiv **1a** $\Delta\delta = \delta - \delta_{free}$	**10** + 1 equiv **1a·2** $\Delta\delta = \delta - \delta_{1a}$
H_i	7.04	0.24	−0.16
H_l	7.23	0.35	−0.25
H_m	7.94	0.32	−0.21
H_n	7.68	0.12	−0.10
H_o	7.22	0.04	–
H_p	2.51	0.08	−0.05

companied by an upfield shift change for the α proton of the alanine moieties. These results indicate that there is an interaction between the guest and **1a**, most likely an inclusion complex of the aniline ring of the guest into the core of

the calix[4]arene as, suggested by the largest upfield shifts experienced by the protons H_i, H_l, and H_m. (In contrast, the addition of one equivalent of **2** to a 1 mM solution of the same guest led only to slight upfield shifts of the guest's resonances.) The subsequent addition of one equivalent of the tetraamidinium calix[4]arene **2** caused a downfield shift of the resonances of the guest towards the position of the free guest (Figure 7), while the tetraamidiniumcalix[4]arene **2** shows the characteristic upfield shifted signals for the protons of the propyl side chains indicative for capsule formation.

The partial downfield shift experienced by the guest's resonances can be ascribed either to a partial dissociation of the complex **1a·10** upon addition of **2** and subsequent formation of the capsule **1a·2** (which results in an increase in the amount of guest free in solution), or simply to a rearrangement of the guest inside the cavity of **1a** upon formation of the complex **1a·2@10** (Scheme 3).



Scheme 3. Schematic representation of the two possible complexes formed upon addition of one equivalent of **2** to **1a·10**.

As a control experiment, an excess of calix[4]arene **2** (up to three equivalents) was added to the 1:1 solution of **1a·2** and **10**. No additional upfield shifts in the resonances of the protons of the guest were observed upon addition of **2**. If the guest was expelled from the cavity all the guest signals should have had the chemical shifts of the free guest, or at least shift further towards the chemical shifts for the free guest. The fact that the upfield shifts are retained at least partly even in the presence of an excess of tetraamidinumcalix[4]arene **2** is a good indication that the guest resides in the capsule's cavity.

A ^{1}H NMR titration was performed by adding increasing amounts of capsule **1a·2** to a solution of **10** in D_2O/$Na_2B_4O_7$·10 H_2O. The titration caused small but reproducible changes in the resonances of the guest.[32] The corresponding isotherm could be fitted to a 1:1 model and gave a binding constant K_a of $2\times10^3\,M^{-1}$ (Figure 8).

Conclusion

The results reported in this paper demonstrate that water-soluble molecular capsules are easily obtained upon introduction of amino acid groups at the upper rim of one of the calix[4]arene components. The strength of the ionic interactions allows the formation of stable molecular assemblies in pure water, with association constants $K_a\sim10^5\,M^{-1}$.

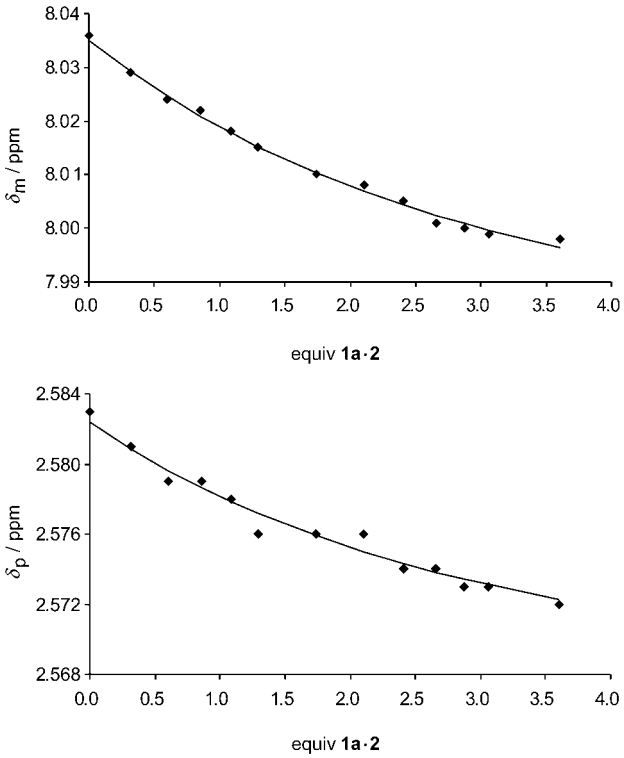


Figure 8. Chemical shift changes for protons H_m (top) and H_p (bottom) experienced by the 6-amino-2-methylquinoline (**10**) upon addition of increasing amount of **1a·2**. The line represents the fit to a 1:1 binding model.

ITC studies have shown that although the association constants for the assembly of the molecular capsules **1a·2** and **1b·2** in water are similar; the length of the side chain of the different amino acid moieties strongly influences the thermodynamic parameters of binding. Docking against **1a·2** allowed the identification of several guest molecules that are encapsulated in the molecular capsule. The ability of **1a·2** to encapsulate guest molecules in water could open new ways for the use of supramolecular structures as molecular receptors or drug delivery systems in physiological media.

Experimental Section

General information and instrumentation: The reagents used were purchased from Aldrich or Acros Chimica and used without further purification. All the reactions were performed under nitrogen atmosphere. Analytical thin-layer chromatography was performed using Merk 60 F_{254} silica gel plates. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA (300 MHz) or a Varian Unity 400 WB NMR spectrometer. ^{1}H NMR chemical shift values (300 MHz) are reported as δ in ppm, with the residual solvent signal as an internal standard (CHD_2OD: $\delta=3.30$; HDO: $\delta=4.67$ ppm). ^{13}C NMR chemical shift values (100 MHz) are reported as δ in ppm using the residual solvent signal as an internal stan-

dard (CD_3OD: $\delta = 49.0$ ppm). Infrared spectra were recorded on a FT-IR Perkin–Elmer Spectrum BX spectrometer and only characteristic absorptions are reported. Fast atom bombardment (FAB) mass spectra were recorded with a Finnigan MAT 90 spectrometer. Electrospray ionization (ESI) mass spectra were recorded on a Micromass LCT time-of-flight (TOF) mass spectrometer. Samples were introduced using a nanospray source. Elemental analyses were carried out using a 1106 Carlo-Erba Strumentazione element analyzer. Compound **3** was synthesized according to a literature procedure.[33]

Calorimetric measurements: The titration experiments were carried out by using a Microcal VP-ITC microcalorimeter with a cell volume of 1.4115 mL. The titrations were performed in a 10 mM solution of $Na_2B_4O_7{\cdot}10H_2O$ in H_2O. The formation of the assemblies **1a,b·2** and **3·2** was studied by adding aliquots of a 1 mM solution of **2** in H_2O/$Na_2B_4O_7{\cdot}10H_2O$ to a 0.1 mM solution of **1a**,**b** or **3** in H_2O/$Na_2B_4O_7{\cdot}10H_2O$, in the calorimetric cell, and monitoring the heat change after each addition. Dilution effects were determined in a second experiment by adding the same 1 mM solution of **2** in $H_2O/Na_2B_4O_7{\cdot}10H_2O$ into the solvent and subtracting this contribution from the raw titrations to produce the final binding curves. The association constants were determined by applying a 1:1 binding model using Microcal Origin®.

^{1}H NMR spectroscopy: The titrations were performed in a 10 mM solution of $Na_2B_4O_7{\cdot}10H_2O$ in D_2O. A solution containing 1 mM of **1a·2** and 200 mM of *N*-methylquinuclidinium chloride in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ was added in numerous aliquots to a 1 mM solution of **1a·2** in D_2O/$Na_2B_4O_7{\cdot}10H_2O$, and the observed chemical shift was recorded after each addition.

In the case of 6-amino-2-methylquinoline (**10**) titrations were carried out by adding increasing amounts of a solution containing 0.3 mM of **1a·2** and 1.0 mM of **10** in $D_2O/Na_2B_4O_7{\cdot}10H_2O$ to a 0.3 mM solution of **1a·2** in D_2O/$Na_2B_4O_7{\cdot}10H_2O$ and following the chemical shift changes of **10**. The binding of **10** to **2** and **1a** was evaluated in separate titrations. The association constants were calculated by fitting the experimental spectral changes to a 1:1 binding model.

Docking: A model of the molecular capsule **1a·2** with *N*-methylquinuclidinium (NMQ) as a guest was created by using Quanta97 (Accelrys, San Diego, USA) and minimized with CHARMm.[34–36] Since NMQ is the largest known ligand of **1a·2**, its inclusion in the capsule prior to docking ensures the cavity in **1a·2** is of a suitable size for ligand binding. The minimized structure was used for docking with DOCK 4.0 with default parameters.[37] The minimized *N*-methylquinuclidinium coordinates were used as spheres to match with non-hydrogen atoms of small molecules in the ACD database of commercially available chemicals (MDL, San Leandro, USA). After filtering out molecules in the ACD database with reactive functionalities or a molecular weight >250, a database of 27999 compounds was obtained. The selection was further refined, retaining only those molecules possessing a 0 or 1 formal charge. This yielded a final database containing 22818 molecules for docking. All ACD compounds were docked in a single, CORINA-generated conformation[38] with Gasteiger–Marsili charges.[39] Scoring of the interactions with the host was done with two different scoring functions: a grid-based version of the AMBER[40] force-field intermolecular energy and a scoring function called "chemical scoring", which penalizes interactions between non-complementary functional groups in the guest/host complex (i.e. polar/nonpolar).[41] The 250 best docking results for the host were visually inspected with VIDA (OpenEye Scientific Software, Santa Fe, USA).

5,11,17,23-(Carbonyl-*N*-L-alanine)-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (1a): Compound **3** (340 mg, 0.38 mmol) was dissolved in CCl_4 (1 mL) and 1 drop of DMF under N_2. $SOCl_2$ (0.15 mL, 2.10 mmol) was added to the solution at 0 °C and the resulting mixture was stirred for 2 h at 50 °C. The solvent was removed under vacuum to afford compound **4**, which, without further purification, was dissolved in dry CH_2Cl_2 (5 mL) and added to a mixture of H-L-Ala-OMe·HCl (222 mg, 1.59 mmol), DMAP (17.7 mg, 0.145 mmol) and Et_3N (0.47 mL, 3.4 mg) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred under N_2 for 24 h at 25 °C. The reaction course was followed by TLC (silica gel, CH_2Cl_2/acetone 5:1). The reaction mixture was diluted with ethyl acetate and washed with citric acid (0.5 M), $NaHCO_3$, and brine. The solvent was then removed under vacuum and compound **5a** was purified by column chromatography with toluene/ethanol (95:5) as the eluent. Compound **5a** (200 mg, 0.16 mmol) was dissolved in MeOH/H_2O/THF (3:1:1; 7 mL) and added to a solution of LiOH (76.6 mg, 3.2 mmol) in MeOH/H_2O (3:1; 4 mL). The reaction was stirred for 24 h at 25 °C. The solvent was then removed under vacuum. Upon re-dissolution in H_2O and addition of 1 N HCl, compound **1a** was obtained as a white precipitate. Yield: 54%; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.38$ (s, 4H), 7.35 (s, 4H), 4.68 (d, $J = 13.2$ Hz, 4H), 4.33 (q, $J = 7.5$ Hz, 4H), 4.26 (t, $J = 4.8$ Hz, 8H), 3.90 (t, $J = 4.8$ Hz, 8H), 3.55 (q, $J = 7.2$ Hz, 8H), 3.33 (d, $J = 13.2$ Hz, 4H), 1.45 (d, $J = 7.2$ Hz, 12H), 1.21 ppm (t, $J = 7.2$ Hz, 12H); ^{13}C NMR (300 MHz, CD_3OD): $\delta = 170.63$, 163.41, 154.51, 130.26, 130.10, 123.57, 123.07, 69.01, 64.98, 61.84, 26,35, 25.10, 12.67, 10.58 ppm; IR (KBr): $\tilde{\nu} = 3365$, 2977, 1732, 1621, 1538, 1456, 1209, 1120 cm^{-1}; MS (ESI-MS): *m/z* calcd for $[C_{60}H_{76}N_4O_{20}Na]$: 1195.5; found 1194.5 $[M+Na]^+$; elemental analysis calcd (%) for $C_{60}H_{76}N_4O_{20}$: C 61.42, H 6.53, N 4.75; found: C 62.07, H 6.83, N 4.21.

5,11,17,23-(Carbonyl-N^{ε}-*t*Boc-L-lysine)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (1b): Compound **3** (340 mg, 0.38 mmol) was dissolved in CCl_4 (1 mL) and 1 drop of DMF under N_2. $SOCl_2$ (0.15 mL, 2.10 mmol) was added to the solution at 0 °C and the resulting mixture was stirred for 2 h at 50 °C. The solvent was removed under vacuum to afford compound **4**, which, without further purification, was dissolved in dry CH_2Cl_2 (5 mL) and added to a mixture of H-L-Lys(*t*-Boc)-OMe·HCl (493 mg, 1.83 mmol), DMAP (20.3 mg, 0.17 mmol), and Et_3N (0.5 mL, 3.90 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred under N_2 for 24 h at 25 °C. The reaction course was followed by TLC (silica gel, CH_2Cl_2/acetone 5:1). The reaction mixture was diluted with ethyl acetate and washed with citric acid (0.5 M), $NaHCO_3$, and brine. The solvent was then removed under vacuum to afford compound **5b**, which was purified by column chromatography using toluene/ethanol (90:1) as the eluent. Compound **5b** (200 mg, 0.11 mmol) was dissolved in MeOH/H_2O/THF (3:1:1; 7 mL) and added to a solution of LiOH (52.7 mg, 2.2 mmol) in MeOH/H_2O (3:1; 4 mL). The reaction was stirred for 24 h at 25 °C. The solvent was then removed under vacuum. Upon re-dissolution in H_2O and addition of 1 N HCl, compound **1b** was formed as a white precipitate. Yield: 45%; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.38$ (s, 4H), 7.36 (s, 4H), 4.69 (d, $J = 10.6$ Hz, 4H), 4.42 (t, $J = 7.2$ Hz, 4H), 4.26 (t, $J = 6.8$ Hz, 8H), 3.91 (t, $J = 6.8$ Hz, 8H), 3.56 (q, $J = 9.2$ Hz, 8H), 3.35 (d, $J = 10.2$ Hz, 4H), 3.04 (t, $J = 8.8$ Hz, 4H) 1.84 (m, 8H), 1.48 (m, 8H) 1.40 (m + s, 16H + 36H), 1.20 ppm (t, $J = 9.6$ Hz, 12H); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 176.01$, 170.05, 160.61, 158.46, 136.13, 129.59, 129.14, 79.82, 75.02, 71.00, 67.40, 54.42, 41.22, 32.15, 30.64, 28.84, 24.58, 15.72 ppm; IR (KBr): $\tilde{\nu} = 3365$, 2977, 1732, 1621, 1538, 1456, 1209, 1120 cm^{-1}; MS (ESI-MS): *m/z* calcd for $[C_{92}H_{136}N_8O_{28}Na]$: 1823.9; found: 1824.5 $[M+Na]^+$; elemental analysis calcd (%) for $C_{92}H_{136}N_8O_{28}$: C 61.32, H 7.61, N 6.22; found: C 60.64, H 7.39, N 6.47.

Acknowledgement

We thank CW-NWO for financial support to F.C. (project number JC 99538).

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[30] No changes were observed upon addition of either one equivalent or an excess of the following compounds: 1,1-diethylpropargilamine, 2,5-dichlorothiophene, 2,6-dihydroxynaphtalene, 2-methylbenzofuran, norbornane, pyridine, *p*-xylene, 1,4-diiodobenzene, ethylpropionate.
[31] ITC calorimetry was used to attempt the determination of the binding constants for the encapsulation. However, for solubility reasons the association constants could not be determined. (Titrations of 1 mM solutions of **1a·2** in H_2O/borate with 10 mM of the guest molecules in H_2O/borate produced either not enough heat or a heat contribution which was much lower than the heat of dilution).
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Received: August 17, 2004
Published online: November 18, 2004

