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Guest Encapsulation and Self-Assembly of Molecular Capsules in Polar Solvents via Multiple Ionic Interactions

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Abstract: Herein we report the formation and characterization of a novel type of capsules resulting from the self-association between oppositely charged complementary building blocks in MeOH/H_2O. The assembly is based on the interaction between tetraamidinium calix[4]arenes **1a**−**d** and tetrasulfonato calix[4]arene **2**. Evidence for the formation of the expected 1:1 assemblies is provided by proton NMR, ESI-MS, and ITC. The association process is fast on the NMR time scale and strongly entropy driven, with association constants in the range of 10^6 M^{-1}. The system **1a·2** shows binding affinity toward acetylcholine, tetramethylammonium, and *N*-methylquinuclidinium cations.

Introduction

Building molecular structures that are able to encapsulate guest molecules in solution is one of the major objectives in supramolecular chemistry.[1−3] Meanwhile, they have been investigated for many different purposes, ranging from the use as molecular reaction chambers in catalysis to their application as sensoring devices or artificial receptors.[4−7] The design of such structures has been achieved using either covalent[8−11] or noncovalent synthesis.[1,12−15] Different approaches have been used to obtain capsules via noncovalent interactions. Multiple hydrogen bonding represents one of the most widely employed tools in the construction of predefined molecular containers.[15−29] Most of these systems, however, are restricted to nonpolar solvents. Nevertheless, capsules that are stable in polar organic solvents[30−32] have recently been obtained by combining hydrogen bonds with other weak noncovalent interactions, like cation−π and $\pi-\pi$ stacking. Metal ions have also been widely used for the self-assembly of supramolecular host capsules.[33−46]

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This has the advantage that guest molecules can be bound through interaction with vacant coordination sites on the metal center.[47,48]

Surprisingly, only few examples of stable cage-like complexes using multiple ionic interactions have been reported.[49−52] In this contribution we report the noncovalent synthesis and characterization of a novel type of capsules resulting from the strong association between oppositely charged calix[4]arenes **1** and **2**.[53] The capsules are stable in solutions containing up to 40 mol % of water, most likely the result of preorganization of the charges on the calix[4]arene scaffold. Formation of the 1:1 complexes has been studied using isothermal titration calorimetry (ITC), ^{1}H NMR spectroscopy and electrospray mass spectrometry (ESI-MS). The capsules show binding of cationic guest molecules, like the tetramethylammonium (TMA), acetylcholine (ACh) and *N*-methylquinuclidinium cation.

Synthesis

Calix[4]arenes **1a**−**d**, with tetraamidinium groups directly attached to the upper rim, were synthesized using a modified one-step literature procedure.[54,55] The introduction of the amidinium moieties was achieved by reacting the corresponding alkylchloroaluminum amide generated from Et_2AlCl and the appropriate alkylamine, in the case of compounds **1a**−**c**, and from Me_3Al and ammonium chloride, in the case of **1d**, with tetracyanocalix[4]arene **3**[56] in fluorobenzene or 1,2-dichlorobenzene. The chloride salts of the amidinium compounds were obtained after reverse phase chromatography and ion-exchange chromatography in 40−60% yield (see Supporting Information).

Compound **2** was obtained as described before by treating the parent 25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene with concentrated H_2SO_4.[57]

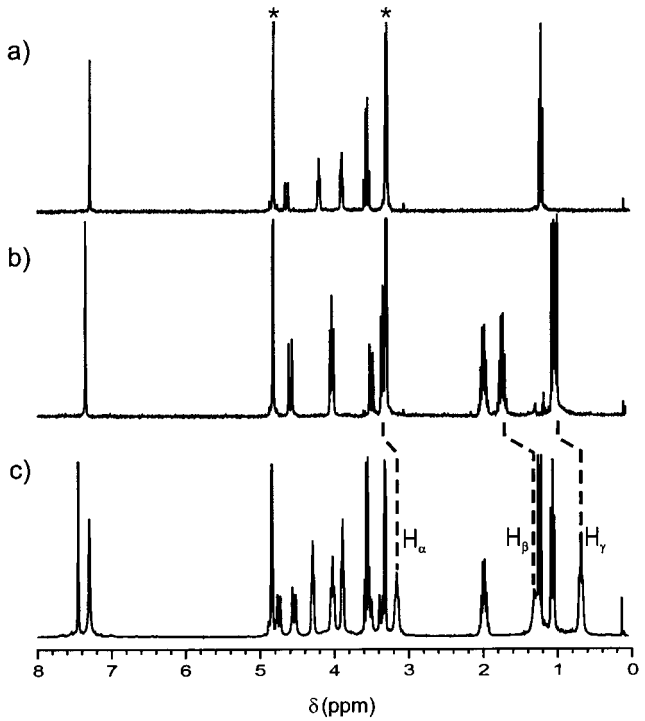

Figure 1. ^{1}H NMR spectra (CD_3OD, 298 K) of (a) **1a**, (b) **2**, and (c) capsule **1a·2** obtained by precipitation upon mixing equimolar solutions of **1a** and **2** in H_2O. Asterisks indicate solvent signals. (For assignment of H_α, H_β, H_γ see Figure 2).

Compounds **1a**−**d** and **2** give sharp ^{1}H NMR spectra in CD_3OD, showing that they do not aggregate in solution, and clearly reveal C_{4v}-symmetry common to tetrasubstituted *cone* calix[4]arenes. All the compounds were found to be well soluble in water and methanol.

Results and Discussion

Isolation of capsule **1a·2** was achieved by precipitation in water. While compounds **1a** and **2** are both water soluble, mixing equimolar solutions (1.0 mM) of the two building blocks in water at room temperature led to the precipitation of a white solid. This precipitate was filtered, washed with water, dried, and redissolved in CD_3OD. Integration of the ^{1}H NMR resonances revealed the 1:1 (or *n:n*) stoichiometry of the complex. Interestingly, the proton signals of the amidinium propyl chains (H_α−H_γ, see Figure 1) had strongly shifted upfield ($\Delta\delta_{H\alpha}$ = 0.23 ppm, $\Delta\delta_{H\beta}$ = 0.53 ppm, $\Delta\delta_{H\gamma}$ = 0.33 ppm), while only small changes were observed for all the other signals (<0.1 ppm chemical shift). This upfield shifting must be due to (partial) self-inclusion of the propyl side chains of calix[4]-arene **1a** in the interior of the cavity formed by the capsule **1a·2** (vide infra). A similar behavior was found by Rebek in the dimeric capsule based on resorcinarene.[58]

Further proof of the stoichiometry of the complex was obtained from a ^{1}H NMR titration in CD_3OD. For solubility reasons the titration was performed by adding compound **1a** to **2**. When less than 1 equiv of **1a** was added, the chemical shift of H_α−H_γ was almost constant and independent of the amount of **1a**, indicating that the calixarene tetraamidinium **1a** is present

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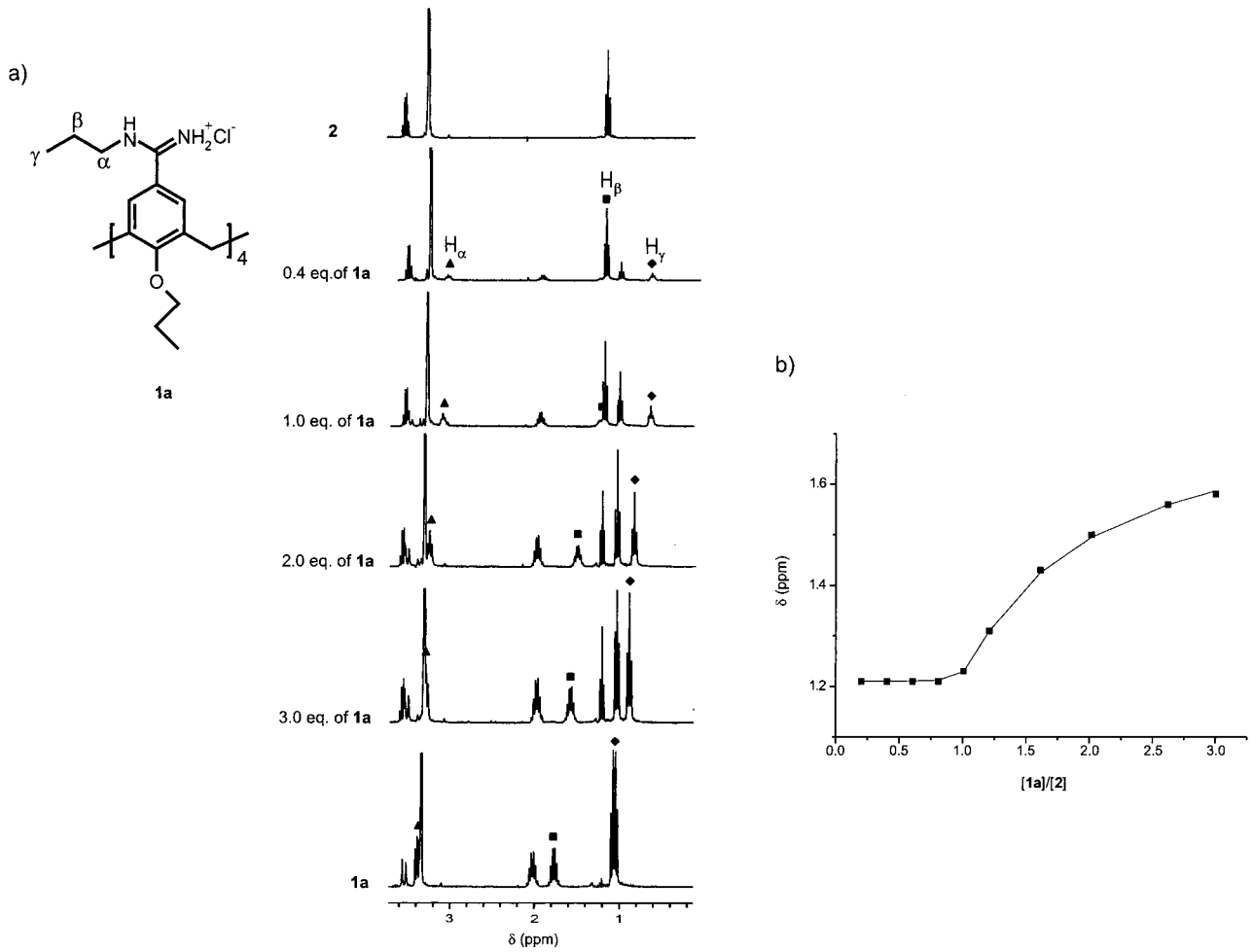

Figure 2. (a) ^{1}H NMR titration of **2** with **1a** in CD_3OD at 298 K. (b) Fitting of the ^{1}H NMR titration data. Data points represent the methyl proton signals of the amidinium chains that shift upon addition of **1a**; line is best-fit curve calculated by nonlinear regression.

mainly as the complex **1a·2** and suggesting that the complex is very stable. Beyond the 1:1 ratio, the chemical shift for $H_\alpha-H_\gamma$ shifts downfield, representing time-averaged values for the free and complexed **1a**. This indicates that the exchange between free and complexed **1a** is fast on the NMR time scale (see Figure 2). A variable temperature (VT) NMR experiment showed that the exchange process is very fast. Even at −30 °C decoalescence of the signals was not observed. The experimental data were fitted to a 1:1 binding model giving a $K_a \sim 10^6$ M^{-1}, the limit of what can be determined via NMR.

Formation of complex **1a·2** was also supported by electrospray mass spectrometry (ESI-MS). The spectrum of an equimolar solution of **1a** and **2** in CH_3OH shows the doubly charged signal of the capsule as the base peak at *m*/*z* 1005.2 (calcd for $[\mathbf{1a}\cdot\mathbf{2} + 2Na]^{2+}$: 1004.4) (see Figure 3). In addition, significant NOE connectivities were observed for the H_α and the H_γ protons of the propyl residues of **1a** with the aromatic proton signals of **2** (see Figure 4).

Most likely, calix[4]arene units **1a** and **2** bind with their opposite charges facing each other. The large upfield shift for $H_\alpha-H_\gamma$ (vide supra) suggests that at least part of the propyl side chains is located inside the cavity where the anisotropic environment of the calix[4]arene aromatic rings leads to shielding of the chain protons. Moreover addition of D_2O (from 0 to 35%) to a 1.8 mM solution of **1a·2** in CD_3OD shifts the signals for H_β and H_γ even further upfield ($\Delta\delta_{H\beta} = 0.07$ ppm, $\Delta\delta_{H\gamma} = 0.25$ ppm), whereas most of the other proton signals remain at their original positions ($\Delta\delta < 0.05$ ppm). This is in good agreement with our assumption that the cavity inside the capsule **1a·2** provides a hydrophobic environment. In addition, significant NOE connectivities were observed for the H_γ protons of the propyl residues of **1a** with the aromatic proton signals of **2** (see Figure 4), which can only mean that (part of) the propyl chains are inside. Molecular modeling studies (CHARMm 24.0) confirm that the internal cavity of the complex is sufficiently large to accommodate one or two propyl side chains. However, with three or more propyl groups inside, the cavity gets severely distorted (see Figure 5 and Supporting Information).

To further study the influence of the side-chain size on the formation of the capsule, we also investigated the assembly of **2** with calix[4]arene tetraamidinium **1b** and **1c** (see Chart 1) having *N*-isopropyl and *N*-heptyl amidinium substituents, respectively. The spectrum of **1b·2** in CD_3OD shows strongly upfield-shifted and -broadened signals for both the H_α and H_β protons ($\Delta\delta_{H\alpha} = 0.17$ ppm and $\Delta\delta_{H\beta} = 0.53$ ppm). However, the proton signals of the heptyl side chains in assembly **1c·2** show almost negligible upfield shifts (max shift observed for H_β, $\Delta\delta_{H\beta} = 0.07$ ppm). This leads us to conclude that the heptyl side chains are too large to be accommodated inside the cavity of the capsule (see Figure 6).

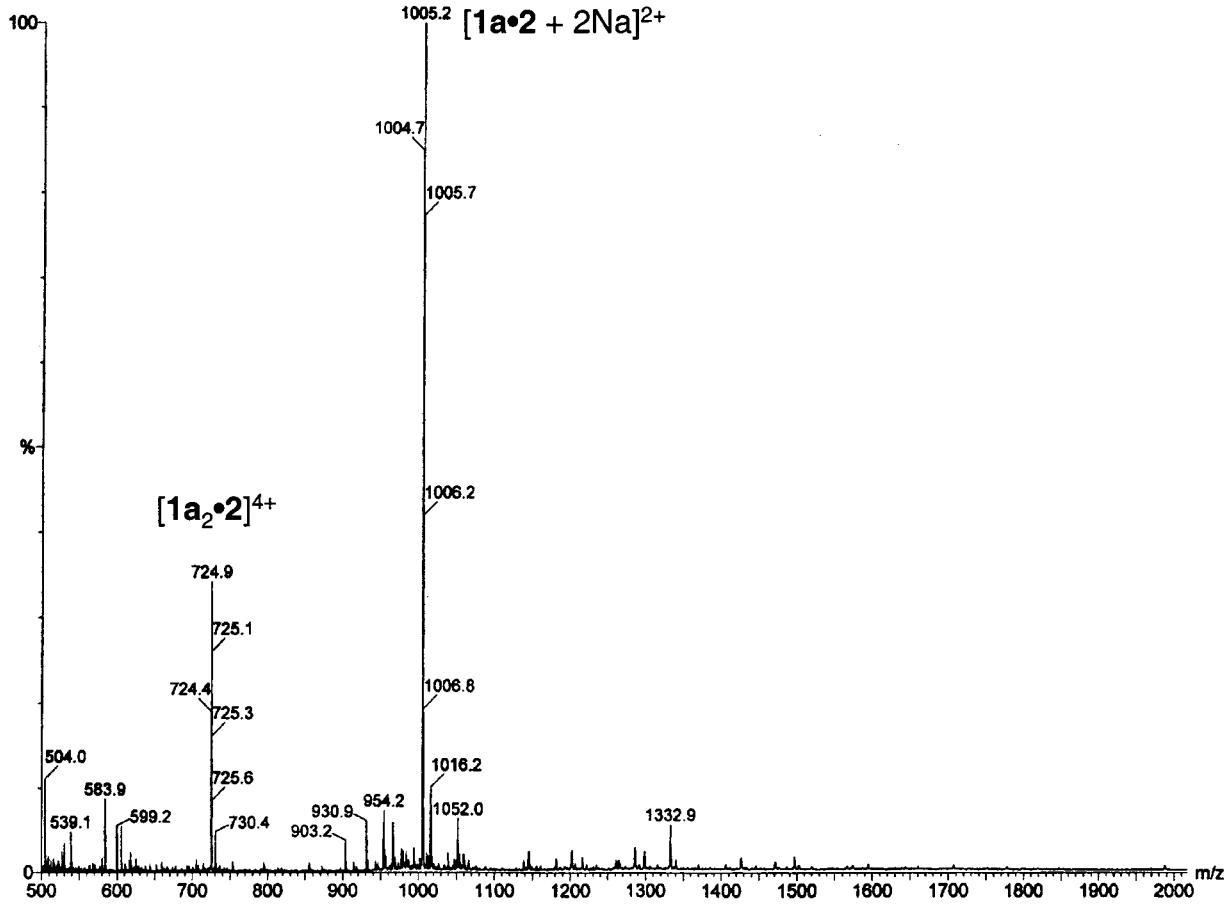

Figure 3. ESI-TOF-MS spectrum for assembly **1a·2**.

Table 1. Thermodynamic Parameters for the Formation of Assemblies **1·2** as Determined by ITC[a]

assembly	K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})
1a·2	$(8.5 \pm 1.4) \times 10^6$	14.1 ± 0.1	180 ± 2
1a·2[b]	$(2.1 \pm 0.5) \times 10^5$	11.6 ± 0.5	141 ± 3
1b·2	$(6.4 \pm 1.7) \times 10^6$	13.7 ± 0.2	176 ± 2
1c·2	$(1.1 \pm 0.1) \times 10^6$	17.9 ± 0.1	176 ± 1
1d·2	$(1.9 \pm 0.3) \times 10^6$	33.3 ± 0.3	231 ± 2

[a] Measured in MeOH/H_2O ($x_{water} = 0.4$) at 298 K, background electrolyte: 1×10^{-2} M Bu_4NClO_4. [b] Background electrolyte: 1×10^{-2} M TMACl.

Isothermal titration calorimetry was utilized to study the thermodynamics of association between **1a**−**d** and **2.** All the titrations were carried out by adding aliquots of calix[4]arene tetrasulfonate **2** to a solution of tetramidinium calix[4]arenes **1a**−**d** at 298 K in MeOH/H_2O ($x_{water} = 0.4$) in the presence of 0.01 M of tetrabutylammonium perchlorate as background electrolyte.

In all cases, the titration curves showed an inflection point around 1.0 equiv of **2** added, which confirms the formation of a complex with 1:1 stoichiometry (see Figure 7). The titration data were fitted to a 1:1 binding model using a nonlinear least-squares fitting procedure. The corresponding association constants and thermodynamic parameters are listed in Table 1. For all the capsules investigated the association constants are in the order of 10^6 M^{-1} with positive $\Delta H°$ and $\Delta S°$ values.[59−62] The association process is driven by entropy most likely due to the release of highly ordered solvent molecules to the bulk solvent. The positive values obtained for the enthalpy is ascribed to the energy needed to desolvate the charged groups, which overrides the negative enthalpic contribution due to the formation of the assemblies. These results are in agreement with the binding of dicarboxylate to diamidinium receptors in methanol.[61] Within the series, assembly **1d·2** exhibits the highest positive values for $\Delta H°$ and $\Delta S°$. This can be attributed to a more favorable solvation of the nonsubstituted amidinium groups of **1d**. Interestingly, increasing the size of the alkyl side chains from propyl to heptyl does not have a dramatic effect on the thermodynamics for capsule formation. There is only a slight increase in the positive $\Delta H°$ for complex **1c·2**, which has probably the best solvated amidinium groups (see Table 1).

As these novel molecular capsules appeared to be well-suited to accommodate guest molecules, we studied the capsule's affinity for different guest molecules including the biological interesting acetylcholine. The encapsulation properties were primarily investigated by ^1H NMR spectroscopy.

Addition of an excess (=15 equiv) of either tetramethylammonium chloride (TMACl) or bromide (TMABr) to the capsule **1a·2** in CD_3OD (1.0 mM) causes a significant downfield shift of the proton signals of the *N*-propyl amidinium side chains ($\Delta\delta_{H\alpha} = 0.23$ ppm, $\Delta\delta_{H\beta} = 0.31$ ppm, $\Delta\delta_{H\gamma} = 0.19$ ppm) (see

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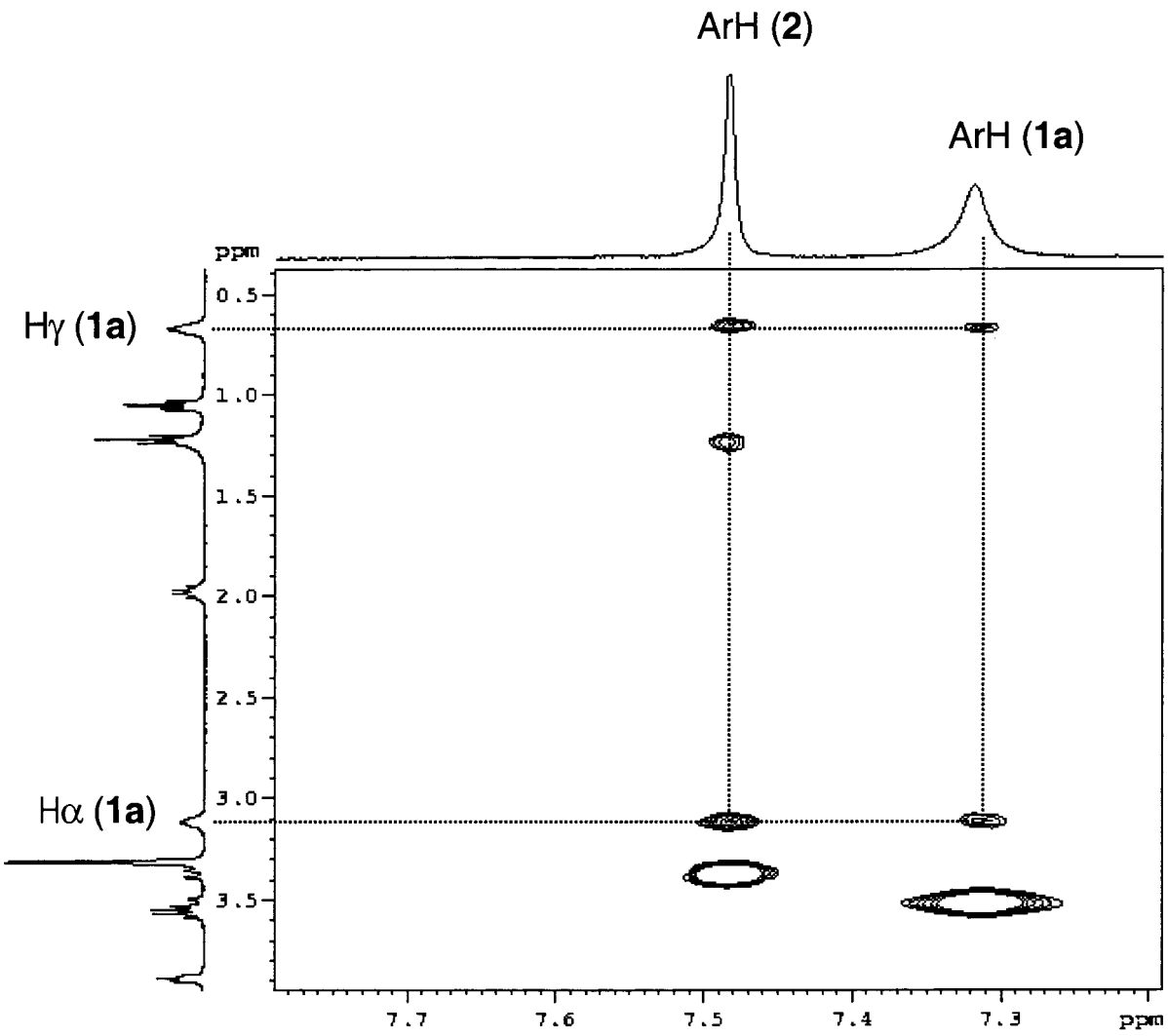


Figure 4. Part of the 2D NOESY NMR spectrum of complex **1a·2** in CD_3OD at 298 K. NOE contacts are observed between the H_α and H_γ protons of the propyl residues and the aromatic protons of either **1a** and **2**.

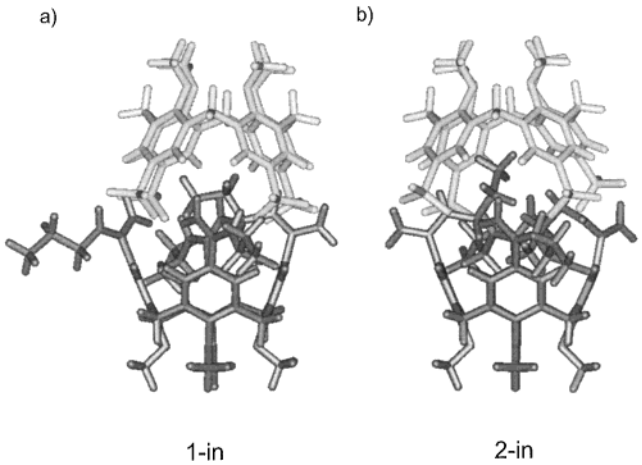


Figure 5. Molecular simulations (CHARMm 24.0) of assembly **1a·2**; (a) one of the alkyl side chains or (b) two of the alkyl side chains are included in the cavity of the capsule.

Figure 8). This finding accounts for the extrusion of the alkyl chains from the cavity as a consequence of a more favorable inclusion of TMA. A control experiment using tetrabutylammonium chloride (TBACl), a molecule too bulky to be accommodated inside the cavity showed no changes in the chemical shift of the chain proton signals. Inclusion of the TMA cation was also confirmed by ESI-MS, clearly showing signals for both the $[\mathbf{1a{\cdot}2} + \text{TMA} + \text{H}]^{2+}$ and the $[\mathbf{1a{\cdot}2} + \text{TMA} + \text{Na}]^{2+}$ complexes.

The TMA resonance itself hardly shifts upon complexation, because it is an averaged signal for the free and complexed guest molecule. As the complex is not extremely strong it can be observed only with a large excess of guest present, which means that the averaged signal does not shift much. However, in a concentrated (10 mM) solution of **1a·2** in CD_3OD containing 1.0 equiv of TMACl the TMA resonance is shifted upfield by 0.08 ppm. Fitting of the ^1H NMR titration data for TMACl to a 1:1 binding model gave a $K_a = 170 \pm 30\ M^{-1}$ in CD_3OD.

Complexation of TMACl was also observed in pure water. However, in this case we were not able to measure complexation directly in solution, because of the inherently low solubility of complex **1a·2** in water. Therefore, we suspended 8 mg of solid **1a·2** in 1.5 mL of a 1.5 M aqueous solution of TMACl and allowed the suspension to equilibrate for 1 h. Then, the solid was filtered and extensively washed with water. NMR analysis (CD_3OD) showed the presence of 50−70% mol equiv of TMACl, which strongly supports encapsulation. A control

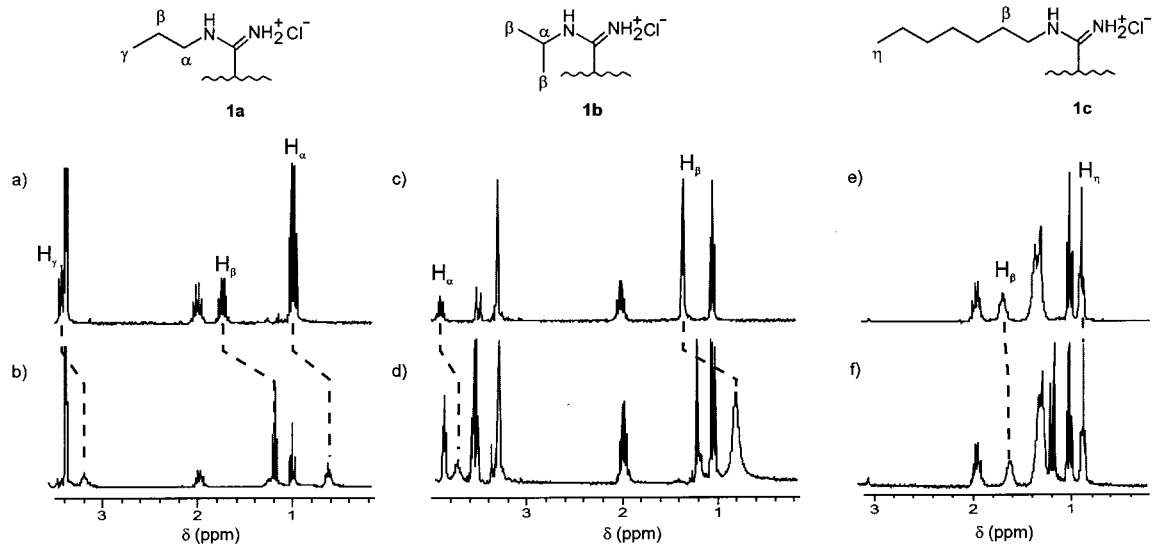
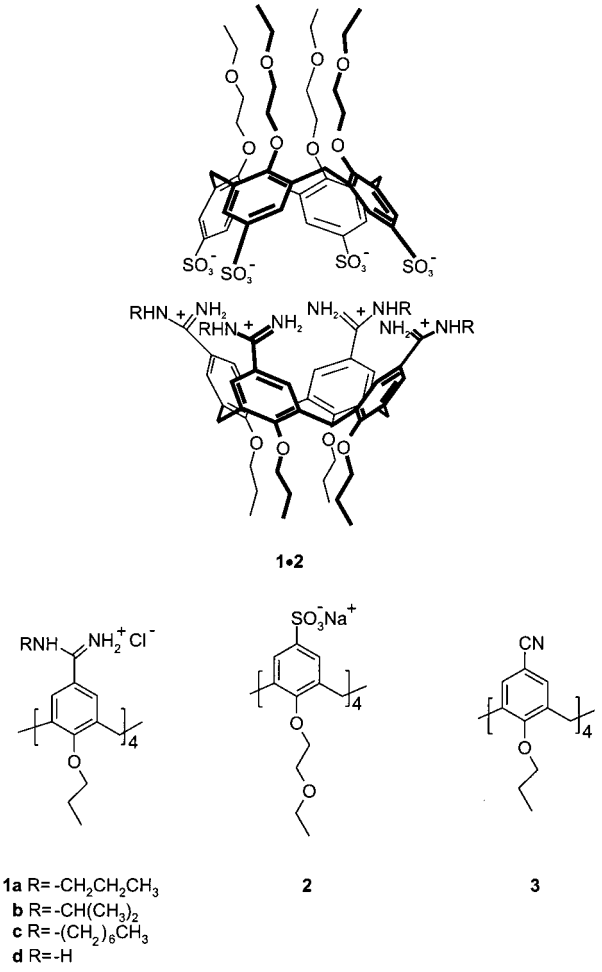


Figure 6. Parts of the ^{1}H NMR spectra (CD_3OD, 298 K) of (a) **1a**, **(**b) a 1:1 mixture of **1a** and **2**, (c) **1b**, (d) a 1:1 mixture of **1b** and **2**, (e) **1c**, and (f) a 1:1 mixture of **1c** and **2**.

Chart 1



experiment with TBACl did not show any of the ammonium salt present, which is too big to fit inside the cavity.

To further prove encapsulation of the guest, new calorimetric experiments were carried out. The calorimetric titration of **2** with **1a** using TMACl as background electrolyte (1×10^{-2} M) produces an experimental curve very similar to that obtained using tetrabutylammonium perchlorate. This result suggests that the presence of TMA chloride does not interfere with the formation of the assembly.

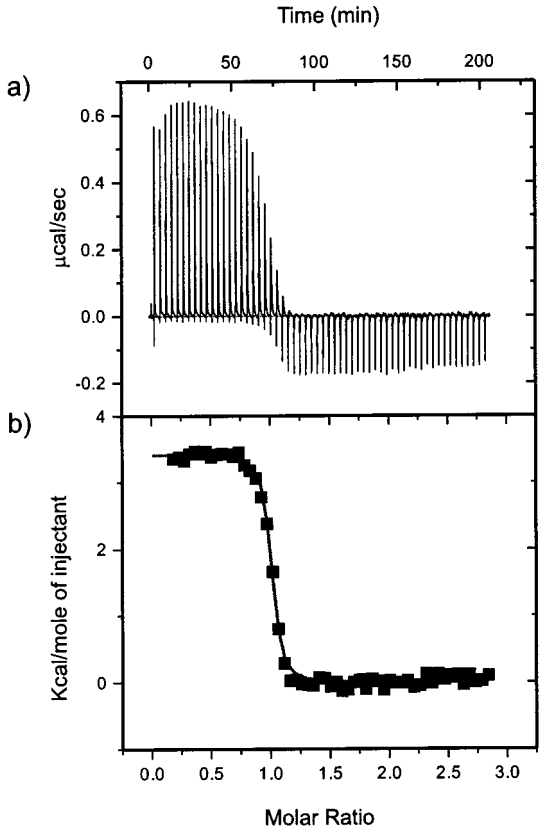


Figure 7. Calorimetric titration of **2** (0.1 mM) with **1a** (1.0 mM) in CH_3OH/H_2O ($x_{water} = 0.4$) at 298 K. (a) Raw data of heat evolution with injection of **1a**, (b) resulting binding curve and best fit curve.

Moreover, as it is known from literature that calix[4]arene tetrasulfonate shows binding affinity toward ammonium salts in aqueous solution,[63−66] a microcalorimetric titration of **2**

(63) Arena, G.; Casnati, A.; Mirone, L.; Sciotto, D.; Ungaro, R. *Tetrahedron Lett.* **1997**, *38*, 1999−2002.

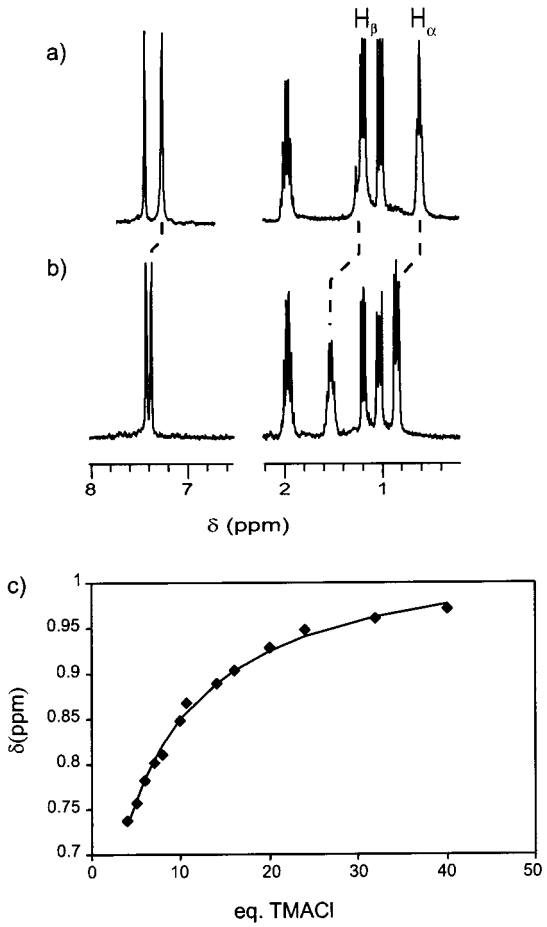


Figure 8. Different regions of the ^{1}H NMR spectra (CD_3OD, 298 K) of (a) **1a·2**, (b) **1a·2** in the presence of 15 equiv of TMACl, and (c) ^{1}H NMR titration of **1a·2** with TMACl in CD_3OD at 298 K. Data points represent the methyl proton signals of the amidinium chains that shift upon addition of the guest. The lines are best-fit curves calculated by nonlinear regression of the titration data. (For assignment of H_α, H_β, H_γ see Figure 2).

(1×10^{-4} M) with TMACl (1×10^{-2} M) has been performed. No heat effect was observed at these concentrations, which allow us to conclude that the experimental curve we observed during the titration is indeed due to the formation of the assembly **1a·2** and not to the formation of the complex **2** with TMA ion.

We then studied the encapsulation of acethylcholine (ACh), an important neurotransmitter. Addition of 15 equiv of ACh chloride also causes significant downfield shifts of the *N*-propyl amidinium protons ($\Delta\delta_{H\alpha} = 0.38$ ppm, $\Delta\delta_{H\gamma} = 0.24$ ppm). Fitting of the data to a 1:1 binding model gave a $K_a = 37 \pm 7$ M^{-1} in CD_3OD. Also in this case upfield shift for the ACh resonances ($\Delta\delta = 0.15$ ppm for the *N*-methyl protons, 0.07 ppm for the α CH_2 protons, $\Delta\delta = 0.13$ ppm for the β CH_2 and $\Delta\delta = 0.07$ ppm for the acetyl protons) was observed in a 10 mM solution of **1a·2** in CD_3OD containing 1.0 equiv of ACh chloride.

Finally, we investigated the complexation of the *N*-methylquinuclidinium ion, which is known to be a suitable guest for molecular capsules based on calix[4]arenes.[67] Similarly to what was observed with the previous guest molecules, addition of 1.0 equiv of methylquinuclidinium chloride to a 10 mM solution of **1a·2** in CD_3OD caused an upfield shift of all the guest proton signals, with the largest shift (0.12 ppm) observed for the methyl protons. The ^{1}H NMR titration produced a curve, which was successfully modeled by a one-site model giving an association constant K_a of 24 ± 14 M^{-1} (see Supporting Information).

Conclusions

In summary, we have shown evidence for the formation of 1:1 calix[4]arene-based molecular capsules **1·2** that are formed via multiple ionic interactions in polar solvents. The capsules have an internal cavity that can accommodate cationic guests, like TMA, ACh, and *N*-methylquinuclidinium. Current work is aimed at improving the kinetic stability of the capsules and their solubility in pure water.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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