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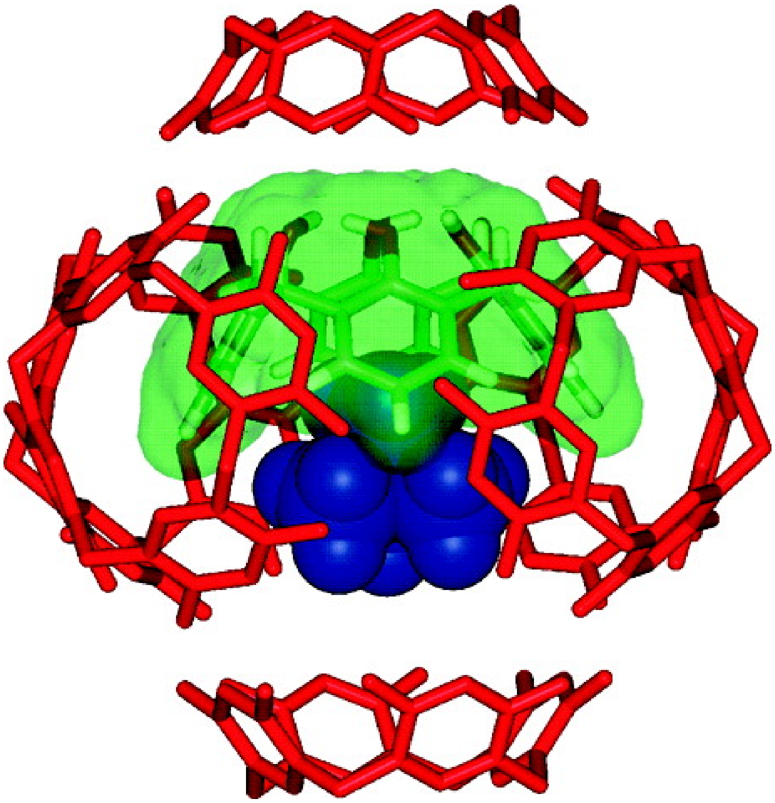

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Nanoencapsulation of Calix[4]arene Inclusion Complexes

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Abstract: A hexameric resorcinarene nanocapsule in wet $CDCl_3$ forms inclusion complexes of calix[4]arene with tetramethylammonium and trimethylsulfoxonium cations to give highly stable Russian-doll-type multicomponent assemblies. The 2D NOESY experiments revealed the size of the assembly, the close proximity of the encapsulated calix[4]arene molecule to the resorcinarene molecules of the capsule, and the inclusion of the tetramethylammonium cation in the calix[4]arene cavity.

Introduction

Large molecular capsules are fascinating self-assembling nanocontainers providing unique microenvironments for the studies of intermolecular interactions.[1] In the crystalline state six molecules of resorcinarene **1a** and eight water molecules form a huge molecular capsule (Figure 1) stabilized by 60 hydrogen bonds.[2] Lipophilic resorcinarene **1b** forms similar assemblies in wet benzene or chloroform.[3] The 1D and 2D NMR techniques revealed that the hexameric capsule can entrap six or eight small molecules (chloroform,[3] benzene,[3] glutaric acid[4]), three medium sized guests (Et_3NHCl),[4] or one bulky species such as R_4NBr, Bu_4SbBr, or a cobaltocenium salt.[5]

In rare cases very large molecular containers have been shown to generate complex-within-complex assemblies.[6] Given the enormous internal space associated with the hexameric resorcinarene capsules these would be expected to encapsulate smaller inclusion complexes. Appropriate conditions were investigated and presented herein for the formation of such Russian-doll (*Matryoshka*) complexes of hexameric resorcinarene capsules.

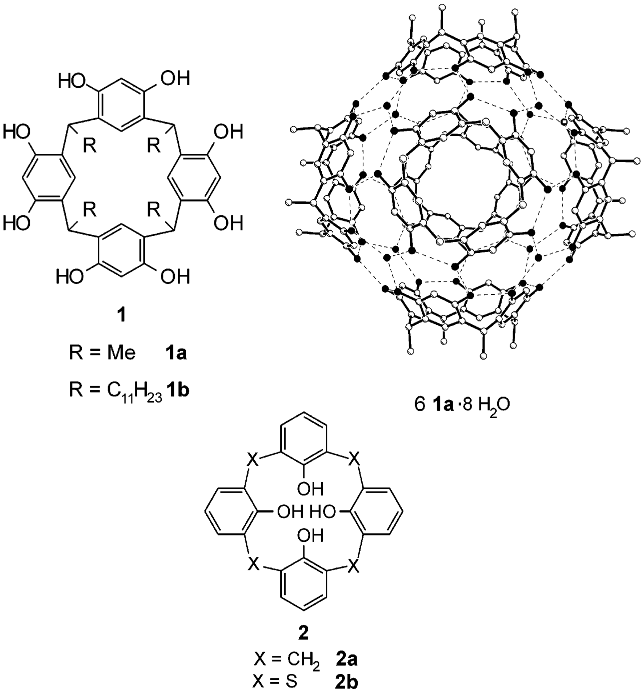

Figure 1. Resorcinarenes **1**, hexameric capsule 6**1a**·$8H_2O$, and calixarene guests **2**.

Results and Discussion

The ^1H NMR spectrum of resorcinarene **1b** in wet $CDCl_3$ contains two singlets for the CH protons of the resorcinol rings and one triplet for the methyne protons of the bridges, whereas the OH groups emerge as a broad singlet at 9.6 ppm (Figure 2a). The broad signal of water protons is located at 4.2 ppm owing to hydrogen bonding to the OH groups of **1b**. This pattern has been shown to correspond to hexameric molecular capsule 6 **1b**·$8H_2O$ including six chloroform molecules.[3]

Calix[4]arene **2a** ($V = 0.36$ nm^3)[7] was anticipated to fit into the hexameric resorcinarene capsule, which has an internal

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(7) The volumes of van der Waals surfaces of the guests were calculated using: *WebLab Viewer Pro 4*; Molecular Simulations Inc: 2000.

10.1021/ja067402c CCC: $37.00

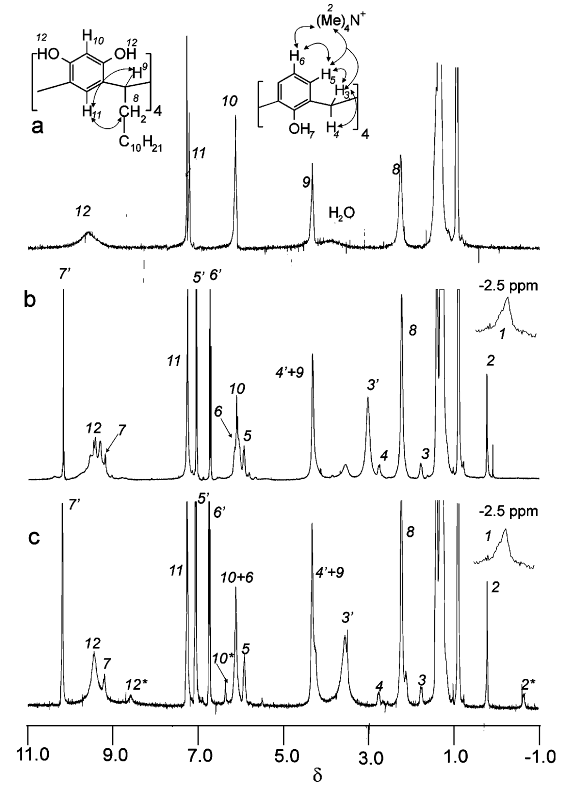

Figure 2. The ^{1}H NMR spectrum (CDCl$_3$, 500 MHz, 308 K, [**1b**] = 10^{-2} M) of (a) **1b** ([H$_2$O]/[**1b**] = 1.5); (b) 6 **1b** + 2 **2a** + $Me_4N^+I^-$ ([H$_2$O]/[**1b**] = 2.5); (c) 6**1b** + 6**2a** + $Me_4N^+I^-$ ([H$_2$O]/[**1b**] = 2). Assignments: *1* is the encapsulated water molecule; the signals of free **2a** are indicated by dashed numerals. The signals of 2**1b**·Me_4N^+ are indicated by asterisks. Integral ratios *1:2:3:4:5:*(*6*+*10*) = 2:12:4:4:8:28 (24+4). Selected intermolecular NOESY correlations 2−3, 2−4, 2−9, 2−5, 2*−10*, exchange cross-peak 2−2*.

volume of about 1.4 nm^3. Unfortunately, ^{1}H NMR spectroscopy revealed no evidence for such an encapsulation in wet CDCl$_3$ since the spectra of mixtures of **1b** and **2a** were identical to the superimpositions of the signals for the individual components. It seemed plausible that addition of tetramethylammonium cation, which is complementary to the calix[4]arene cavity,[8] might favor the formation of self-assembling hexameric capsules confining an inclusion complex **2a**·Me_4N^+.

Mixtures of resorcinarene **1b** and calix[4]arene **2a** extract insoluble $Me_4N^+I^-$ into wet CDCl$_3$, whereas the individual components *do not*. This is a macroscopic manifestation for the formation of highly lipophilic complex(es) between **1b**, **2a**, and $Me_4N^+I^-$. The ^{1}H NMR spectra reveal that the complexation does not affect considerably the position of the resorcinarene signals resulting only in the broadening of the OH resonances and the signals for protons at 2-positions of the resorcinol rings.

The NMR titration experiments[9] and 2D NOESY spectra revealed that the protons of calix[4]arene **2a** emerge as *two* sets of signals due to the formation of kinetically stable complex with **1b**. The axial protons of the methylene bridges of bound **2a** emerge as a broadened doublet (J = 14 Hz) at 2.7 ppm shifted upfield by 1.6 ppm (Figure 2b,c) likely as a result of the shielding effects of molecules **1b.** The broadened doublet for the equatorial protons of **2a** is centered at 1.7 ppm being shifted upfield by 2.0 ppm. The methylene protons of the bound **2a** give intermolecular NOEs to the protons of the methyne bridges of **1b**, alluding to their spatial proximity in the complex.

The 2D NOESY correlations and the H−D exchange experiments revealed that the broadened OH singlet of the encapsulated **2a** is located at 9.2 ppm upfield by 1.0 ppm from its normal position. The protons of the aromatic rings of the complexed **2a** emerge at 5.92 ($\Delta\delta = -1.2$ ppm) and 6.20 ppm ($\Delta\delta = -0.5$ ppm) according to NOESY correlations with the protons of the methylene bridges. The COSY technique revealed spin−spin interactions between these signals. The intensities of the NOESY cross peaks indicate that the more strongly shifted signal corresponds to the protons in ortho positions to the methylene bridges. Thus, the above results strongly suggest that the encapsulated calix[4]arene molecule exists in a cone conformation which is, most probably, stabilized by a cyclic seam of intramolecular hydrogen bonds.[10] It should be noted that the signals for the methylene protons of the encapsulated **2a** remained virtually unchanged upon rising the temperature to 323 K, whereas the signals of the external **2a** transformed into a broad singlet due to the fast conformational interconversion. This suggests that the encapsulation within the hexameric capsule results in the conformational stabilization of calix[4]arene molecules.

The broadened ^{1}H NMR signal of water ([H$_2$O]/[**1b**] = 2) is located at 3.5 ppm (NOESY assignment) suggesting that water molecules link the resorcinarene subunits by hydrogen bonds. As expected, this signal shifts up field upon an increase of water concentration. The NMR titration experiments revealed that *six* molecules of resorcinarene **1b** bind *one* calix[4]arene **2a**.[11] Thus, the above results provide strong evidence to the inclusion of one calix[4]arene molecule in the hexameric capsule, which is similar to the crystalline state assembly 6 **1a**·8H$_2$O (Figure 1). This conclusion is further strengthened by the fact that the neighboring protons of **1b** show strong negative NOEs characteristics for large slowly tumbling aggregates.[12,13] The dependence of the intramolecular NOEs on mixing time (0.2−0.6 s)[14] is identical to that of chloroform-filled hexameric capsules, whose size has been independently established by gradient spin echo experiments.[3] The NOEs between H5 and H6 are also negative (30%, tm = 0.4 s) apparently due to the tight binding of encapsulated **2a** to slowly tumbling hexameric nanocapsule. Quickly tumbling free calixarene is characterized by week positive NOEs between H5 and H6 (2%, tm = 0.4 c).

Titration experiments also revealed that the molecule of **2a** shares the hexameric cavity with one tetramethylammonium cation. The slightly broadened ^{1}H NMR signal for the encapsulated Me_4N^+ is centered at 0.18 ppm indicating strong magnetic shielding ($\Delta\delta = -3.0$ ppm), likely resulting from spatial proximity of the methyl protons to the shielding regions

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(8) Atwood, J. L.; Barbour, L. J.; Junk, P. C.; Orr, W. *Supramol. Chem.* **1995**, *5*, 105−108.

(9) The ^{1}H NMR spectra were measured at different ratios between **1b** and **2a**. Solid $Me_4N^+I^-$ was extracted into chloroform solution upon sonication for 8 h.

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(11) The stoichiometry was determined through integration of the ^{1}H NMR signals assigned via NOESY correlations. Automatic correction of integrals was used in MestreC2.3a program.

(12) Neuhaus, D.; Williamson, N. *The nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH Publishers Inc.: Weinheim, Germany, 1989.

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(14) Mestrec 4.7.0.0 program was used for integration of NOESY spectra.

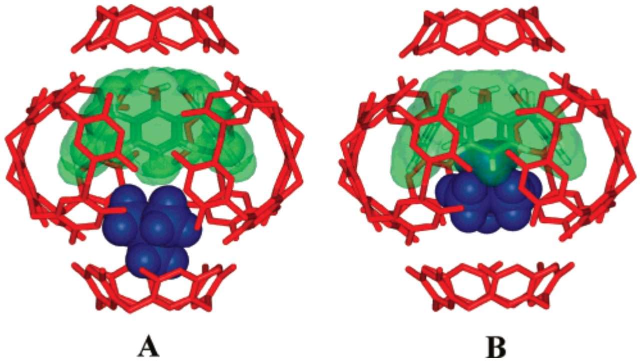

Figure 3. Energy optimized structures of **2a**·Me_4N + @6**1b**·$8H_2O$. Water molecules are omitted for clarity; transparent van der Waals surface of **2a** is shown.

of the capsule. Such a strong induced chemical shift is characteristic for the inclusion in the cramped cavities of dimeric molecular capsules[15] or monomeric resorcinarene based cavitands[16] rather than in large quarters of the nanosized hexamers.[3-5]

Molecular mechanics calculations[17] predict that one molecule of **2a** can be readily accommodated in the hexameric capsule in a face-to-face arrangement with one of the resorcinarene molecules (Figure 3). In such a disposition the protons of the aromatic rings in ortho positions to the methylene bridges of **2a** should reside within the shielding regions of molecules **1b**, a prediction corroborated by the experimentally observed induced chemical shifts (see above).

The residual free volume in the capsule ($V = 450$ Å^3) would permit the inclusion of Me_4N^+ ($V = 130$ Å^3). Apparently, the encapsulated Me_4N^+ can reside within either the resorcinarene (structure **A**) or calix[4]arene (structure **B**) cavities. The protons of the encapsulated Me_4N^+ give strong NOESY correlations with the protons of the aromatic rings and methylene groups of the encapsulated **2a**. The latter are 1.5 times weaker than the NOESY cross-peaks between protons of entrapped Me_4N^+ and the methyne protons of the resorcinarene bridges. These results suggest that the time-averaged structure in solution is represented more accurately by arrangement **B**. Thus, it can be concluded that the inclusion complex **2a**·Me_4N^+ is encapsulated by the hexameric resorcinarene nanocapsule.

The formation of the coencapsulation complexes was independent of the counterion whether $Me_4N^+Br^-$, Me_4N^+ BF_4^-, or $Me_4N^+TsO^-$ were used as guests. This result and the fact that there is no observable induced upfield shift for the ^{19}F NMR signal of BF_4^--anion suggest that the counterions are situated outside of the hexameric capsule. The residual space in the nanocavity is filled with one molecule of water, whose protons emerge in the ^1H NMR spectrum as a broadened singlet at −2.48 ppm (Figure 2b), which disappears upon H−D exchange with D_2O suspended in a chloroform solution of the assembly. Such a strong magnetic shielding ($\Delta\delta = -4.1$ ppm relative to the signal of "free" water in $CDCl_3$) indicates a very close proximity of the encapsulated water molecule to the diamagnetic currents of the capsule.[18] It should be noted that the signal of the encapsulated water gives weak 2D NOESY correlations with the protons at 2-positions of the resorcinol rings of **1b**. The NMR studies in $CHCl_3$ failed to prove the coencapsulation of chloroform within the hexameric capsule.[3]

As expected, the formation of the Russian-doll complexes is sensitive to the size of the calixarene component and guest cation. Namely, thiacalix[4]arene **2b**, which is nearly isosteric to calix[4]arene **2a**, forms the co-encapsulation complex in the presence of $Me_4N^+I^-$, whereas molecules of *p-tert*-butylcalix[4]arene and calix[4]arene tetramethylether are too bulky to be entrapped in the resorcinarene hexamer. On the other hand, Me_3SO^+ cation, which is isosteric to Me_4N^+, forms the coencapsulation complex with **2a**, whereas the larger Et_3NH^+ does not. In the latter case the hexameric capsule including three ion pairs $Et_3NH^+Cl^-$ is formed.[4a]

The addition of polar methanol-d_4 (10%) causes total disruption of the Russian-doll assemblies which is evidenced by the disappearance of the ^1H NMR signals for the encapsulated **2a**, Me_4N^+, and water. Tetrabutylammonium iodide *does not* substitute the encapsulated **2a**, Me_4N^+, and H_2O apparently owing to the lower stability of its hexameric capsular complex.[5a-c] Very high stability of the Russian doll complexes and insolubility of $Me_4N^+I^-$ in $CDCl_3$ hampered quantitative thermodynamic studies of the encapsulation.

The peaks of chemical exchange in NOESY spectra revealed a minor set of signals for resorcinarene **1b** and Me_4N^+ (Figure 2c) at [**1b**]/[**2a**]=1. The broadened ^1H NMR singlet of the cation is centered at −0.65 ppm ($\Delta\delta = -3.83$ ppm) apparently due to very close proximity of the methyl protons to the diamagnetic currents of the resorcinol rings. Integration of signals 2* and 10* revealed that one tetramethylammonium cation is bound by two molecules of resorcinarene. It seems plausible that the second set of signals corresponds to dimeric resorcinarene capsules entrapping Me_4N^+ in the cramped π-basic cavities. Such assemblies have been observed in the crystalline state;[18b,19] however, their solution studies were hampered by low solubility of the tetramethylammonium salts in nonpolar solvents.

In conclusion, the highly stable hexameric resorcinarene capsule reversibly entraps inclusion complexes of calix[4]arene and thiacalix[4]arene with Me_4N^+ and Me_3SO^+. Such an encapsulation provides the calixarene complexes with kinetic stability at ambient temperatures. Apparently, the simple approach described above provides an easy access to nanosized *Matryoshka* complexes, which might eventually include inorganic cations, small hydrocarbons or biologically active compounds like choline, cisplatine, etc. The improved kinetic stability of the encapsulated **2a**·Me_4N^+ demonstrates the potential of molecular encapsulation in providing a unique environment for investigations of molecular interactions and highlights the possibilities presented by supramolecular chemistry within nanospaces.[20]

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(17) The MM calculations have been carried out on the basis of crystallographic coordinates of 6**1a** ·$8H_2O$ (ref 2). MMX force field was used as implemented in : Gilbert, K. E.; *PCMODEL 7.50.00*; Serena Software: P.O. 3076, Bloomington, IN 47402.

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Experimental Section

Preparation of Assemblies. ^{1}H NMR spectra and 2D NOESY and COSY spectra were measured on Bruker DRX 500 spectrometer. To a solution of resorcinarene **1b** (33 mg) and calixarene **2a** (variable amount) dissolved in $CDCl_3$ (0.7 mL), one drop of water was added, and the solution was sonicated for 10 s. The chloroform layer was carefully separated from water by a pipet and was placed into an NMR tube. Solid tetramethylammonium salt was added, and the sample was sonicated for 8 h.

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

JA067402C

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(20) For recent example of inclusion of calixarenes and cavitands in the open-ended nanosized cavities see: Botana, E.; Da Silva, E.; Benet-Buchholz, J.; Ballester, P.; de Mendoza, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 198−201.