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Exploring the Self-Assembly of Polar Dimeric Capsules Using Molecular Rulers

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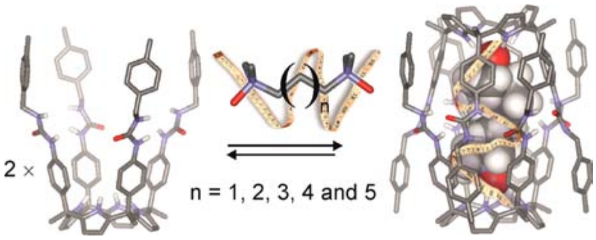
ABSTRACT



A homologous series of *N,N,N′,N′*-tetramethylalkyl-*N,N′*-dioxides is used to probe the level of guest induced fit attainable in the assembly of a dimeric capsule based on tetraurea benzyl calix[4]pyrrole components. A sensible enhancement of the capsule's volume was observed in response to guest size for *n* = 2, 3, and 4. In turn, larger guests adopted folded conformations to adapt to the capsule's dimensions.

In solution, the emergence of self-assembled molecular capsules stabilized by hydrogen bonding interactions requires the appropriate filling of their inner spaces. Mecozzi and Rebek formulated the 55% packing coefficient rule (the ratio of the guest volume to the host volume) to explain and predict efficient encapsulation processes.[1] Molecular modeling studies and X-ray crystallography data are useful for producing estimates of available volumes in the internal cavity of molecular containers.[2] However, variable results can be obtained depending on the parameters used to determine empty spaces, i.e. probe radii and grid spacing. The use of a homologous series of molecules, so-called molecular rulers, constitutes an experimental approach to probe the available space inside a molecular container.[3] One of us recently described the dimerization of tetraurea calix[4]pyrrole **1•1** induced by the pairwise encapsulation of trimethyl-*N*-oxide **2** (Figure 1).[4] The oxygen atom of each of the two included *N*-oxides **2** is hydrogen bonded to the four pyrrole NHs located at opposite ends of the capsule. The capsular assembly is stabilized by the formation of a belt of 16 hydrogen bonds between the eight urea functions, which are all oriented in the same direction. Previously, the self-assembly of **1•1** was also achieved using just one molecule of 4,4′-bipyridine-*N,N*′-dioxide **3** as a template.[5] We observed a significant variation in the estimated volumes for the empty internal cavities of **1•1**$\supset$**2**$_2$ (MM3 minimized structure, 359 Å^3) and **1•1**$\supset$**3** (X-ray structure, 312 Å^3), which suggested to us the existence of cavity size modulation through guest-induced fit. In the work described herein, we determine the level of guest induced fit achievable by the cavity of the capsular assembly **1•1**. To this end, we selected a homologous series of *N,N,N′,N′*-tetramethylalkyl-*N,N′*-dioxides **4** as potential encapsulation guests.

Guest series **4** represents a set of molecular rulers because, although its components are conformationally flexible, they possess easily measurable oxygen–oxygen distances that can be progressively varied by a single methylene unit. The dimethyl-*N*-oxide knobs placed at both termini of the rulers provide an ideal fit in terms of size, shape, and function for interacting with the inner functionalized

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[1] Mecozzi, S.; Rebek, J. *Chem.—Eur. J.* **1998**, *4*, 1016–1022.

[2] Swiss-PdbViewer 4.0.1 (Cavity) http://spdbv.vital-it.ch/ and Mercury CSD 3.0 (Voids) http://www.ccdc.cam.ac.uk/mercury/.

[3] Korner, S. K.; Tucci, F. C.; Rudkevich, D. M.; Heinz, T.; Rebek, J. *Chem.—Eur. J.* **2000**, *6*, 187–195.

[4] Gil-Ramirez, G.; Chas, M.; Ballester, P. *J. Am. Chem. Soc.* **2010**, *132*, 2520–2521.

[5] Ballester, P.; Gil-Ramirez, G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10455–10459.

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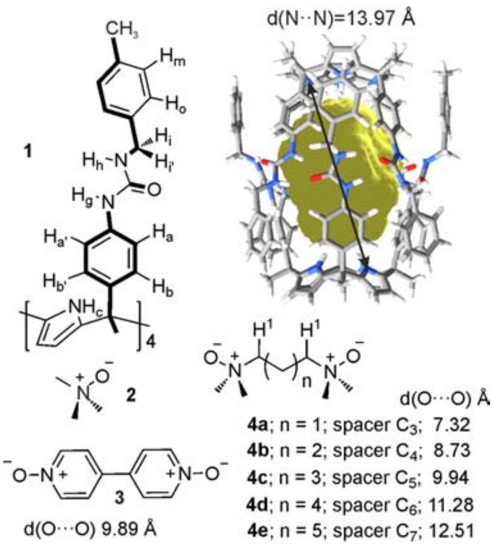

Figure 1. Line drawing structures of the tetraurea calix[4]pyrrole **1** and mono- and bis-*N*-oxides. Rendering of the MM3 minimized structure of the **1•1⊃2**$_2$ complex with the shape of the inner space calculated using Swiss-Pdb Viewer (~359 Å^3) after guest deletion.

cavities (poles) defined by the *meso*-phenyl calix[4]pyrrole units in **1•1**. From these studies, we expected to gain additional information on the conformational malleability of the bis-*N*-oxide series **4** caused by encapsulation in **1•1**. The induced fit of the host's cavity and the guest adaptability seem to be inevitably linked.[6−8]

N,N'-Dioxides **4a**, **4b**, and **4d** were obtained by oxidation of the corresponding commercially available diamines. Using literature procedures, the diamine that leads to **4c** was obtained by $LiAlH_4$ reduction of *N,N',N''*,*N'''*-tetramethylglutaramide (prepared by reacting dimethyl glutarate with dimethylamine and sodium methoxide), whereas the diamine that leads to **4e** was obtained by double nucleophilic substitution of 1,7-dibromoheptane with dimethylamine. The tetraurea calix[4]pyrrole **1** was prepared following a described procedure.[5]

Initial encapsulation experiments were performed by simply adding 0.5 equiv of bis-*N*-oxide **4a**−**e** to individual NMR tubes containing 1−2 mM suspensions of tetraurea **1** in $CDCl_3$. Only the addition of bis-*N*-oxides **4b**, **4c**, and **4d** (C_4−C_6) resulted in the rapid dissolution of the mixtures. Analyzing the resulting solutions by ^{1}H NMR spectroscopy revealed the existence of sharp and well resolved proton signals having the earmarks for the formation of the capsules **1•1⊃4b-d** with S_8 symmetry (Figure 2).[4]

Guest **4a** is too short to establish a simultaneous ditopic interaction with the two endohedral calix[4]pyrrole binding sites in **1•1**. Assuming a typical hydrogen bonding distance of 3.0 Å, the sum of the distance between the oxygen atoms in **4a** plus two hydrogen bonds (7.32 + 6.0 = 13.32 Å) is not enough to bridge the gap between the two binding sites (*d*(N··N) ≈ 14 Å, Figure 1). Similar back-of-the-envelope calculations suggest that **4b** and **4c** are suitable fits because, in their fully extended conformation, the sum of their O−O distance plus two hydrogen bonds matches the capsule's dimensions. The other two bis-*N*-oxides **4d**−**e** must adopt some folded conformation to fit into the capsule. Capsular assembly **1•1⊃4e** is not observed by experiment. The folding required for **4e** to adapt to the capsule's dimensions is probably too energetically costly and cannot be compensated by the gain in host−guest interactions provided by the self-assembly.

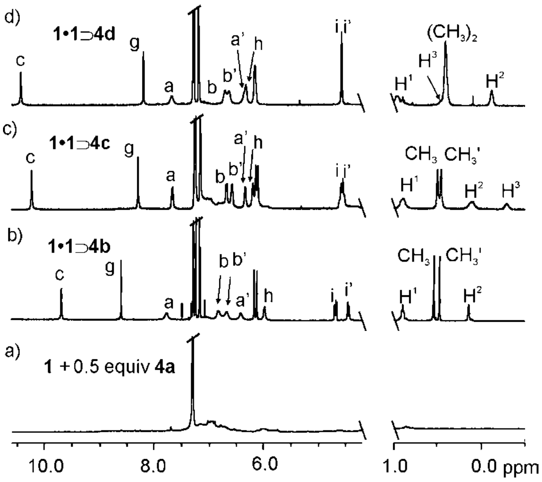

Figure 2. Selected upfield and downfield regions of the ^{1}H NMR spectra acquired from the $CDCl_3$ solutions obtained by mixing: (a) urea **1** and 0.5 equiv of **4a**; (b) urea **1** and 0.5 equiv of **4b**; (c) urea **1** and 0.5 equiv of **4c**; and (d) urea **1** and 0.5 equiv of **4d**. See Figure 1 for proton assignment. H^2 and H^3 correspond to methylene protons β and γ with respect to the N-atoms of **4**.

In the formed capsular assemblies, the observation of two sets of two separated doublets for the *meso*-phenyl *ortho* (a,a') and *meta* (b,b') protons with respect to the urea group is general; it originates from the asymmetry inherent in the unidirectional orientation of the eight urea groups and the slow interconversion between the two possible directional senses on the ^{1}H NMR time scale.[9] The difference in broadening between the proton signals of the *meso*-aromatic protons in the assemblies **1•1⊃4b**−**d** is due to slightly different rate constant values for interconverting the sense of direction of the capsules' urea belts. By means of EXSY experiments we determined k_{ex} = 20 ± 2 s^{-1} ($\Delta G^{\#}$ = 15.6 kcal/mol) for **1•1⊃4b**, k_{ex} = 10 ± 1 s^{-1} ($\Delta G^{\#}$ = 16.0 kcal/mol) for **1•1⊃4c**, and k_{ex} = 14 ± 1 s^{-1} ($\Delta G^{\#}$ = 15.8 kcal/mol) **1•1⊃4d**. These data suggested that the urea belt in **1•1⊃4c** is stabilized by slightly stronger hydrogen bonding interactions than in the other two capsules.[10]

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(6) Ajami, D.; Rebek, J. *Acc. Chem. Res.* **2012**10.1021/ar300038r.

(7) Mirtschin, S.; Slabon-Turski, A.; Scopelliti, R.; Velders, A. H.; Severin, K. *J. Am. Chem. Soc.* **2010**, *132*, 14004–14005.

(8) Gibb, C. L. D.; Gibb, B. C. *Chem. Commun.* **2007**, 1635–1637.

(9) The 3D nature of guests **4** makes the interconversion between aromatic *meso*-phenyl protons through rotation of the C_{meso}−C_{phenyl} bond to be sterically congested with a very high associated energy barrier.

We also noticed that, as the length of the guest was increased, the benzylic (H_h) and phenylic (H_g) NH protons of the urea groups in the **1•1** capsules shifted in opposite directions (Figure 2 and Table 1). How can this be? Molecular modeling (MM3) showed that longer guests must fold in order to fit into the **1•1** capsular assembly and bridge the gap between the two binding sites. This folding must somehow apply pressure to the ends and sides of the self-assembly forcing the two tetraurea units to slip partially in order to increase the dimensions of the capsule.[11] The separation of the urea units shortens and enhances the linearity of the hydrogen bonds between the carbonyl oxygens and the benzylic NHs but has the opposite effect for the hydrogen bonds with the phenylic NHs (Figure 3 and Table 1). Based on the energy barriers calculated for the interconversion of the urea belt direction and taking **1•1⊃4b** as reference, a putative increase in the strength of the benzylic NH hydrogen bond (shorter distance and better linearity) compensates for the potential weakening of the phenylic one (longer distance) in **1•1⊃4c–d** assemblies.

Interestingly, the pyrrole NH protons (H_c) showed a monotonic trend of downfield shifts in response to the guest's length. The longer the guest's alkyl chain, the deeper the terminal *N*-oxide knobs are positioned into the aromatic cavities of the two calix[4]pyrrole poles yielding shorter NH··O hydrogen bonds.

2D COSY experiments were very useful for the assignment of the proton signals of the bound guests. Possible conformations for the encapsulated guests in assemblies **1•1⊃4b, 1•1⊃4c**, and **1•1⊃4d** were derived from molecular modeling studies (SI). Guest **4b** (C_4 spacer) possesses a length compatible with the capsule's dimensions and can be accommodated in **1•1** in an almost fully extended conformation although a conformation with one gauche interaction also fits (Figure 3B).

Table 1. Chemical Shift Values (ppm) of Hydrogen Bonded Protons in the Urea Belt of the Assemblies and Averaged Geometric Parameters (distance, Å; angle, deg)

	1•1⊃4b	**1•1⊃4c**	**1•1⊃4d**
$\delta(H_h)$	5.91	6.20	6.29
$d(C{=}O \cdot\cdot H_hN)^a$	2.97 ± 0.01	2.95 ± 0.01	2.96 ± 0.01
angle $(O \cdot\cdot H_hN)^a$	160.0 ± 0.2	162.4 ± 0.4	162.2 ± 0.4
$\delta(H_g)$	8.57	8.28	8.15
$d(C{=}O \cdot\cdot H_gN)^a$	2.99 ± 0.01	3.02 ± 0.01	3.01 ± 0.02
angle $(O \cdot\cdot H_gN)^a$	159.0 ± 2.3	157.4 ± 0.9	158.3 ± 0.6
$d(Cent{-}Cent)^b$	13.54	13.54	13.67

[a] Measured in the MM3 energy minimized structures. [b] Distance between the two centroids defined by the four C_{meso} of each calixpyrrole.

Encapsulated **4b** showed four upfield shifted signals: two multiplets, one corresponding to the methylene protons (H^1) α to the guest nitrogen (δ = 0.88 ppm; $\Delta\delta$ = 2.42 ppm; 4H) and another β to the methylene (H^2) protons (δ = 1.21 ppm; $\Delta\delta$ = 1.95 ppm; 2H), and two singlets for the diastereotopic methyl protons of each *N*-oxide knob (δ = 0.45 and 0.51 ppm; $\Delta\delta$ = 2.8 ppm; 12H).[12] The methyl groups and the α methylene experienced the larger upfield shifts due to increased shielding by the aromatic groups at the poles of the cavity compared to its equator. The dimensions of guest **4c** are compatible with encapsulation in a fully extended conformation; however, molecular modeling suggested that the presence of one gauche interaction provides a more complementary guest conformation for hydrogen bonding with the polar ends in **1•1** (Figure 3B). Guest **4d** and **4e** are definitely too long to be encapsulated in an extended conformation and must fold in order to fit in the capsule. Coiling into a helix has been shown to be a suitable mechanism for long alkanes to decrease their length when included in cylindrical spaces related to the inner cavity of **1•1**.[13–15] The NOEs observed between the terminal methyl groups and H^3 provided a clear indication of the existence of gauche conformations in the four and five carbon atom chains of encapsulated **4c** and **4d**, respectively (Figure 3B). In the ^1H NMR spectra, the chemical shift change of the H^3 methylene protons is noticeably smaller for the longer spacer ($\Delta\delta$(**4c**) = 1.8 vs $\Delta\delta$(**4d**) = 1.0 ppm). This observation is in agreement with both the placement of the C_3 methylene protons (H^3) (Figure 2) of **4d** close to the center of the cavity, due to coiling of the alkyl spacer into a helical conformation, and the concomitant elongation and widening of the cavity induced by guest fitting.[16] It is worth noting that the induction level of diastereotopicity caused by the unidirectional orientation of the urea groups diminishes gradually with longer guests. In other words, the larger guest **4d** causes the disappearance of diastereotopic signals for both the methyl protons of the guest and the benzylic ones in the host. Most likely, the elongation and widening of the cavity are associated with a steady reduction of the "in-line" placement of urea carbonyl groups, decreasing the induced local anisotropy.

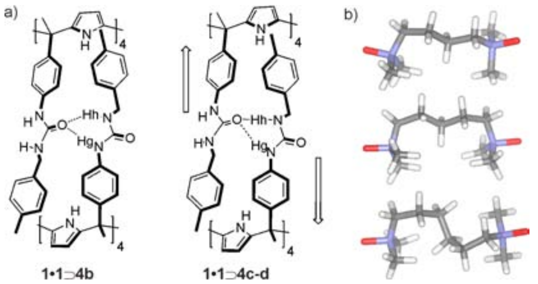

Figure 3. (a) Schematic representation of the effect caused by the capsule's enlargement on the urea belt. (b) Folded conformations adopted by the encapsulated guest in the energy minimized capsules **1•1⊃4b–d**.

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(10) We assume here that an increase in the rotational energy barrier correlates with an energy stabilization in the hydrogen bonded state. Cozzi, F.; Ponzini, F.; Annunziata, R.; Cinquini, M.; Siegel, J. S. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1019–1020.

(11) The length and shape of the capsule is slightly modified in response to the size of the guest by sliding and flexing the urea arms, respectively. Both processes have a similar effect on the hydrogen bonding network of the urea belt.

In order to assess this modulation of cavity size induced by guest fitting, we calculated the volumes of the encapsulation complexes **1•1⊃4** using Swiss-Pdb-Viewer. We calculated a linear increase in both volume size (354, 359, 365 Å^3) and the packing coefficient (PC) values (54, 60, 61) with the length of the spacer (**4b**C_4, **4c**C_5, **4d**C_6).[17] Addition of one methylene unit requires a volume increase of 5–6 Å^3. The calculated parameters for the **1•1⊃4e** complex (i.e., hydrogen bonds lengths, binding energy and PC, Figure S5) do not clearly exclude its experimental observation; however, we did not detect it. We surmise that the volume modulation exhibited by **1•1** could easily cope with the 6 Å^3 increase required for the encapsulation of **4e** but that the problem may be the conformation that **4e** must adopt to fit the cavity. The folding of the C_7 spacer of **4e** into a helical conformation suitable for encapsulation in **1•1** demanded the presence of up to five gauche interactions. The energy cost for adopting this twisted conformation of **4e** can be estimated as 3.0 kcal/mol. Favorable interactions in **1•1⊃4e** should compensate for this extra cost for the species to assemble. Differences in the energies provided by the 16 hydrogen bonds of the urea belts in the capsules **1•1⊃4b-c** accounts for less than 0.5 kcal/mol *(vide supra)*. Other gains in binding free energy arise from hydrogen bonding each of the two oxygen atoms of the *N*-oxide knobs to the NH pyrrole protons and other favorable capsule–guest CH–π interactions. In order to rank the binding affinities of guest **4b**–**d** and to gain some insight into their energy differences, we performed competitive experiments. We prepared $CDCl_3$ suspensions containing 0.5 equiv of the two competitive guests and 1 equiv of tetraurea **1**. To ensure that thermodynamic equilibria had been reached and to minimize solubility problems, we heated the mixtures at 60 °C for 2.5 h and then analyzed the solutions by ^{1}H NMR spectroscopy at room temperature. Integration of characteristic proton signals for the two competing capsular assemblies allowed us to derive the following relationships of relative binding affinities or stabilities: 100*K*(**1•1⊃4b**) = *K*(**1•1⊃4c**) = 45*K*(**1•1⊃4d**). In terms of energy, assembly **1•1⊃4c** was 2.7 and 2.2 kcal/mol more stable than **1•1⊃4b** and **1•1⊃4d**, respectively. The relative strength estimated for the hydrogen bonding of the urea belt (EXSY) correlates with the comparative binding affinity of guests. The drop of 2 kcal/mol in binding stability between **1•1⊃4c** and **1•1⊃4d**, after correcting for the energy difference in the hydrogen bonded belt, reflects an energetic penalty corresponding to roughly three gauche interactions for the encapsulated **4d** guest compared to **4c**. Despite the positive hints obtained from molecular modeling, the data account for why an increase in the number of gauche interactions combined with weakening of the hydrogen bonds in the urea belt effectively disrupts the formation of **1•1⊃4e**. In addition, the assembly encapsulating the flexible bis-*N*-oxide that is thermodynamically more stable (i.e., **1•1⊃4c**) was observed to be 84% of the equilibrium species in competition with trimethyl *N*-oxide **2**. However, it was not detected when bis-*N*-oxide **3** was used as a competitor. The fact that the four-particle aggregate **1•1⊃2**$_2$ is still assembled in competition with **4c** provided additional support for the presence of gauche interactions in this encapsulated guest.

We have shown that the assembly of dimeric capsule **1•1** can be templated by a series of bis(dimethyl-*N*-oxides) having alkyl chain spacers of different lengths. The capsule elongates and widens to accommodate the guest length. In turn, longer guests fold to adapt to the limiting dimensions of the capsule. The self-assembly adaptation process takes place as long as favorable binding interactions can compensate for the cost of a high-energy guest conformer to reside in the capsule's interior. The subtle balance of energies is not easy to deduce from simple molecular modeling studies.

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Supporting Information Available. CAChe minimized structures of **1•1⊃4a-e**, ^{1}H NMR spectra of the encapsulation complexes, and the pairwise competitive binding experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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(12) EXSY experiments revealed that the activation barrier for the interconversion between directional senses of the urea belt coincides with the interconversion barrier between diastereotopic methyl groups of encapsulated guests **4b** and **4c**, thus confirming the source of this latter asymmetry.

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(15) Jiang, W.; Ajami, D.; Rebek, J. *J. Am. Chem. Soc.* **2012**, *134*, 8070–8073.

(16) The coiling of the encapsulated guest probably has a reduced effect on its effective width due to the bulky *N*-oxide terminal knobs.

(17) Volumes and packing coefficients after energy minimization of the structures.