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Self-Sorting Dimerization of Tetraurea Calix[4]arenes

Damien Braekers,[†] Christelle Peters,[†] Anca Bogdan,[‡] Yuliya Rudzevich,[‡] Volker Böhmer,*[,‡] and Jean F. Desreux*[,†]

Coordination and Radiochemistry, University of Liège, Sart Tilman B16, B-4000 Liège, Belgium, and Abteilung Lehramt Chemie, Fachbereich Chemie, Pharamazie und Geowissenschaften, Johannes Gutenberg-Universität, D-55099 Mainz, Germany

jf.desreux@ulg.ac.be; vboehmer@uni-mainz.de

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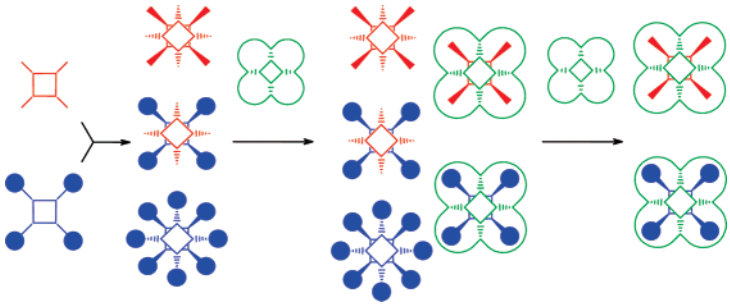

Calix[4]arenes substituted by four urea functions are self-complementary molecules that spontaneously combine in apolar solvents in the presence of an ammonium salt to form dimeric capsules held together by a belt of hydrogen bonds. In the presence of tetraethylammonium salts, the Et_4N^+ cation is included as a guest. The sorting between dimeric capsules formed in a mixture of calix[4]arenes directly depends on the steric crowding of the substituents grafted on the urea groups whether aromatic derivatives or aliphatic chains linking urea functions in mono-, di-, or tetraloop structures. Simple rules allow one to anticipate which capsules will be exclusively formed when calix[4]arenes are mixed in different proportions. The stabilization of the dimeric structures by hydrogen bonds is thwarted by the overlaps of aliphatic loops and/or by bulky groups that cannot pass through these loops. Despite the structural similarity of the calixarenes, the exclusive formation of dimers of well-defined compositions and clear titration breaks are observed by electrospray mass spectrometry. This technique yields reliable information on stoichiometries and composition despite measurements in the gas phase rather than in solution and it does not suffer from excessive peak overlaps in contrast with NMR.

Introduction

Molecular recognition is one of the basic principles of all forms of life and is the first step in fundamental reactions such as replication of DNA or transcription of RNA, etc. Nature combines in a highly sophisticated way a comparatively small number of basic recognition motifs (a surprisingly simple set of complementary structures) to reach an incredible variety/diversity of species. Therefore it is a particular challenge to develop an artificial set of very similar molecules which are able to distinguish themselves and to express this distinction into a result that can be monitored.

Calix[4]arenes substituted on their wide rim by four urea functions are self-complementary molecules. In apolar solvents they form quantitatively dimeric capsules held together by a belt of intermolecular hydrogen bonds involving alternately the urea groups of both calixarenes[1] (see Figure 1).

It has been shown that tetraurea calixarenes do not interact with another similar self-complementary system formed by triurea derivatives of triphenylmethanes.[2] This may not be very surprising since there is a mismatch between both molecular systems concerning the number of urea functions and the shape of the molecules.[3,4] However, there are already some selectivities

* Address correspondence to this author. Phone: +3243663501. Fax: +3243664736,
[†] University of Liège.
[‡] Johannes Gutenberg-Universität.

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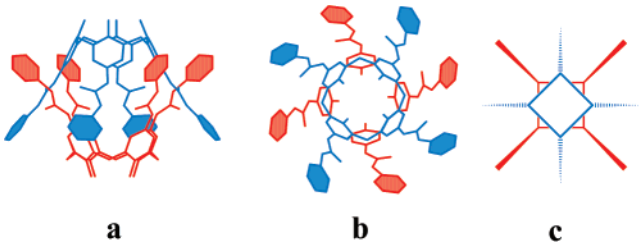

FIGURE 1. Side view (a) and top view (b) of a dimer and its sketchy representation (c) with aromatic arms of the lower and upper calix[4]-arenes pointing respectively upward and downward.

known for the dimerization of tetraurea calix[4]arenes themselves (see Chart 1).

Both, tetraaryl (**1**) and tetratosyl (**2**) ureas form homodimers, but a 1:1 mixture of both contains only the heterodimers **1**−**2**.[5] Tetraureas **3** derived from a rigidified calix[4]arene do not form heterodimers with **1** and a 1:1 mixture contains exclusively the two homodimers **1**·**1** and **3**·**3**.[6] Although not understood in detail in each case,[7] these observations have been used to synthesize di- and tetraloop tetraureas **5** and **6**, using **2** as template,[8] and to build up uniform dendritic structures via self-assembly.[9] **5** and **6**, on the other hand, do not form homodimers as this would require an overlap of the loops of the two calixarene units. The formation of ill-defined aggregates only is indicated by broad, featureless NMR spectra. However, **5** and **6** readily form heterodimers when mixed with **1a**, since this is the only possibility to incorporate each urea group into the cyclic hydrogen-bonded belt. This observation was the basis for the synthesis of various bis[2]catenane,[10] bis[3]catenane, and cyclic [8]catenanes[11] in high yields. In addition, the monoloop compound **4** forms only a single homodimer, in which again the two loops do not overlap.[10] Thus, the rational synthesis of well-defined bis[2]catenanes became possible, in which the two loops of each calix[4]arene are different.[12]

As outlined above the formation of dimers with a tetraurea calix[4]arene featuring aliphatic loops (**4**−**6**) requires the penetration of these loops by the linear urea residues of the other calixarene. This should be impossible for bulky residues of sufficient size. The incorporation of loops and bulky substituents could thus lead to novel selectivities for the dimerization of tetraurea calix[4]arenes. The following rules can be tentatively formulated: if adjacent urea functions are covalently linked by an aliphatic loop, only those dimers are formed (1) which do not require an overlap of loops and (2) for which the respective urea residues of one calixarene are small enough to pass through the loop(s) of the second calixarene.

Encouraged by preliminary results, we tried to develop a set of tetraurea derivatives with a gradual system of selectivities that can be understood by such simple rules. NMR spectroscopy proved to be the ideal method for unraveling the species distribution in a mixture of calixarene−urea macrocycles.[2,5,13−17] However, it becomes more and more difficult, if not impossible, to analyze the NMR spectra completely and to prove that only certain dimers are present if three, four, or even more different tetraurea molecules are mixed. We therefore developed an electrospray-mass (ESI-MS) spectroscopy protocol for the analysis of complex systems. In contrast to NMR spectra this approach should have the advantage that the presence of each species/dimer should be indicated by a single *m*/*e* peak that does not overlap with signals/peaks of similar compounds. A similar approach has already been used by Rebek et al.[5b] in supramolecular studies on calixarenes.

Results and Discussion

General Considerations. Two tetraurea calix[4]arenes are combined in a dimer via their wide rims and are turned by 45° around their common axis with respect to each other as shown in Figure 1. The hydrogen-bonded belt involves alternately the urea groups of the two calixarenes which act simultaneously as donor and acceptor.[14] Normally the NH_α attached to the urea residue forms a stronger hydrogen bond as indicated by a low field shift of the NMR signal.[17] Intermediates between dimer and monomer have not been observed, although the dissociation/association most likely does not occur in one step.[18]

Analysis by mass spectrometry requires (cat)ions which are often produced from neutral molecules by ionization directly in the mass spectrometer. The dimeric capsules of tetraurea calix[4]arenes necessarily include a guest, most often a solvent molecule, and are thus electrically neutral before being injected. However, organic cations are preferred as guests by calix[4]-arenes because of cation−π interactions. Therefore all ESI-MS experiments were conducted in the presence of tetraethylammonium hexafluorophosphate, which ensures that only dimeric capsules are charged (monomers do not include a guest) and that all capsular cations have the same guest and charge. For the present study we have chosen five tetraurea calix[4]arenes: two open chain compounds **1a** and **1b**, which differ by the size of their urea residues, a monoloop compound **4**, where two urea residues are connected to a macrocyclic ring, and two tetraloop compounds **6a** and **6b**, which differ by their ring size. Four series of "titration experiments" were performed in which ESI-MS were recorded for various amounts of one tetraurea added to another tetraurea or to a mixture of two ureas (see the formula survey in Chart 1).

Series I (Addition of 1a to 6a). This combination allows one to compare the results obtained by ESI-MS and ^{1}H NMR.

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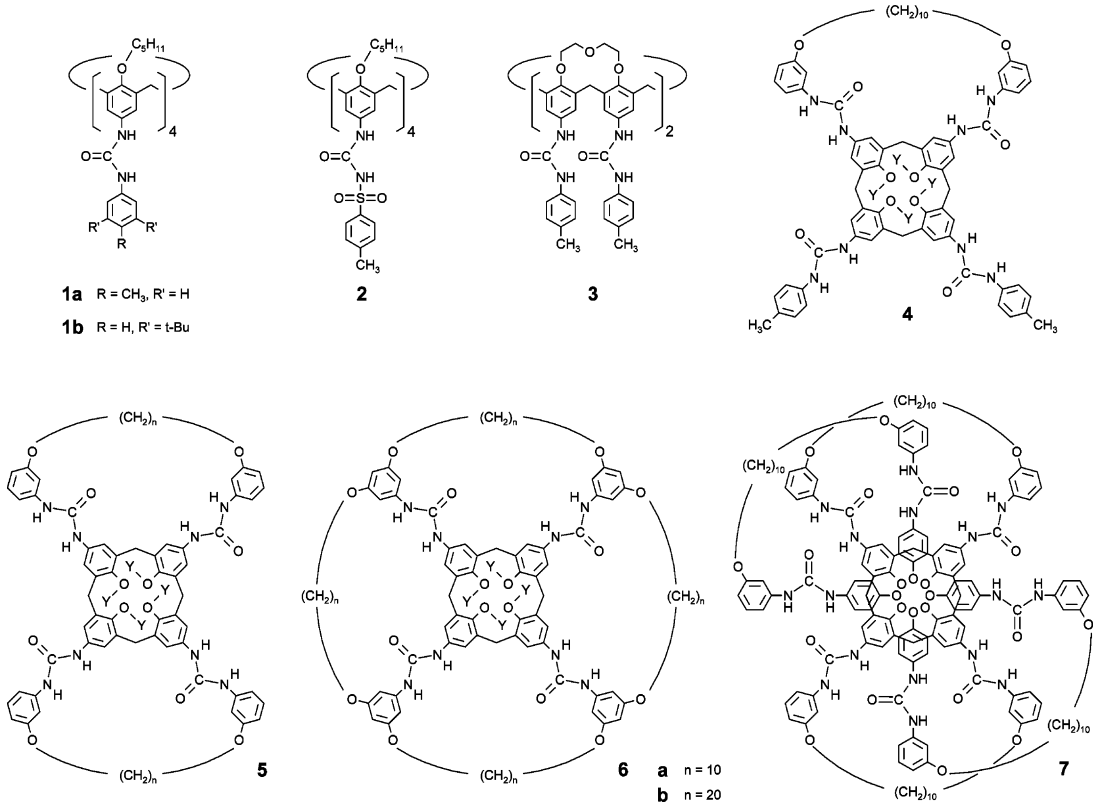
CHART 1. Structure of the Tetraurea Calix[4]arenes 1−6 and of the Bis[2]catenane 7 (Y = Pentyl)



Bis- or tetraloop tetraureas **5** or **6** do not form homodimers because the aliphatic chains connecting adjacent urea groups would have to overlap in a sterically very unfavorable arrangement (Rule 1). However, they easily form heterodimers with **1a** since the tolyl residues can pass through the macrocyclic rings. Accordingly, when **1a** is added to a solution of a tetracyclic calix[4]arene such as **6a** (Figure 2), the ^{1}H NMR signals for the homodimer **1a·1a** appear only after the addition of more than an equal amount of **1a** relative to **6a**. This NMR titration also proves the exclusive formation of the heterodimer **1a·6a** and the presence of the homodimer of **6a** can be safely excluded.

An ESI-MS titration of a **6a** solution by **1a** in chloroform is presented in Figure 3. The tetraethylammonium ion was the guest in this titration experiment while the solvent was most likely the guest in the NMR study. In addition, the ESI-MS measurements were conducted at much lower concentrations (about 10^{-7} M rather than 1 mM). Despite these differences, the results obtained by the two spectroscopic techniques are in complete agreement: the heterodimer [**1a·6a**] is the only species present at low concentration ratios c(**1a**)/c(**6a**) $\leq$ 1. The homodimer [**1a·1a**] is only observed when this concentration ratio becomes higher than one and no peak was found for the homodimer of tetraloop **6a** in the whole titration range.

It is noteworthy that the ESI-MS spectrum is exceedingly simple in all concentration conditions (see the Supporting Information). By contrast, the NMR spectra of systems such as **1a/6a** are rather complicated, and analogous peaks are not always as well separated as in Figure 2, especially in the presence of a tetraethylammonium salt. NMR spectra then become difficult to interpret although they can afford information that is impossible to obtain by ESI-MS such as the rate of reorientation of the intermolecular hydrogen bonds between the urea groups.[19] Both compounds, **1a** and **6a**, are C_{4v} symmetrical. If dimers are formed by less symmetrical tetraurea calixarenes the NMR spectra will be even more complicated.

It thus appears that ESI-MS can compete with NMR when one wants to unravel the stoichiometries and the nature of the dimeric capsules formed by calix[4]arene urea ligands despite the known difficulties associated with mass spectrometry, for instance perturbation of the equilibrium state and removal of solvent molecules during the ionization process or reactions in the gas phase.[20] Similar conclusions have been reached with other systems featuring metal ions.[20,21]

Series II (Addition of 6a to a Mixture of 1a and 4). More complex mixtures of calix[4]arene tetraureas lend themselves to ESI-MS analyses using tetraethylammonium as guest, provided special care is paid to kinetic phenomena (see the Experimental Section). A titration of a chloroform solution of **1a** and monoloop **4** by the tetraloop **6** is presented in Figure 4.

The starting mixture contains the heterodimer [**1a·4**] and similar concentrations of the homodimers [**1a·1a**] and [**4·4**]. The former is the most abundant species most probably for entropic reasons (it should, however, be remembered that each species has its own response in ESI-MS and that relative peak areas are not strictly proportional to concentrations). The addition of **6a** brings about a progressive decrease of the [**1a·4**] and [**1a·1a**] peaks while the [**4·4**] peak increases slightly and the new heterodimer [**1a·6a**] appears. At concentration ratios c(**6a**)/c(**1a**) equal or higher than one, [**1a·1a]** and [**1a·4]** are

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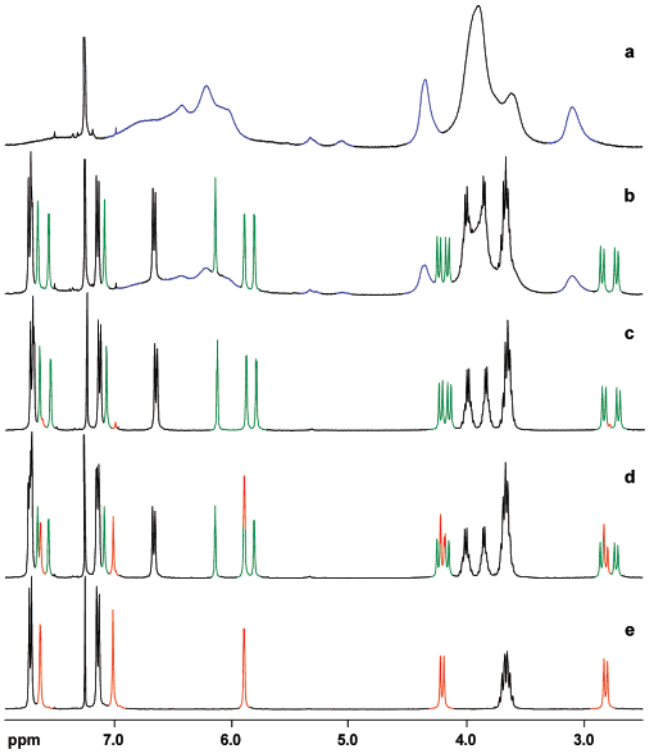

FIGURE 2. Sections of the ^{1}H NMR spectra (400 MHz, $CDCl_3$) of tetraloop **6a** (a), a 2:1 mixture of tetraloop **6a** and tetratolyl **1a** (b), a 1:1 mixture of **6a** and **1a** (c), a 1:2 mixture of **6a** and **1a**, (d) and **1a** alone (e). Peak assignments for compounds **1a** and **6a** are reported in refs 15 and 8, respectively.

entirely consumed by the formation of the heterodimer [**1a·6a**] in which the total amount of **1a** is incorporated while **4** forms exclusively a homodimer. This species distribution is entirely in keeping with the fact that all tetraurea compounds want to form hydrogen-bonded dimers in apolar solvent. Tetraloop compound **6a** can form neither homodimers nor heterodimers with monoloop compound **4** since the overlapping loops would violate Rule 1 mentioned above. Thus it has no choice but to form an heterodimeric capsule with **1a**. Monoloop **4** is then left alone and dimerizes. Should any [**1a·4**] remain as in the starting mixture, some **6** would then also be free and as it cannot homodimerize, it would remain "unpaired".

Series III and IV (Addition of 6a or 6b to a Mixture of 1a and 1b). The nature and distribution of the dimeric capsules drastically depend on the size of the loops grafted onto the calixarene ring and can be anticipated on the basis of the simple rules enunciated above. The tetraloop compounds **6a** and **6b** form neither homodimers nor heterodimers with each other because this would force two aliphatic chains to be in close contact ("overlapping loops"). Furthermore, the *tert*-butyl substituents of **1b** strongly augment the bulkiness of the urea residues attached to this calixarene and it seems unlikely that these groups will be able to intercalate through the small loops of **6a** while they might do so in the case of the larger loops of **6b**.

Figures 5 and 6 present the ESI-MS titrations of a mixture of **1a** and **1b** by **6a** and **6b**, respectively. As expected, the starting mixtures contain homodimers of **1a** and of **1b** as well as the heterodimer [**1a·1b**], which is the major component most likely because it is entropically favored. The addition of **6a** brings about the rapid growth of a peak due to the [**1a·6a**] capsule until the concentration ratio *c*(**6a**)/*c*(**1a**) equals one. Moreover, the peak due to the homodimer [**1b·1b**] also increases

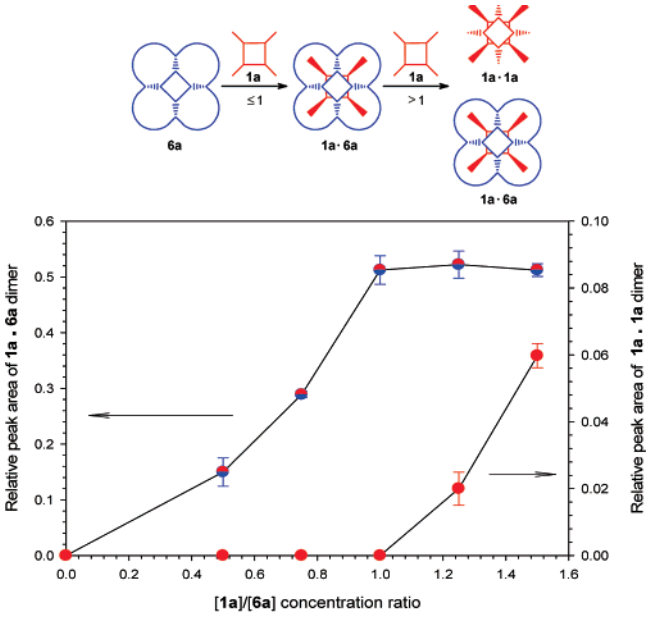

FIGURE 3. Sorting process and electrospray mass spectrometry titration of **6a** by **1a** in chloroform (red and blue circles: [**1a·6a**] dimer; red circles: [**1a·1a**] dimer). The peak area of each species was divided by the total ion charge. The sketchy presentation of the sorting process follows the scheme illustrated in Figure 1. The same colors have been used for each capsule and for the corresponding data points.

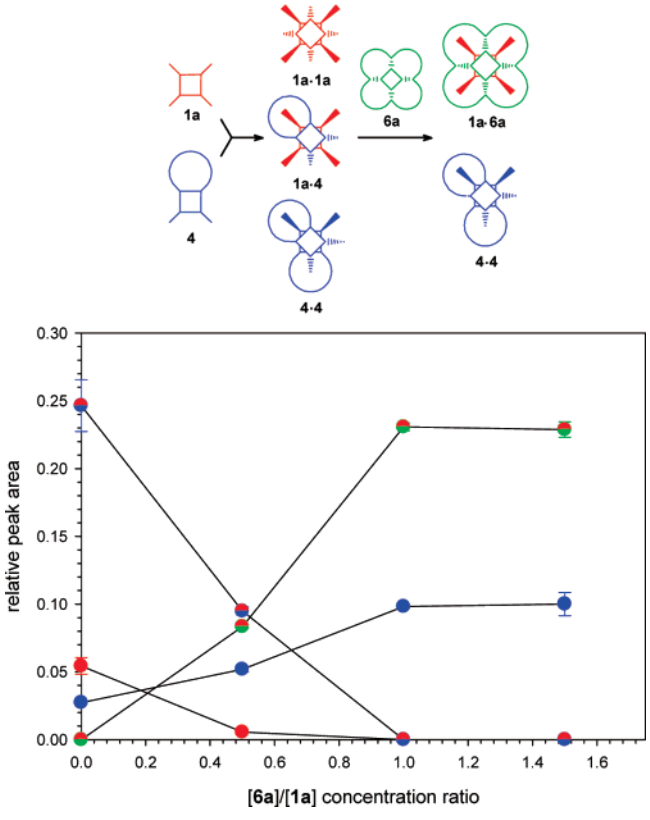

FIGURE 4. Sorting process and electrospray mass spectrometry titration of a mixture of **1a** and **4** by **6a** in chloroform (red and blue circle: [**1a·4**] dimer; red circle: [**1a·1a**] dimer; blue circle: [**4·4**]; red anf green circle: [**1a·6a**] dimer). The peak area of each species was divided by the peak area of reference **7**.

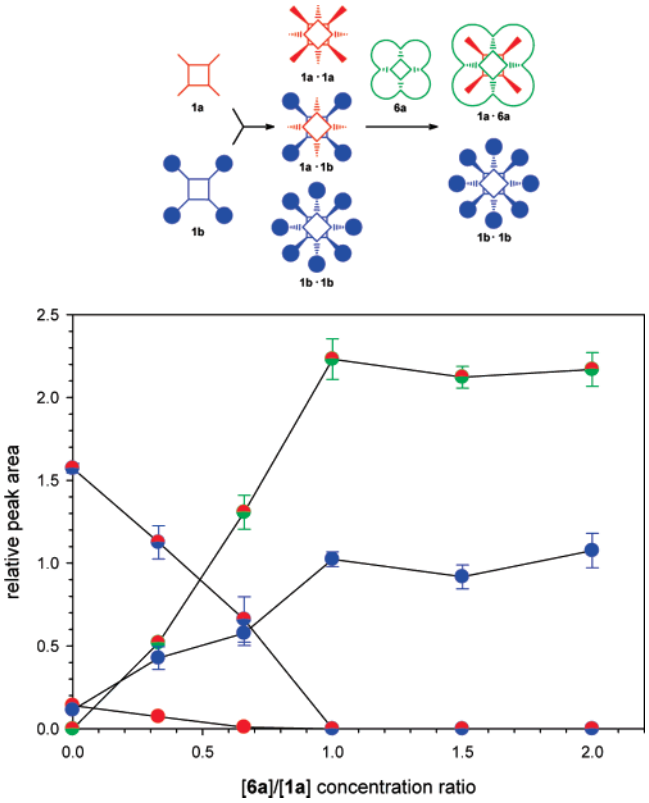

FIGURE 5. Sorting process and electrospray mass spectrometry titration of a mixture of **1a** and **1b** by **6a** in chloroform (red and blue circle: [**1a·1b**] dimer; red circle: [**1a·1a**] dimer; blue circle: [**1b·1b**] dimer; red and green circle: [**1a·6a**] dimer). The peak area of each species was divided by the peak area of reference **7**.

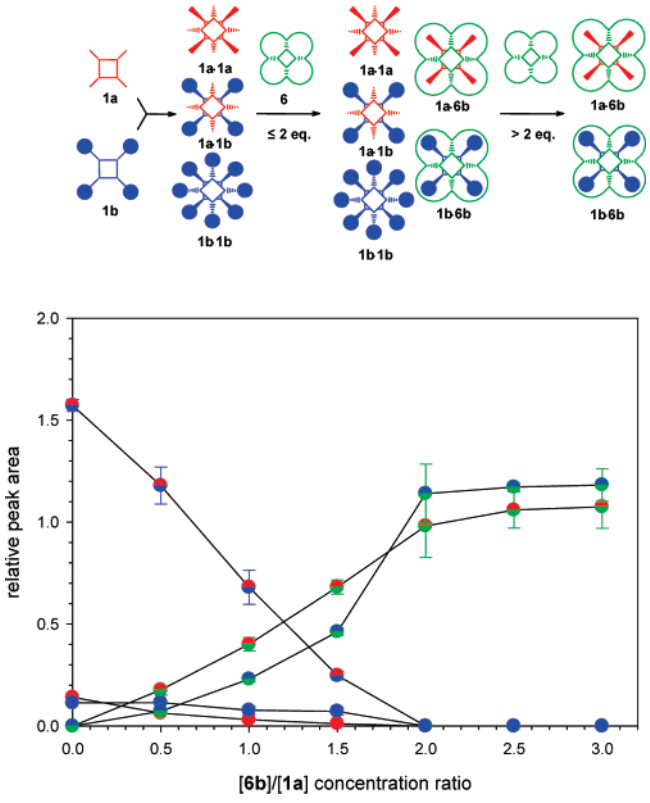

FIGURE 6. Sorting process and electrospray mass spectrometry titration of a mixture of **1a** and **1b** by **6b** in chloroform (red circle: [**1a·1a**] dimer; blue circle: [**1b·1b**] dimer; red and blue circle: [**1a·1b**]; red and green circle: [**1a·6b**] dimer; blue and green circle: [**1b·6b**] dimer). The peak area of each species was divided by the peak area of reference **7**.

but no heterodimeric capsule [**1b·6a]** is formed. This system can only evolve in one direction: **6a** cannot homodimerize nor can it form heterodimers with **1b**. Its only choice is to form a heterodimer with **1a**. Left alone, **1b** associates with itself. In principle this combination is analogous to the previous example: the starting mixture contains all three dimers while the added tetraloop **6a** cannot homodimerize. This shows that the combination of bulky residues with loops can indeed prevent the formation of dimers, as well as the combination of overlapping loops. These results fully confirm the validity of Rule 2.

As shown in Figure 6, the outcome of the ESI-MS titrations is quite different if the aliphatic loops are larger and more flexible but the line of reasoning is the same. Because of its much larger loops, **6b** can form mixed dimers with both **1a** *and* **1b** and as in the preceding cases, it will form dimers so as to have all its urea groups involved in the most favorable arrangement for hydrogen bonding. **6b** will thus associate with any suitable molecule, namely **1a** and **1b**, at the expense of their homo- and heterodimers present in the starting mixture. In contrast to experiment III, plateaus will only be obtained for a concentration ratio c(**6b**)/c(**1a**) ≥ 2 because **6b** cannot form a homodimer and associates with both simple calix[4]arenes **1a** and **1b**.

Conclusions

Tetraurea calix[4]arenes have the remarkable ability to form highly stable capsules in chloroform even in highly diluted solutions. These capsules are stabilized by an intricate network of intermolecular hydrogen bonds between the urea groups. In the presence of an ammonium salt they contain a Et_4N^+ cation as guest. Relatively small structural changes profoundly modify self-sorting processes in which the steric crowding of the urea substituents seems to play the major role. Rigidification with ether chains,[6] crowding with one or several aliphatic loops, or replacing methyl groups by bulkier *tert*-butyl moieties prevent or favor the formation of homo- or heterodimeric structures according to simple rules. One can thus anticipate the outcome of simply mixing together urea calix[4]arenes featuring the same macrocyclic core and obtain capsules of well-defined composition.

The sorting processes investigated here emulate biological systems in the sense that they are based on a spontaneous self-assembly approach combined with a host−guest interaction with one single substrate that takes place between entities differing only by their substituents. Electrospray mass spectrometry proved particularly suitable for the investigation of these sortings. This technique yields the same speciation of the capsules as NMR for simple mixtures but also allows unraveling more complex systems for which the overlapping of NMR resonances would preclude any detailed study.

Experimental Section

Chloroform (Chromasolv Plus for HPLC) was dried over molecular sieves. Tetraethylammonium hexafluorophosphate and all calixarenes were dried under vacuum at 80 °C over 24 h. Stock solutions of the calix[4]arenes (8×10^{-4} M in chloroform) were

prepared by accurate weighing using an analytical balance in a glovebox under argon atmosphere. All dilutions were performed with an automatic pipet for organic solvents.

Samples of the stock solutions were mixed together in 1.5 mL glass vials with Teflon caps in the proportions that were needed to cover the full ESI-MS titration curves. Two equivalents of tetraethylammonium hexafluorophosphate per calix[4]arene was added to each sample and all experiments remained at room temperature until the equilibrium was reached (between 1 and 25 days depending on the experiment). Solutions were diluted 1600 times before injection to reach a 10^{-7} M concentration in each species. Three different samples were prepared for each data point in the titration experiments in order to take into account dilution and mass spectrometer experimental errors. The ammonium salt of bis[2]catenane **7**[11,14] was used as an internal reference when the kinetics of the sorting process was slow. Catenane **7** is a rigid entity that does not take part in exchanges with **1**−**6**.[11,14] The concentration of **7** was adjusted to obtain a peak intensity comparable to the intensities of the various dimeric species and was kept constant throughout each titration experiment. All peak areas were divided by the peak area of the bis[2]catenane **7**. Although this procedure is not absolutely necessary, it leads to an improvement of the titration curves.

Samples were analyzed by direct injection into an electrospray mass spectrometer with a syringe pump (glass syringe with a stainless steel needle) at a 3 μL/min flow rate. All spectra were recorded in the positive mode. The nitrogen flow rate was set to 4 L/min and its temperature was 200 °C. Optimization over a mass range *m*/*z* 2000−6000 was performed using the following parameters: capillary voltage 4500 V, capillary exit voltage 300 V, first skimmer voltage 100 V, transfer and prepulse storage times 124 and 40.6 μs, respectively, repetition rate 8000 kHz. A total of 2×10^5 scans were recorded per measurement to reach a high signal-to-noise ratio. Spectra were recorded only when the total ion charge (TIC) was stabilized (between 5 and 10 min). The total area of each dimer peak and of reference **7** was calculated by adding all the peak areas in each isotopic pattern. The average relative peak areas were computed for each set of three measurements by dividing the peak area of each dimer by the area of reference **7**.

Derivatives **1a**,[15] **2**,[13] **3**,[6] and **5**[16] were obtained as reported. Compounds **1b**,[15] **4**,[10] and **6a**,**b**[8] were prepared in analogy to described examples.

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Supporting Information Available: Representative electrospray mass spectra for each ESI-MS titration experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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