Structural Examination of Supramolecular Architectures by Electrospray Ionization Mass Spectrometry

Christoph A. Schalley,[a] José M. Rivera,[a,b] Tomás Martín,[a] Javier Santamaría,[a] Gary Siuzdak,[a] and Julius Rebek, Jr.*[a]

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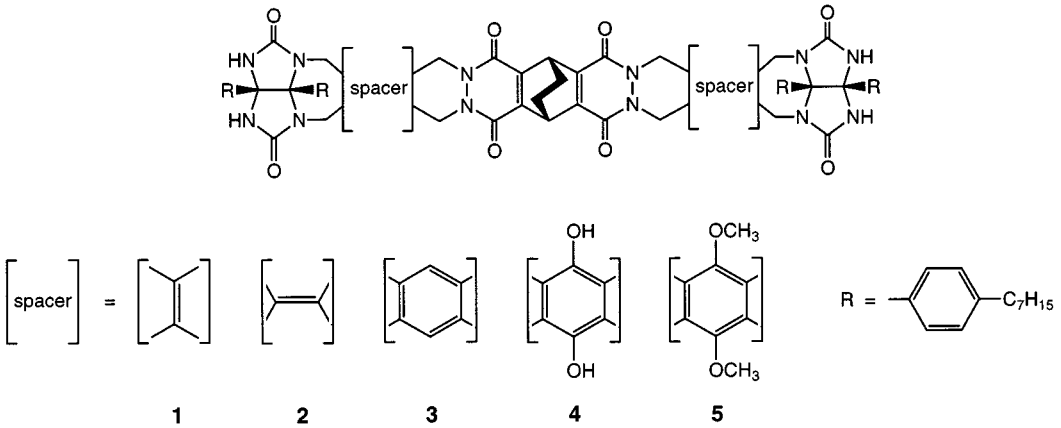
Reversibly self-assembling host–guest complexes of the softball type are characterized by electrospray ionization mass spectrometry. Quaternary ammonium ions serve simultaneously as guests and ion labels. Isotope pattern analysis, inclusion of labeled guests, heterodimer experiments, size and shape dependence, and collision-induced fragmentation reveal that the capsular structure of these hydrogen-bonded complexes is retained in the gas phase with the guests inside their cavity. The results parallel findings from solution-phase NMR experiments and show that the guests are encapsulated inside the cavity. These results also demonstrate the value of mass spectrometry for high-order structural examination of supramolecular architecture.

Introduction

Self-complementary molecules **1**−**4** (Scheme 1) assemble to form dimeric capsules **1•1**−**4•4** in solution. They feature reversible encapsulation of smaller organic guest molecules to give molecule-within-molecule complexes.[1] Extensive NMR studies have revealed many details about the encapsulation process in solution,[1][2] including catalytic processes[3] and chiral recognition.[4] In view of larger[5] and less symmetrical capsules[6] currently under study, the detection of these inclusion complexes by NMR alone becomes more and more difficult due to the complex spectra. A second, independent analytical method for structure determination is therefore desirable.

teractions and supramolecular complexes of a large variety have been intensively studied,[9] including protein/protein interactions,[10] enzyme/substrate and enzyme/inhibitor complexes,[11] assemblies of DNA with drugs, proteins, and oligonucleotides,[12] supramolecular metal complexes,[13] knots and catenanes,[14] carcerand/guest assemblies,[9a−9c] and cyclodextrin complexes.[15] Even gas-phase micelles[16] and whole viruses[17] have been studied. Consequently, a mass-spectrometric approach seems promising for the characterization of our capsules. However, in the area of organic self-assembly only a limited number of applications has been reported with ion-labeling methods such as the attachment of crown ether−Na^+ complexes,[18] metal ions,[19] or anions[20] for the characterization of aggregates



Since the introduction of electrospray ionization mass spectrometry (ESI-MS)[8] in the late 1980s, noncovalent interactions by MS. This may be due to the lower binding constants of these species than of, for example, protein/substrate complexes. Furthermore, common ESI-MS solvents like methanol or water interfere with the hydrogen bonds of the assemblies.

In this paper, we present an MS-based protocol which allows for the generation of gaseous ionic, noncovalently bound encapsulation complexes and also helps to charac-

[a] The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute,
10550 North Torrey Pines Road, La Jolla, CA 92037, USA
Fax: (internat.) +1-619/784-2876
E-mail: jrebek@scripps.edu
[b] Department of Chemistry, The Massachusetts Institute of Technology,
Cambridge, MA 02139, USA

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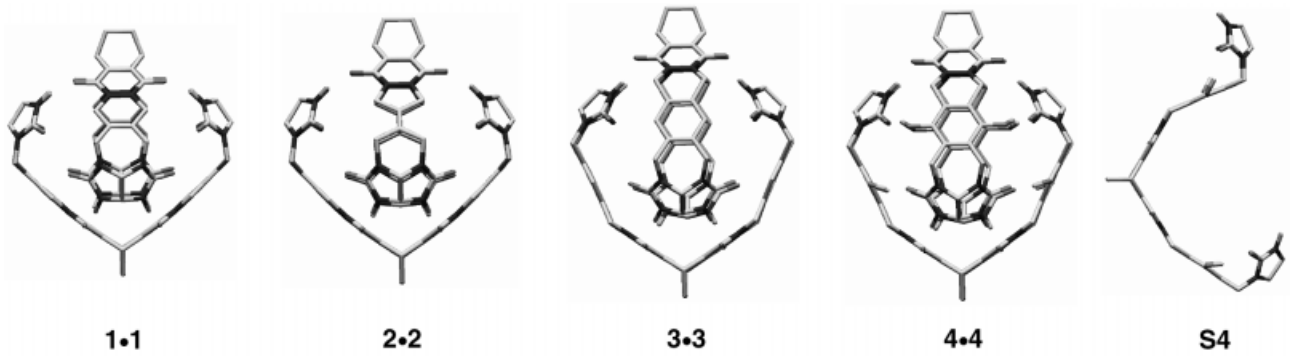


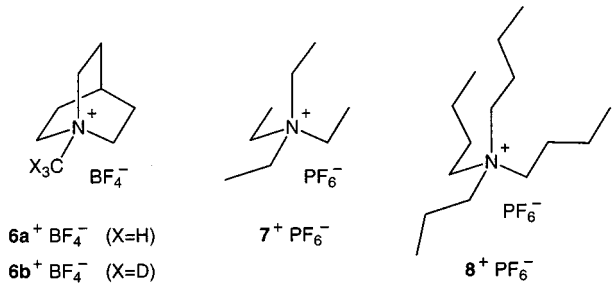
Scheme 1. Series of softball monomers **1**−**5** and optimized (Amber* force field, MacroModel 5.5)[7] geometries of dimeric capsules **1•1**−**4•4** and S-shaped isomer **S4** of the hydroxy softball **4**; R groups and carbon-bound hydrogen atoms are omitted for clarity

terize the capsular gas-phase ion structure.[21] Clearly, these results indicate that mass spectrometry is an independent technique capable of offering valuable information about the structures of supramolecular architectures.[22]

Results and Discussion

As ionization technique, electrospray ionization was chosen, because it is one of the softest methods available to date.[8] Strictly speaking, mass spectrometry only reflects the properties of gas-phase species. Nevertheless, the correlation between gas phase and solution is often good for ESI-MS and, consequently, this method has recently been used to analyze solution-phase aggregation processes.[19b,23] As a prerequisite for the generation of capsule ions, standard solvents commonly employed in ESI-MS, i.e. methanol and water, have to be replaced by solvents that do not act as hydrogen-bridge donors or acceptors. While methanol and water produce ions by protonation, this is not possible with aprotic solvents. Thus, the use of non-competitive organic solvents (e.g. $CHCl_3$, CH_2Cl_2, benzene) for ESI-MS requires an ion-labeling strategy and the presence of preformed ions in solution prior to the electrospray process. The most elegant way of ion-labeling the softballs under study is the encapsulation of charged guests,[24] e.g. quaternary ammonium ions $\mathbf{6}^+$−$\mathbf{8}^+$ (Scheme 2). In size and shape $\mathbf{6}^+$ and $\mathbf{7}^+$ are comparable to adamantane which was found earlier to be a guest for the softballs.[25] In contrast, $\mathbf{8}^+$ is expected to be too large to fit into the cavity and thus serves as a control. This ion-labeling strategy makes sure that the seam of hydrogen bridges remains intact. Finally, the counterions must be carefully chosen, because they have to provide solubility of the ammonium salts in organic solvents like chloroform without interfering with the seam of hydrogen bonds. Therefore, weakly coordinating anions like BF_4^- and PF_6^- are used in this study.

Before discussing the results obtained with this approach, let us briefly outline the three-step protocol for the determination of the capsules' gas-phase ion structures: (i) The correct elemental composition and charge state is established from a comparison of the measured isotope pattern with that calculated on the basis of natural abundances. Repetition of the experiment with a deuterium-labeled guest should lead to a mass shift which corresponds to the product of the number of deuterium atoms present in the guest times the number of guests present in the assembly (e.g. $\Delta m = 3$ for encapsulation of one guest $\mathbf{6b}^+$ instead of $\mathbf{6a}^+$). Thus, the numbers of guest ions can easily be determined. From the difference between the observed mass and the mass of the guest, the number of softball monomers incorporated in the assembly can be derived. In conclusion, these experiments ensure the correct elemental composition and number of each subunit. (ii) Once the correct composition is established, evidence for the hydrogen-bonded nature of the complexes can be gained from control experiments with methanol added to the sample, which should disrupt the hydrogen bonds and make the capsule signals disappear. The formation of heterodimers from a mixture of two different softball monomers is another strong indication for the formation of hydrogen-bonded species and has earlier been used as a criterion for capsule formation.[25b,26] (iii) The final and most important step is to distinguish the capsular structure with the ammonium ion as a guest *inside* the cavity from any other unspecific complex of two softball monomers with the guest cation, e.g. an empty cavity with the ammonium ion attached on the *outside*. Although structure determination by mass spectrometry is indirect, the following experiments should provide insight: In case of true host−guest complexes, the corresponding signals should be sensitive to the shape of the softball monomers as well as the size of the guest cation. Thus, **5**, which is known not to dimerize,[3b] and the S-shaped monomer **S4** (Scheme 1)



Scheme 2. Tetrafluoroborate and hexafluorophosphate salts of guests **6a**,**b**$^+$, **7**$^+$, and **8**$^+$

should not give rise to 2:1 complexes of monomers and guest. Furthermore, due to its large size, $\mathbf{8}^+$ (Scheme 2) is expected to be a bad guest or no guest at all. These experiments use the different geometrical requirements of capsules and unspecific complexes to distinguish these structures from each other. Another criterion might be the energy demand for guest release from the inner cavity or the outside of the complex. Thus, additional support for the capsular structure may come from collision-induced fragmentations of the ions.[15b,27] In these experiments, the ions are collided with gas molecules inside the instrument and part of the kinetic energy is converted into rovibrational excitation finally leading to fragmentations of a fraction of the ions.

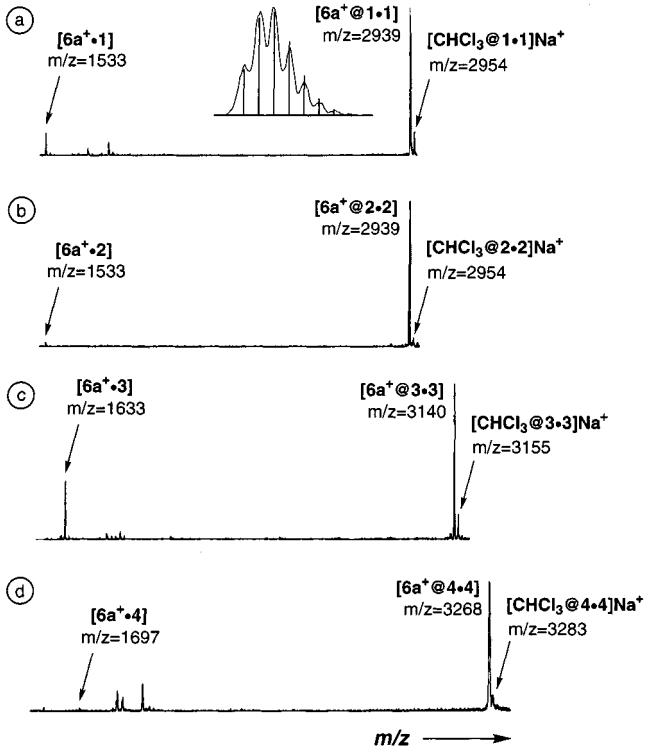


Figure 1. Electrospray mass spectra of 50 μM $CHCl_3$ solutions of (a) **1**, (b) **2**, (c) **3**, and (d) **4** with $\mathbf{6a}^+BF_4^-$ as guest salt (75 μM); the inset shows the measured and calculated isotope pattern for ions **[6a$^+$@1•1]** (the "@" sign indicates encapsulation of the guests); note that not only the ammonium ions under study, but also chloroform can act as a guest; this is indicated by signals for **[$CHCl_3$@1•1]Na$^+$** − **[$CHCl_3$@4•4]Na$^+$**

Figure 1 shows the mass spectra obtained by electrospray ionization of chloroform solutions of $\mathbf{6a}^+BF_4^-$ and softballs **1**−**4**, respectively. A pattern common to all four of these spectra emerges. The base peaks correspond to complexes of $\mathbf{6a}^+$ with two units of **1**−**4** (**[6a$^+$@1•1]**, *m/z* = 2939; **[6a$^+$@2•2]**, *m/z* = 2939; **[6a$^+$@3•3]**, *m/z* = 3140; **[6a$^+$@4•4]**, *m/z* = 3268). In addition smaller signals are visible for monomer−guest complexes (**[6a$^+$•1]**, *m/z* = 1533; **[6a$^+$•2]**, *m/z* = 1533; **[6a$^+$•3]**, *m/z* = 1633; **[6a$^+$•4]**, *m/z* = 1697).[28] Surprisingly, the signals for the charged complexes are accompanied by peaks for capsules containing $CHCl_3$ as the guest with background sodium ions providing the charge. The appearance of these ions in the spectra suggests that capsules with neutral guests can also be observed by MS. The elemental composition of ions **[6a$^+$@1•1]** and **[6a$^+$@2•2]** has been confirmed by the analysis of the isotope patterns, which are in line with those calculated from natural isotope abundances (Figure 1a). The distance between two isotope peaks of $\Delta m = 1$ demonstrates that the ions are singly charged. Together with this information, mass shifts of $\Delta m = 3$ with $[D_3]$-labeled $\mathbf{6b}^+$ instead of $\mathbf{6a}^+$ as the guest clearly establish the 2:1 complexes of softball monomers and guest cation.

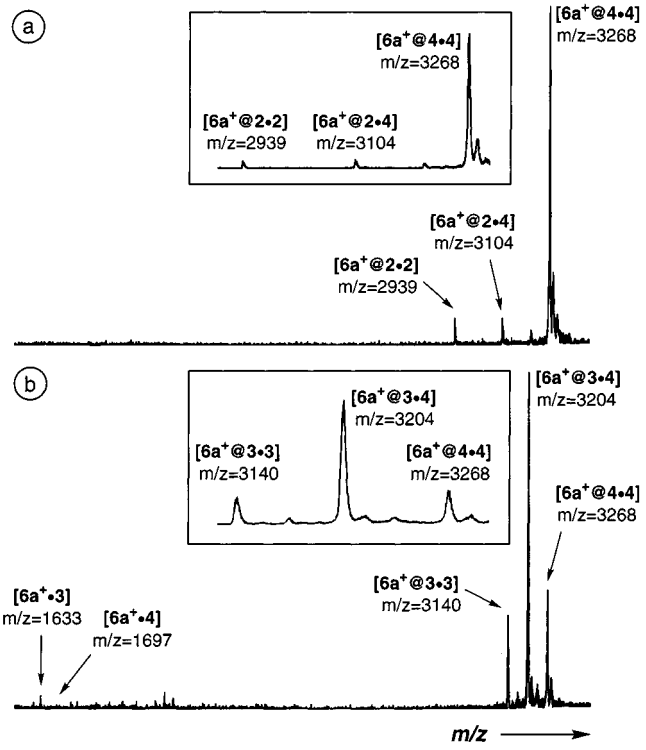


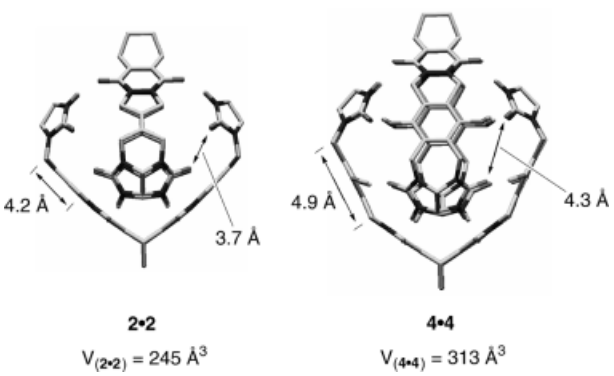
Figure 2. Electrospray mass spectra of chloroform solutions of (a) **2** (25 μM) and **4** (25 μM) and (b) **3** (25 μM) and **4** (25 μM) with $\mathbf{6a}^+BF_4^-$ as guest salt; the inset shows the dimer regions of these spectra separately scanned for higher quality

Upon addition of methanol, the signals for the capsules vanish completely. Intense peaks for the protonated monomers and much less intense signals for proton-bridged dimers without any guests are observed instead. Ionic guests would, of course, be disfavored by charge repulsion with the proton. The absence of neutral guests such as $CHCl_3$ in the proton-bridged dimers suggests that the structure of these aggregates is different from a capsule. Rather unspecific dimerization may be the case which is not unusual in the type of MS experiments discussed here. To further support the hydrogen-bonded nature, heterodimer experiments have been performed. Indeed, the ESI mass spectra of equimolar mixtures of **2** and **4** (Figure 2a) or **3** and **4** (Figure 2b) with $\mathbf{6a}^+$ confirm the presence of heterodimers **[6a$^+$@2•4]** (*m/z* = 3104) and **[6a$^+$@3•4]** (*m/z* = 3204). The relative intensities of the homo- and heterodimers show effects which are related to the geometrical properties of the softballs. Although the samples contain equimolar amounts of the two softballs, the abundances of ions **[6a$^+$@2•2]**,

[6a$^+$@2•4], and **[6a$^+$@4•4]** (Figure 2a) are far from the statistical 1:2:1 ratio. Seemingly, the assembly of the larger capsule **4•4** is favored over **2•2**. Three possible explanations may account for the lower intensity of **[6a$^+$@2•2]** than of **[6a$^+$@4•4]**. (i) According to an earlier report,[25b] the dipole-dipole repulsion between the glycoluril carbonyl oxygen atoms of the two monomers (Scheme 3) may disfavor the assembly of **[6a$^+$@2•2]**. Modeling results[25b] show the distance between these oxygen atoms to amount to 3.7 Å for **2•2** in contrast to 4.3 Å for **4•4**. The larger distance results in reduced repulsion and more facile assembly for the latter. The dipole-dipole repulsion in **4•4** is further reduced by the involvement of the carbonyl oxygen atoms in hydrogen bridges. (ii) The different volumes of **2•2** and **4•4** (Scheme 3) may also play a role. While **2•2** is smaller and gives a defined capsule with $CHCl_3$ as guest (see the sharp signals in the NMR spectrum below), **4•4** probably is too large to accomodate $CHCl_3$ and assembles in an unspecific manner. This is indicated by broader signals in the ^{1}H-NMR of a $CHCl_3$ solution of **4**. Consequently, the charged guest competes with the solvent to a larger extent for **2•2** than for **4•4**, which would result in a lower intensity of the **[6a$^+$@2•2]** signal. (iii) The larger number of hydrogen bonds in **4•4** as compared to **2•2** could account for the observed effect, because they stabilize the capsule. However, a ca. 1:1 ratio of the two homodimers **[6a$^+$@3•3]** and **[6a$^+$@4•4]** (Figure 2b) is observed, which bear the same number of hydrogen bonds as **[6a$^+$@2•2]** and **[6a$^+$@4•4]**. If **[6a$^+$@4•4]** were more stable than **[6a$^+$@3•3]** because of the larger number of hydrogen bonds, we would expect a similar intensity distribution as found for the **2/4** couple. Thus, the almost statistical ratio of **[6a$^+$@3•3]** and **[6a$^+$@4•4]** in Figure 2b rules out this possibility. As an additional effect, which does not affect the homodimers, the difference in size of **2** and **4** (Scheme 3) disfavors the heterodimer **[6a$^+$@2•4]** due to a bad fit of the complementary hydrogen-bonding sites.

In contrast, the process of heterodimerization should be more or less thermoneutral for **3** and **4** and thus lead to a statistical mixture. Both monomers are of the same size and should fit together well. Cleavage of 8 and 16 hydrogen bonds in **3•3** and **4•4**, respectively, is balanced by the formation of 2 × 12 hydrogen bonds for the two heterodimers **3•4**. Indeed, the intensities of **[6a$^+$@3•3]**, **[6a$^+$@3•4]**, and **[6a$^+$@4•4]** (Figure 2b) are close to the statistical 1:2:1 ratio.[29] The slightly higher abundance of the heterodimer might be rationalized with a favorable interaction of the charged guest with the inherent dipole of the heterodimer. Accordingly, these observations strongly support the presence of hydrogen-bonded complexes in the gas phase and already reflect properties which are in better agreement with the formation of capsules than with unspecifically bound complexes.

While the lower relative intensities of the monomer-cation complexes **[6a$^+$•1a]**−**[6a$^+$•4]** (Figure 1) already indicate that the dimers bind the cations more strongly than the monomers, several control experiments were carried out in order to provide further evidence for a capsule-like structure of the ions: (i) All attempts to generate signals for dimeric host−guest complexes with the non-assembling S-shaped isomer **S4** of monomeric **4** failed. (ii) Similarly, **5** is known from previous studies not to form capsules due to its sterically too demanding methoxy substituents.[3b] As expected, no signal was observed for **[6a$^+$@5•5]** in the ESI mass spectrum. (iii) Competition experiments with **3** and the guests **7**$^+$ and **8**$^+$, which should have similar (non-specific) binding properties as compared to **6a**$^+$, showed that both **6a**$^+$ and **7**$^+$ are good guests of the softball **3•3**. In contrast, the signal for **[8$^+$@3•3]** ions vanishes within the noise. These studies indicate the formation of the capsule ions to require a strict complementarity in both size and shape between the two host monomers and the guest cation.



Scheme 3. Some geometrical properties of **2•2** and **4•4**;[25b] The cavity volumes of Amber*-optimized structures were calculated with the GRASP program[30]

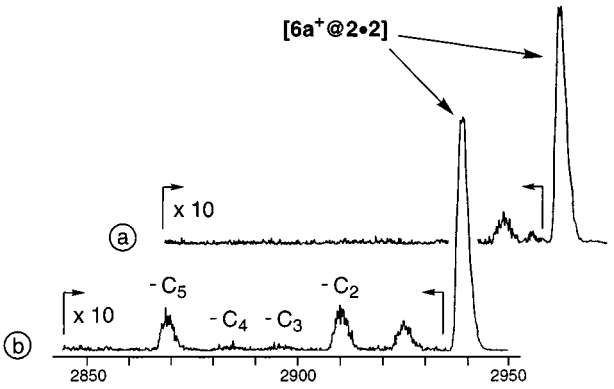
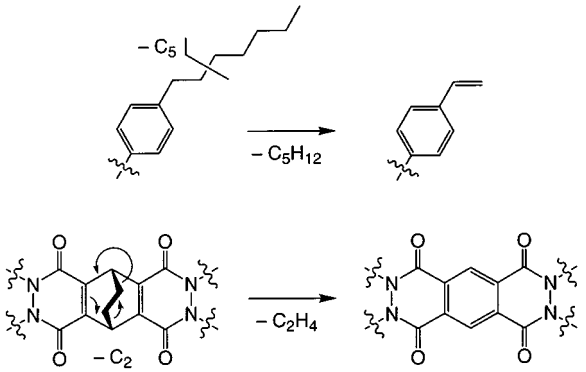


Figure 3. (a) Detail of the electrospray mass spectrum of a 50 μM $CHCl_3$ solution of **2** with **6a**$^+$$BF_4^-$; (b) same experiment with an additional acceleration voltage of −100 V on the octapole of the LCQ instrument (source-CID experiment); the same mass range (m/z = 2850−2950) is shown for both spectra

The most conclusive evidence for the capsular structure, however, is provided by collisional activation of **[6a$^+$@2•2]** ions in the ion source. In these source-CID experiments, the ions are accelerated and collide with gas molecules present in the region between the ion source and the octapole of the LCQ ion trap instrument (see Experimental Section). In these collisions part of the kinetic energy is converted into internal energy, which finally leads to fragmentations.[31] It is important to note that all ions produced in the ion source



Scheme 4. Collision-induced processes which lead to the losses of C_2 and C_5 fragments

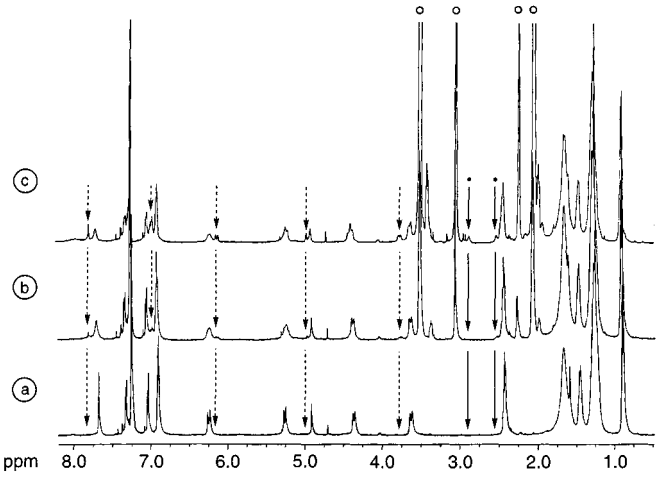


Figure 4. ^{1}H-NMR spectra (600 MHz, $CDCl_3$) of (a) **2** (ca. 1 mM), (b) **2** with 15 equiv. **6a**$^+$$BF_4^-$, and (c) **2** with a large excess of **6a**$^+$$BF_4^-$; dashed arrows indicate signals of the softball which increase in intensity upon addition of the ammonium salt, solid arrows (•) indicate signals tentatively assigned to the encapsulated guest; the signals for the free guest are labeled with open circles (o)

undergo this process. For **[6a$^+$@2•2]**, losses of C_2-C_5 units are observed (Figure 3b), which are not present when the acceleration voltage is switched off while all other conditions are kept constant (Figure 3a). Assuming that the cleavage of more than one covalent bond is unlikely, these fragmentations must occur in one of the *n*-heptylphenyl side chains of the capsules. All other parts of the capsule and the guest consist of cyclic systems and cleavage of, for example, a C_5 unit from these parts would require an even higher internal energy for the cleavage of more than one covalent bond. Tentatively, we assign the two predominant fragmentations leading to the C_2 and C_5 losses to the two processes outlined in Scheme 4. Both pathways leave quite favorable structures, i.e. a double bond conjugated to the aromatic phenyl group of one of the *n*-heptylphenyl side chains and an aromatic ring in the softball′s central unit. The charge, and thus the guest, is still present in the complex. Consequently, the cleavage of C−C bonds is competitive with the release of the guest. This indicates a much higher energy demand for guest release than would be expected for any unspecific binding of the guest by noncovalent forces. Since the guest must be bound to the monomer in **[6a$^+$•2]** by weak interactions instead of encapsulation, this complex may serve as a control. Indeed, no covalent fragments are observed for **[6a$^+$•2]** upon collisional activation. Here, guest release is the only low-energy pathway available for fragmentation. Evidently, these results rule out structural possibilities in which the guest is bound to the outer surface of any dimer by cation-π interactions[32] or other weak forces and we safely conclude that **[6a$^+$@2•2]** bears the capsular structure.

At this point, it is clear that mass spectrometry provides qualitative structural information about the softballs without any help from other analytical techniques. Nevertheless, as the protocol presented here is new, a substantiation of the MS results by NMR experiments is necessary.[1,25,26] Figure 4 shows the ^{1}H-NMR spectra of chloroform solutions of **2** without guest (Figure 4a), with 15 equiv. of **6a**$^+$$BF_4^-$ (Figure 4b) and a large excess of this guest added (Figure 4c). The spectrum is sharp as long as no guest is present and suggests the formation of a defined assembly such as a solvent-filled softball **2•2**. Upon addition of **6a**$^+$$BF_4^-$, the signals broaden, probably due to the change of polarity; and new signals appear close to the former capsule peaks which indicate a new softball species (dashed arrows). At least two peaks appear, which, according to their integration, do not correspond to other nearby capsule signals (solid arrows). Their upfield chemical shift relative to the signals of the free guest′s $N-CH_3$ and $N-CH_2$ groups suggests that these signals correspond to protons of the encapsulated ammonium ion, which are shielded by the capsule walls. The other two signals of the encapsulated guest are probably buried under the large peaks associated with the aliphatic protons of the capsule. These results are in good agreement with the MS data presented above in that **6a**$^+$ is acting as a guest. However, the new species represents only a minor fraction of monomer **2**. It is not possible to determine the binding constant from these NMR experiments, but − taking into account the large excess of the guest salt − it must be rather low. This points to another aspect of the mass-spectrometric protocol presented here. As none of the neutral species present in solution − e.g. the monomer or the dimer containing a solvent molecule as guest − can be detected, the ESI-MS procedure also acts as a filter. Only charged particles can be observed, and those are the free guest, the monomer−guest complex and the charged capsule. In conclusion, the sensitivity of the MS approach is better than that of the NMR experiments.

Conclusions

The encapsulation of cations in the softballs can be detected by ESI-MS which provides a method for their characterization independent from and complementary to NMR studies. Encapsulation of **6a**$^+$ could be detected by

NMR for **2•2** in chloroform solution, although only a small amount of the capsules containing the charged guest is present in solution. Accordingly, mass spectrometry is more sensitive for the detection of these complexes, since no neutral species are detected. However, the most important aspect of this report is that the architecture of the capsules can be determined from mass-spectrometric experiments. Two sets of results helped to distinguish the capsular structure from nonspecifically bound complexes: (i) the dependence of the signal for the capsules on the size and shape of host and guest subunits and (ii) the particular covalent and noncovalent fragmentations observed upon collisional activation. In the past few years, mass spectrometry has become a valuable tool for the characterization of biopolymer noncovalent interactions. We believe that − as these studies show − mass spectrometry will also become equally important in characterizing supramolecular architecture.

Experimental Section

The syntheses of the softballs **1**−**5** have been described previously.[2b,3b,25b] Salts $\mathbf{7}^+PF_6^-$ and $\mathbf{8}^+PF_6^-$ are commercially available from Aldrich Chemical Company; $\mathbf{6a},\mathbf{b}^+BF_4^-$ were prepared by methylation of quinuclidine with CH_3I (CD_3I) followed by exchange of the iodide against BF_4^- with $AgBF_4$.

The ESI-MS experiments were performed on a single quadrupole Perkin−Elmer API-100 Sciex (mass range < 3000) and a Finnigan MAT LCQ ion trap instrument (mass range < 4000). The samples were introduced as chloroform solutions at flow rates of 4−6 μL/min. The ion intensities increased with the ion spray and the orifice potentials, which were set to 4−5 kV and 100−200 V, respectively. Changes in the flow rate and the gas flows of nebulizer, auxiliary, or sheath gases did not change the ion intensities substantially. To improve the signal-to-noise ratio, 50−200 scans were accumulated. Due to the lower resolution of the LCQ spectrometer meaningful isotope patterns could only be obtained with the API-100 instrument, i.e. for capsules **[G$^+$@1•1]** and **[G$^+$@2•2]** only.

The control experiments with **S4** and **5** were performed by optimizing the conditions for **[6a$^+$@4•4]** and, after purification of the inlet system, introducing the sample of **S4** or **5**, respectively. In order to make sure that the conditions did not change during these measurements, the experiments with **[6a$^+$@4•4]** were repeated afterwards and showed excellent reproducibility.

For competition experiments, equimolar mixtures of two different guests (each 25 μM) with the capsule monomer **2** (50 μM) were introduced to the ion source of the Perkin−Elmer instrument and the experiments performed as described above.

Collision (source-CID) experiments[15b,27] were carried out with the LCQ mass spectrometer in the region between the skimmer and the octapole. For collision-induced decay (CID) the potential of the octapole was set to a voltage of −100 V relative to the grounded skimmer. The solvent or air molecules present in this region provided the collision gas. It should be noted that all ions produced in the ion source are subjected to the same collision conditions in these experiment.

^{1}H-NMR spectra were recorded with a 600-MHz Bruker DRX-600 spectrometer with $CDCl_3$ as solvent, internal lock, and internal reference (δ = 7.26).

Acknowledgments

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