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Review

Supramolecular chemistry goes gas phase: the mass spectrometric examination of noncovalent interactions in host–guest chemistry and molecular recognition

Christoph A. Schalley*

Kekulé-Institut für Organische Chemie und Biochemie der Universität, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany

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Abstract

This review deals with the examination of supramolecular architectures by mass spectrometric methodology. Aspects of ion generation, structure determination, thermochemical data determination, the analysis of stereochemical features, and the mechanistic pathways of ion generation and fragmentation in the gas phase are discussed. (Int J Mass Spectrom 194 (2000) 11–39) © 2000 Elsevier Science B.V.

Keywords: Supramolecular chemistry; Mass spectrometry; Host–guest complexes

1. Introduction

Nature makes extensive use of weak interactions such as π–π forces, cation–π interactions, and hydrogen bonding in order to control and to fine tune structures and functions of the species involved in many important biological processes. With the development of soft ionization techniques—fast atom bombardment (FAB) [1–4], matrix-assisted laser desorption ionization (MALDI) [5–8], and in particular electrospray ionization (ESI) [9–11]—the mass spectrometric examination of large molecules and their noncovalent interactions with binding partners became feasible. An increasing number of studies on biomolecules deals with topics such as protein–protein, protein–substrate, and enzyme-inhibitor binding as well as DNA base pairing or DNA-drug complexation [12–16]. Even whole viruses have been studied by mass spectrometry, of which the tobacco mosaic virus constitutes a highlight in this area with its 2130 noncovalently bound capsid proteins and its mass of more than 40 mil Dalton [17,18].

Supramolecular chemistry [19,20] has been defined by Lehn [20] as "the chemistry of the noncovalent bond" and may thus be considered as the nonnatural, man-made equivalent of this area of chemistry. This chemistry involves the study of the binding of substrates to synthetic receptors, catalysis by artificial enzymes and enzyme models, self-assembly, self-

* E-mail: c.schalley@uni-bonn.de

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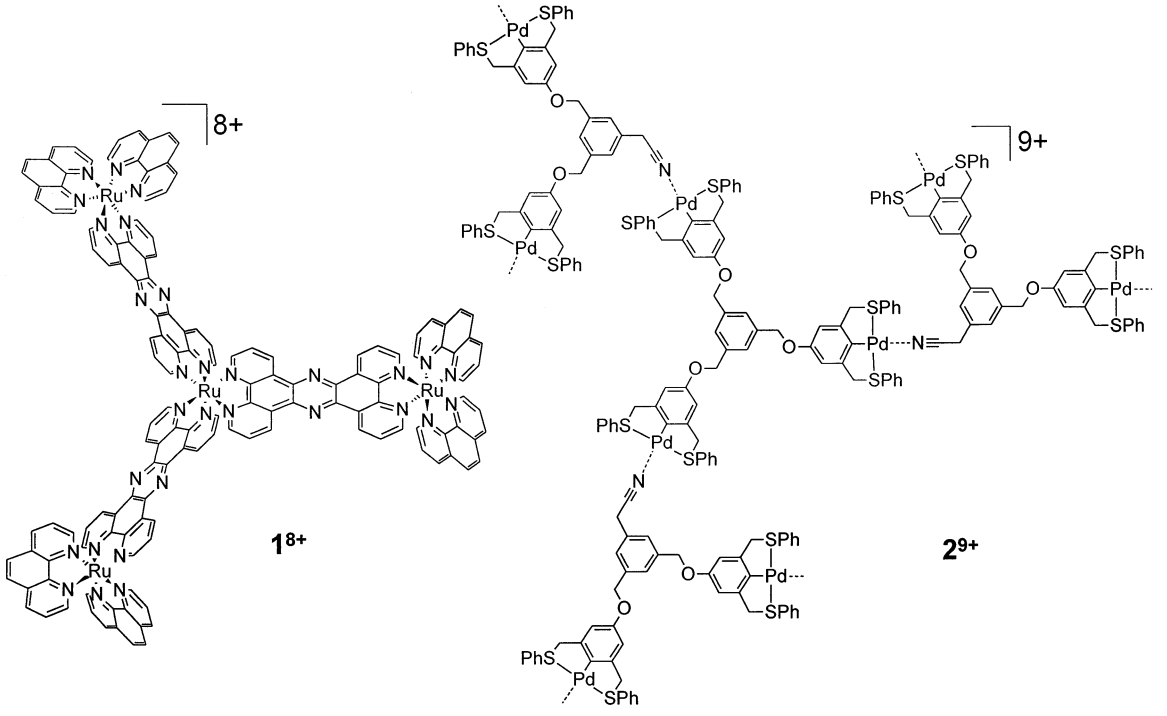
replication, and other processes that involve noncovalent interactions. It is an explosively growing field that combines a large variety of methods for the characterization of the species involved. During the past decade, mass spectrometry has become an increasingly important tool in a growing number of such studies that frequently made use of the soft ionization methods mentioned previously [13,21–25]. The present article intends to give an overview over this fascinating area of gas-phase chemistry and discusses the topics of ion generation and ion labeling strategies, structure determination, ways of gathering thermochemical data in the gas phase, stereochemical features, and gas-phase reactions with the corresponding mechanistic implications. The discussion of mass spectrometry methodology is kept brief throughout and the reader is referred to the original literature. Instead, the focus will be on chemical problems in the area of supramolecular chemistry that can be approached with the large potential of mass spectrometric experiments.

2. Ion generation: determination of mass, elemental composition, and charge state

One of the prerequisites for a mass spectrometric characterization of supramolecular entities is that they can be successfully charged and transferred into the gas phase without destruction. This section discusses ion labeling techniques and gives an overview of studies that used mass spectrometry as an analytical tool for determining the molecular masses of the aggregates. In most of these studies, the structural features and other aspects were investigated by methods like UV or NMR spectroscopy and x-ray crystallography. Nevertheless, molecular weight data are quite important, because they allow one to determine stoichiometry and often yield information on the numbers of each subunit in the complex. Mass spectrometry is capable of determining accurate masses and thus has a clear advantage over other methods such as vapor-phase osmometry, size exclusion chromatography (SEC) or gel permeation chromotography (GPC), or light scattering, which give only rough estimates for the molecular weights. In addition, it is possible to confirm mass spectrometrically the elemental composition of a certain ion by comparing the experimental isotope pattern to that calculated on the basis of natural abundances or by measuring the exact mass within an error range of a few parts per million. Furthermore, the charge state of a certain ion can be derived from the peak distances of the isotope pattern which are $\Delta m = 1/n$, if n represents the number of charges in the complex. One of the most severe problems is that unspecific binding might occur, which gives rise to complexes larger than those found in solution. In other words, the mass spectrometric data must be interpreted carefully. Any structural assignment, for example, necessarily remains speculative, if only the mass-to-charge ratio and the elemental composition are known. Other experimental methods should be—and usually are—used to determine the exact structure of the complexes under study. That said, let us now discuss some examples roughly in the order of decreasing binding energies—a brief list that is by no means complete and represents just a selection of interesting and beautiful molecules.

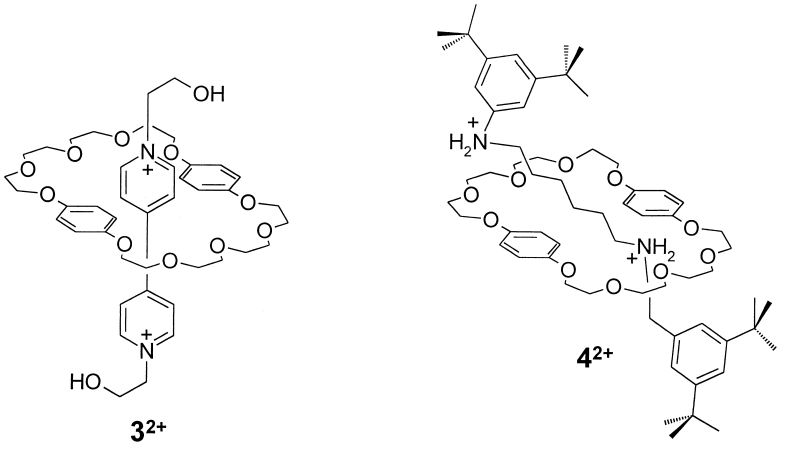
Although dendrimers are covalent species, most chemists would count them as valid supramolecular entities due to their nanosized, hyperbranched structure allowing guest substances to be bound noncovalently in interior niches, at peripheral functions or mechanically inside “dendritic boxes.” MALDI-MS is a convenient way to produce protonated dendrimer ions [26–35] and is often used to provide evidence of their purity in terms of a narrow molecular weight distribution and the completeness of their functionalization in each generation. Scheme 1 shows two examples of metallodendrimers [36–39]. Both give rise to intense signals for the molecular ions with several counterions in the complex. Generation of these ions with MALDI or ESI-MS is quite straightforward. The tetranuclear ruthenium complex $\mathbf{1}^{8+}$ [36] has been specifically designed from kinetically inert, chiral subunits as a chiral D_3 symmetric dendrimer. In contrast, the same building principles that were used to prepare the first generation dendrimer $\mathbf{2}^{9+}$ [39] can be applied to synthesize larger dendrim-



Scheme 1.

ers up to the fifth generation, where the first four could be detected by mass spectrometry.

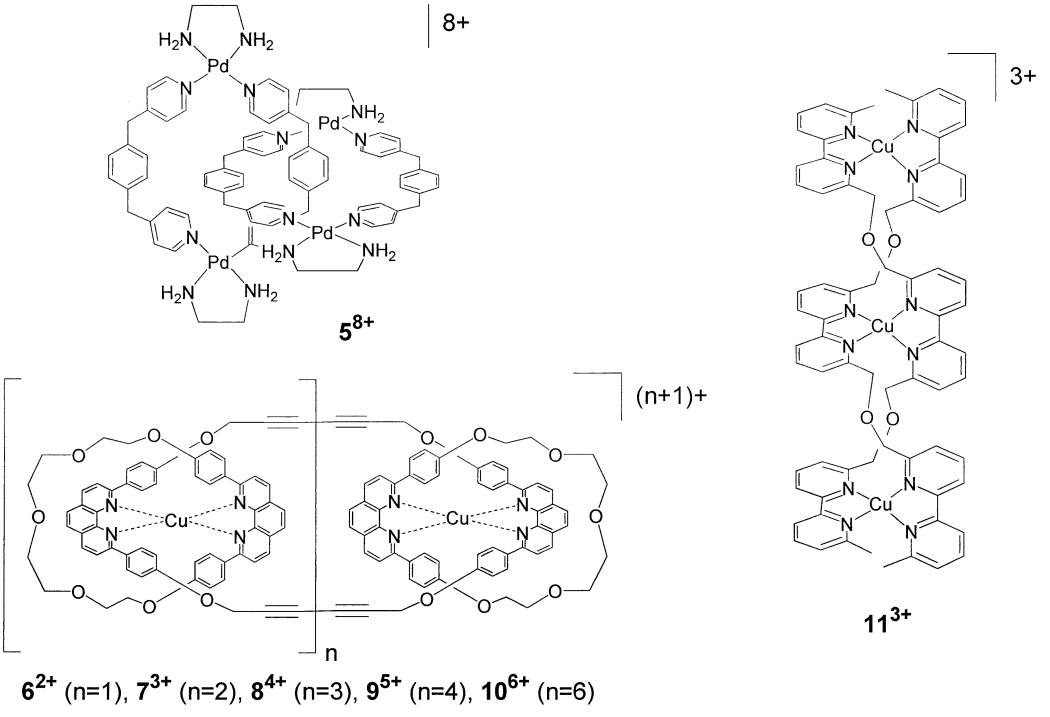
Catenanes are molecules that consist of two or more mechanically interlocked macrocycles. For the separation of the two rings it is necessary to break a covalent bond in one of the cycles. Although the two rings are noncovalently bound to each other, one of them has to be opened before de-threading can occur and consequently quite a large amount of energy is needed for this process. Similar arguments apply to rotaxanes—macrocycles that are penetrated by an axle—if the stopper groups at the ends of their axles are large enough to prevent de-threading. Due to this special situation, ion generation is usually quite straightforward and many examples exist for the characterization of catenanes [40–44] and rotaxanes [45–54] by mass spectrometry. Even the early catenanes prepared by the groups of Wolovsky [55], Wassermann and co-workers [56], and Schill and co-workers [57,58] in the 1960s and 1970s have been the subject of mass spectrometric studies, which used the conventional ionization methods [electron and chemical ionizations (EI and CI, respectively)] available at that time. Besides fragmentation at the periphery of the catenane leaving intact the interlocked structure, cleavage of a bond within one of the macrocycles leads to dethreading and loss of “half” the catenane [59,60]. A comparison of the mass spectra of catenanes and their individual components reveals the operation of an interannular hydrogen transfer in the catenane radical cations. This process is followed by ring cleavage and de-threading and does not occur for macrocyclic or open chain analogues so that it is indicative for the presence of a catenane. A different situation is found for pseudorotaxanes where an axis with small or no stopper groups at all is threaded through a macrocyclic ring. The axis is held inside the wheel by noncovalent interactions and can reversibly dethread in solution. Mass spectrometric ion generation and the observation of signals corresponding to the pseudorotaxanes has nevertheless been possible for quite a number of these species



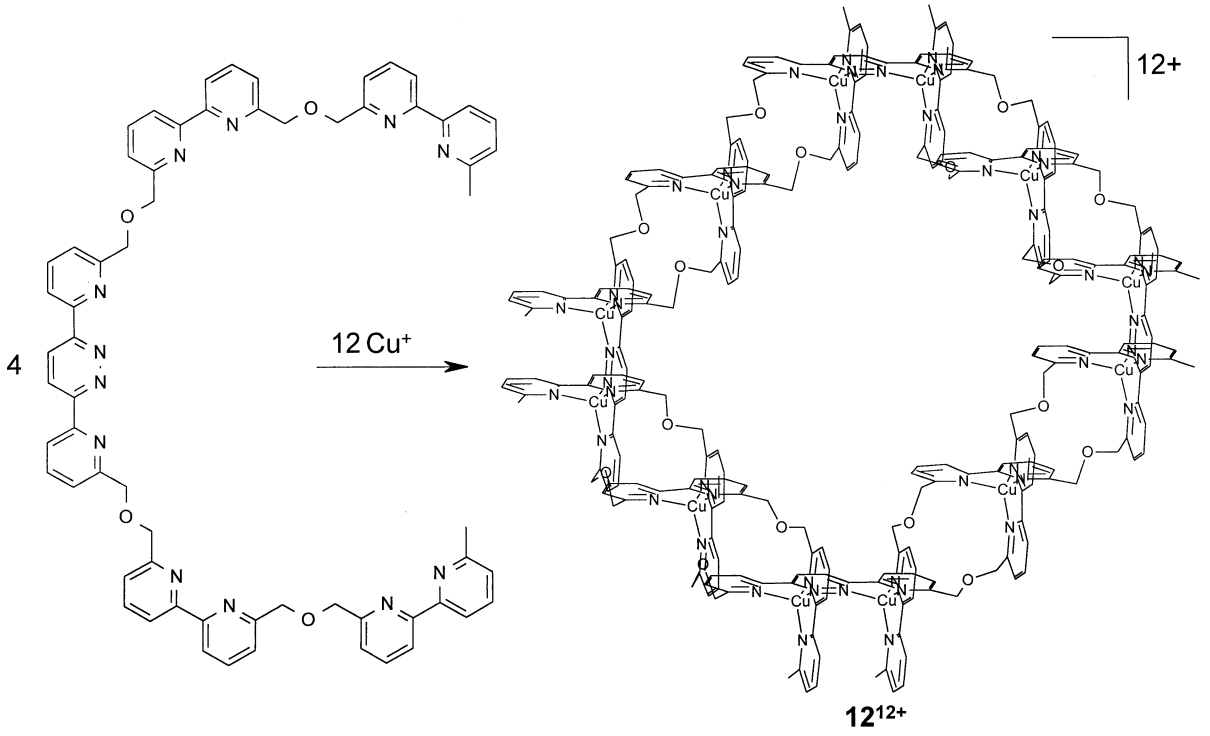
Scheme 2.

[30,61–68]. In $\mathbf{3}^{2+}$ for example (Scheme 2), donor–acceptor interactions and C–H–O hydrogen bonds bind the axis in the cavity of the ring with a binding energy of ~4 kcal/mol, whereas in $\mathbf{4}^{2+}$ mainly N–H–O hydrogen bonds are responsible for the recognition of ring and axis.

Among the many kinds of supramolecular metal complexes [69–76] are catenanes such as $\mathbf{5}^{8+}$ [77,78] (Scheme 3) and catenates like the multiring species $\mathbf{6}^{2+}$–$\mathbf{10}^{6+}$ with their (n + 1) peripheral rings interlocked with a central macrocycle [79]. Double- and triple-helical compounds like prototypical $\mathbf{11}^{3+}$ have



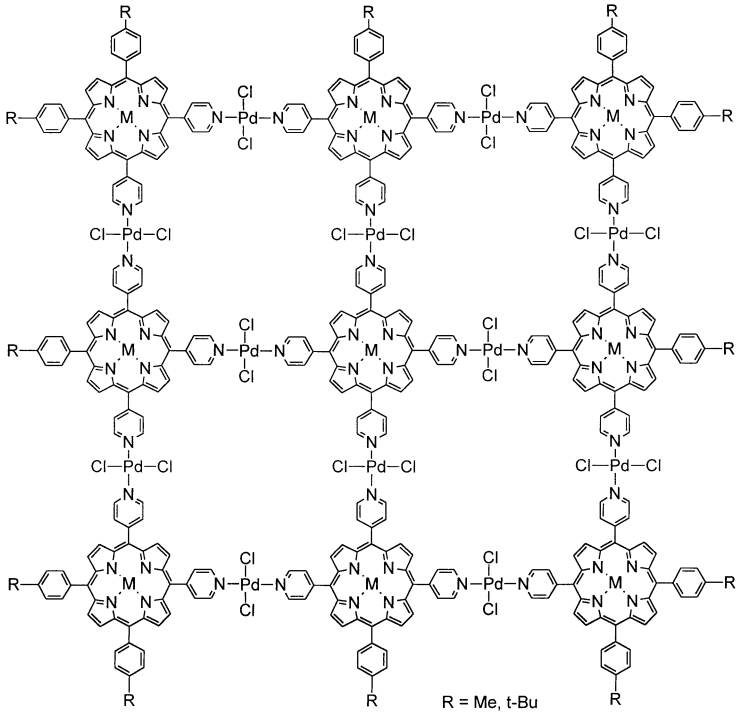
Scheme 3.



Scheme 4.

been prepared and characterized [80–87]. The self-assembly pathway of a pentanuclear helicate [88] and the heterotrimerization of two different triple helical homotrimers could be followed by electrospray mass spectrometry [89]. A detailed tandem mass spectrometry analysis of the fragmentation pattern of such complexes suggests that the nitrogen–metal bond is rather strong in the gas phase [90]. Larger aggregates such as $\mathbf{12}^{12+}$ [91] (Scheme 4) and the porphyrin grid **13** [92,93] (Scheme 5) have been prepared and signals for the corresponding ions have been observed in the mass spectra. Several metal containing cage molecules [94–97] could be vaporized and ionized, among them assemblies that contain a guest molecule inside the cavity [98] or where two cages penetrate each other in a catenane-like fashion [99]. Finally, the ruthenium complex $\mathbf{14}^{4+}$ (Scheme 6) represents a special case, because it dimerizes to $(\mathbf{14}^{4+})_2$ through π–π stacking interactions that are strong enough to transfer the intact dimer into the gas phase by electrospray ionization [100]. Since all of these metal complexes are inherently positively charged, it is usually not difficult to generate singly or multiply charged ions consisting of the metal complex cation surrounded by the appropriate number of counterions such as BF_4^- or PF_6^-. Thus, the charging mechanism is simply the loss of one or more counterions from the neutral aggregate, although examples exist where redox processes might play a role [36].

Calixarene and resorcinarene-based container molecules with encarcerated guest molecules have been synthesized. Guest encapsulation during shell closure has been observed as well as penetration by guest molecules of the carcerand's walls upon heating the mixture of empty container and guest. In their FAB mass spectra, signals for the host–guest complexes can be found that are usually accompanied by signals for the empty host [101–109]. A particularly interesting example is depicted in Scheme 7 [110]. Benzocyclobutanedione can be encarcerated in the carcer-



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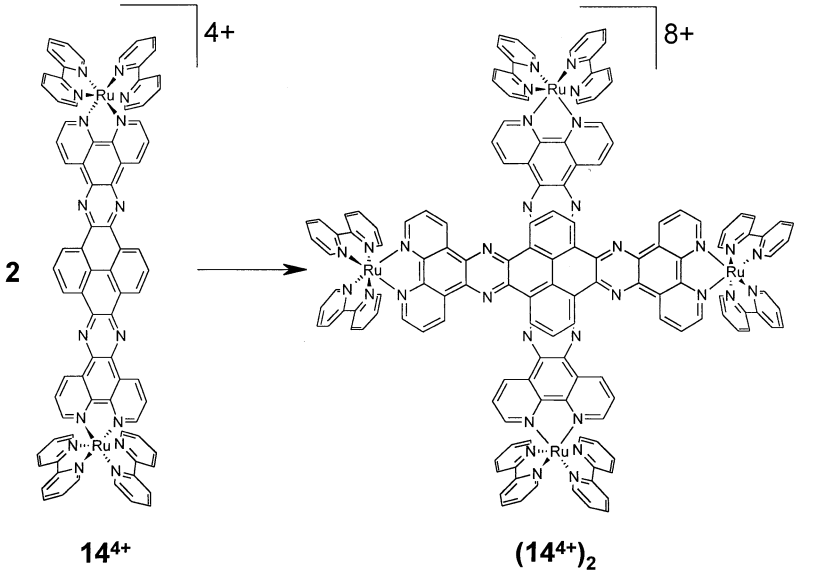
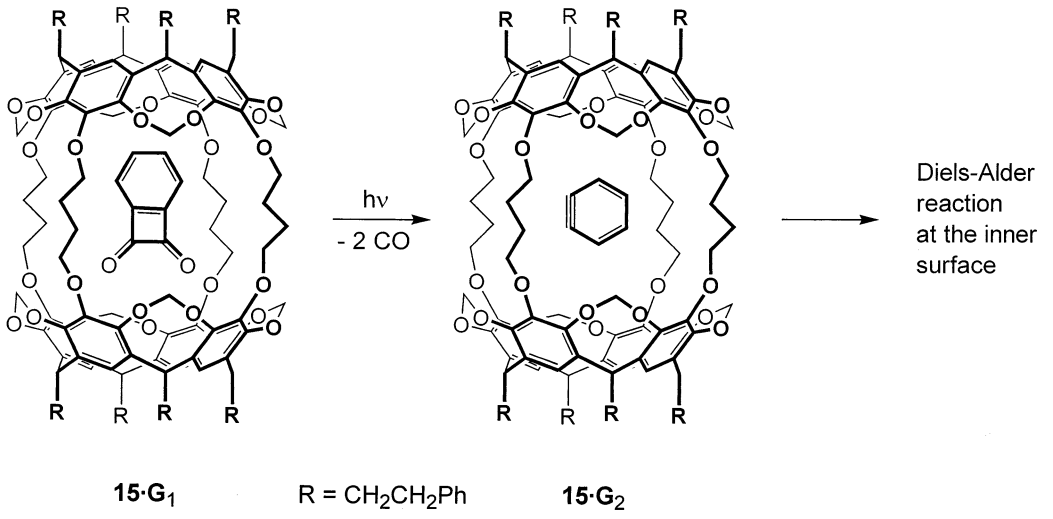
Scheme 5.



14^{4+} **$(14^{4+})_2$**

Scheme 6.

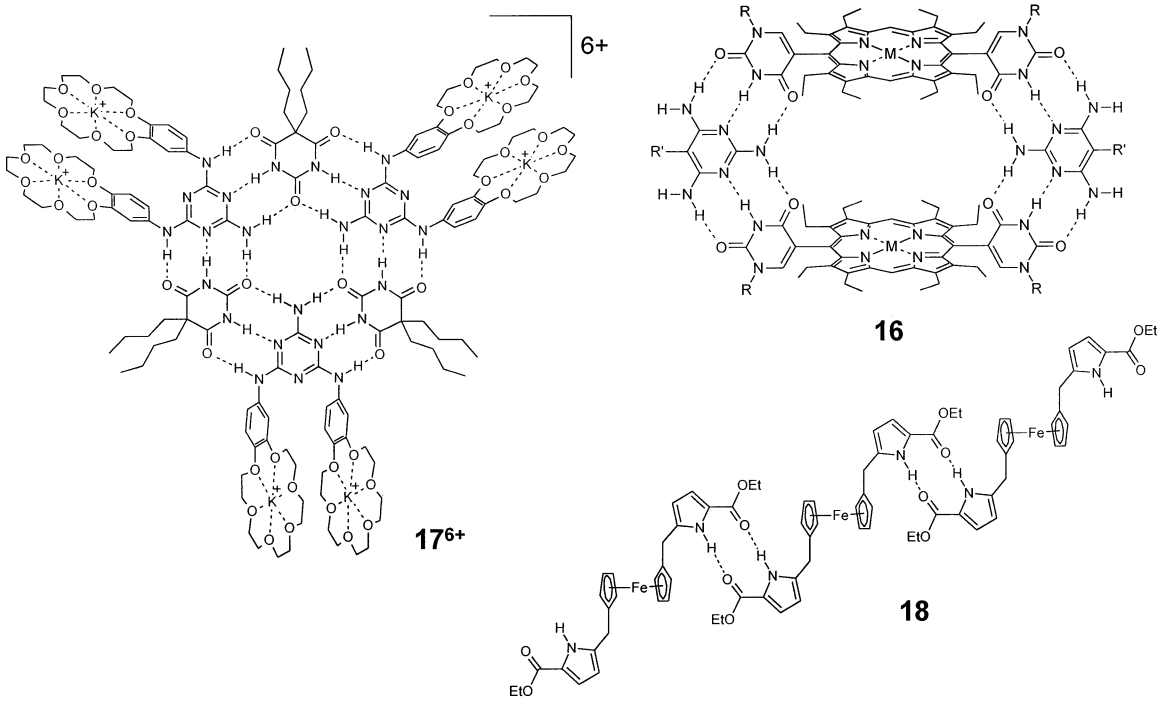


Scheme 7.

and **15** by "melting" the two components together. Irradiation at a wavelength of >400 nm and—in time-delayed—of 297 nm provokes a twofold carbon monoxide extrusion and yields benzyne trapped and isolated inside the host. Interestingly, the FAB mass spectrum did not show any signal for the empty host, pointing to covalent attachment of the benzyne to the capsule. Indeed, NMR experiments confirmed the occurrence of an inner-phase Diels-Alder reaction of the benzyne with one of the aromatic rings of the host [111]. The mass spectrometric observation of alkali and silver cations binding to calixarenes that do not bear any functional groups [112] indicates cation–π interactions to be operative in such systems as observed before for alkali ion/benzene complexes [113,114]. The formation of host–guest complexes with open cavitands as the hosts has been observed in the gas phase. Under chemical ionization conditions, the cavitand is evaporated by desorption chemical ionization and forms complexes with guest molecules in the ion source, where the pressure is high enough to cool the newly formed complexes by collisions [115,116].

Many more examples for different supramolecular systems could be discussed here, among them are inclusion complexes with cyclodextrins as the hosts [117–124], different kinds of receptor–substrate complexes [125–134], perfluoro crown ethers binding molecular oxygen [135,136], supramolecular pigments [137], or gas-phase micelles [138]. A particular challenge for mass spectrometry is, however, the characterization of hydrogen-bonded complexes. FAB and MALDI matrices are usually protic and the standard solvents for ESI-MS—methanol, water, or mixtures of these two with organic acids—are an environment where hydrogen bonds become weak or even destroyed. There exists some examples for hydrogen-bonded complexes strong enough to survive these conditions [139–141], but usually the use of aprotic media, which are also weak hydrogen-bond acceptors, is necessary [142–144]. As in such solvents or matrices, protonation is not feasible anymore, an ion labeling strategy must be developed for those aggregates that are neutral in solution.

The first example of such an approach was reported in 1993 by Lehn and co-workers [145] who prepared the hydrogen-bonded aggregate **16** (Scheme 8). Based on a similar hydrogen-bonding motif, the rosette $\mathbf{17}^{6+}$ equipped with crown ethers to provide a binding site for cations was characterized by ESI-MS from dichloromethane which gives rise to ionic complexes such as $[\mathbf{17}^{6+}\cdot 3PF_6^-]$ and several similar species [146]. The crown ether ion labeling strategy has the disadvantage to require synthetic modifications of



Scheme 8.

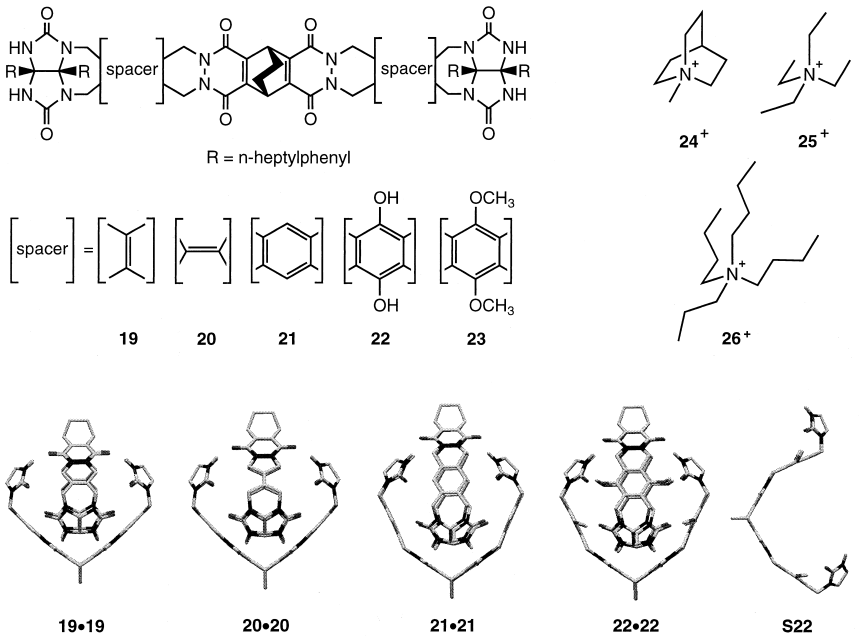
the systems under study. Therefore, Cheng et al. [147,148] developed another method for ionizing a series of larger aggregates based on the same rosette motif. They used chloroform-soluble Ph_4PCl as a charge carrier. In the electrospray process ionization occurs by the transfer of a Cl^- ion to the rosette assemblies which then can easily be detected in the negative mode. The rosettes have also been used by Reinhoudt and co-workers [149–151] to assemble molecular boxes bearing two rosettes that are capped by three calixarene moieties. Ion labeling could be afforded by complexing a silver ion with cyano groups or two aromatic rings in a sandwich-type manner. The complexes are stable enough to be ionized by MALDI from a 2,5-dihydroxy benzoic acid matrix.

Another elegant approach was chosen by Scherer et al. [152] for the ionization of the pyrrole-ferrocene hybrid complex **18** and similar species. In order to pre-generate an ion in dichloromethane solution prior to the spray process, a small amount of iodine was added to the sample solutions. Oxidation of the ferrocene subunit provided the charge. The ionic complex could then easily be transferred into the gas phase and mass analyzed.

The broad range of systems discussed so far clearly demonstrates that ion generation is possible, although it is not mere routine yet. Even if the development of a particular procedure for a given sample sometimes requires some creativity, ion generation is not an invincible barrier anymore. Consequently, the huge potential of mass spectrometric methodology and gas-phase chemistry can be applied to supramolecular systems.

3. Structure examination of supramolecular architectures

As noted in Sec. 2, most studies use methods other than mass spectrometry for the structural investigation of the supramolecular systems under study. Clearly,



Scheme 9.

structure determination using mass spectrometry methodology is a challenging task, in particular because structural evidence from mass spectrometry experiments necessarily remains indirect and comes from energetic considerations or fragmentations indicative of certain structural elements. Although the examination of the connectivities of small ions with a few atoms is usually possible [153,154], large supramolecular aggregates pose problems that cannot be solved so far. However, the structural features of the building blocks of supramolecular complexes are usually known. Since the subunits themselves are covalent structures, it can be assumed that they do not undergo any rearrangement reactions when transferred into the gas phase by one of the milder ionization methods. Consequently, the secondary structures of the aggregates under study are of interest, not the primary structures that are given by the atom connectivities, bond lengths, and angles. It would be a great progress, if mass spectrometry could be used to determine what kind of aggregate is formed, how the building blocks are connected to each other, and whether the binding of the subunits in the complex is specific in terms of molecular recognition.

As an example, let us discuss the mass spectrometric experiments aimed at the structure determination of the "softballs" of Rebek and co-workers **19·19**–**22·22** (Scheme 9) [155]. These complexes are built from two identical, concave monomers that bear self-complementary hydrogen-bonding sites at their periphery. The information for the construction of the dimers is programmed into the monomers by chemical synthesis which determines the curvature and the positions of the hydrogen-bonding sites. The four softballs **19·19**–**22 · 22** form capsules in aprotic organic solvents like chloroform or xylene and surround an inner cavity which can be filled by a guest molecule appropriate in size and shape. The sizes of the four cavities differ from ∼190 $Å^3$ for **19·19** to ∼310 $Å^3$ for **21·21** due to the length of the spacers that connect the center piece and the two glycoluril arms. In order to produce charged capsules with a guest inside by electrospray ionization, a simple ion labeling strategy is applied. If encapsulation of neutral molecules is possible (which could be shown by

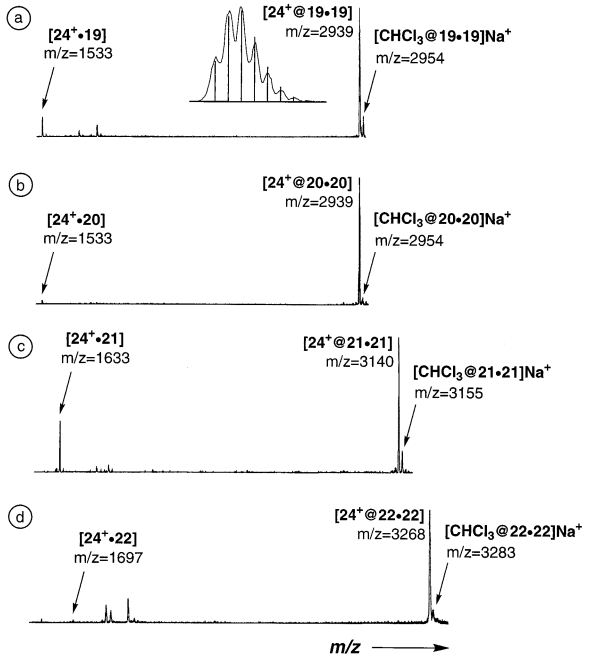


Fig. 1. ESI mass spectra of $CHCl_3$ solutions of (a) **19**, (b) **20**, (c) **21**, and (d) **22** with $\mathbf{24}^+BF_4^-$ as guest salt. The inset shows the measured and calculated isotope pattern for ions [$\mathbf{24}^+$@**19**·**19**] (the '@' sign indicates encapsulation of the guests).

NMR), inclusion of quaternary ammonium ions of similar size, e.g. $\mathbf{24}^+$ and $\mathbf{25}^+$ (Scheme 9) should also easily be feasible. This approach has the advantages that (1) no synthetic modification of the capsules is necessary, (2) the use of organic solvents such as $CHCl_3$ is possible, (3) the seam of hydrogen bonds is thus not destroyed, and (4) the charge is inherent in the complex and species which do not contain a guest ion would be neutral. Consequently, they are not observed in the mass spectra. Mass spectrometry thus provides a filter and even a small fraction of charged capsules in solution can be detected among an excess of neutral species as, e.g. solvent-filled capsules or nonspecific aggregates. The choice of the counterion for the ammonium cation is quite important. In contrast to the halogenides or carboxylates, weakly coordinating anions like BF_4^- or PF_6^- provide sufficient solubility of the salt in organic solvents and do not interfere with the seam of hydrogen bonds. Electrospray ionization of $CHCl_3$ solutions of the corresponding ammonium salt and the capsule monomers gives rise to intense signals (Fig. 1) for complexes of one guest ion and two capsule monomers. The experimental isotope patterns confirm the correct elemental composition. Isotopically labeled guest ions produce signals shifted in *m/z* by the amount expected from the number of deuterium atoms incorporated.

With the elemental composition confirmed, one can think about structural possibilities for the assembly. There are several different arrangements possible: an empty, but closed capsule with the guest attached to the outer surface by, e.g. cation–π interactions could be realized as well as a central ammonium ion which binds two diverging monomers that are bound to the ion by charge–dipole forces. They may be in contact with each other just by a few or even no hydrogen bonds at all. By intuition, of course, a closed capsule that is filled with the ammonium ion seems to be energetically most favorable. In this arrangement, all hydrogen bond donors match with the corresponding acceptors. In addition, the cation–π or cation–dipole forces can be maximized with the guest ion on the concave inside instead of the convex surface of the capsule.

As all dimer-guest signals vanish in favor of the protonated monomers upon addition of methanol, it can safely be assumed that hydrogen bonds play an important role in stabilizing the assembly. Further evidence for the intact capsular structure comes from experiments with deformed capsule monomers and guest ions inappropriate in size. Control compounds like the methoxylated softball **23** or the S-shaped monomer **S22**, which are both unable to form capsules, do not show any signals for dimer–guest complexes in their mass spectra. Similarly, competition experiments with equimolar amounts of guests $\mathbf{25}^+$ and $\mathbf{26}^+$ give signals for encapsulated $\mathbf{25}^+$, whereas no capsule is found that contained $\mathbf{26}^+$ as the guest. For the unspecifically bound complexes mentioned previously, signals should be seen in these experiments. That the geometric fit of the hydrogen-bonding sites is important can also be seen, when two different softball monomers are mixed. Signals for the two homodimers are observed accompanied by a signal for the mixed, heterodimeric capsule. If the

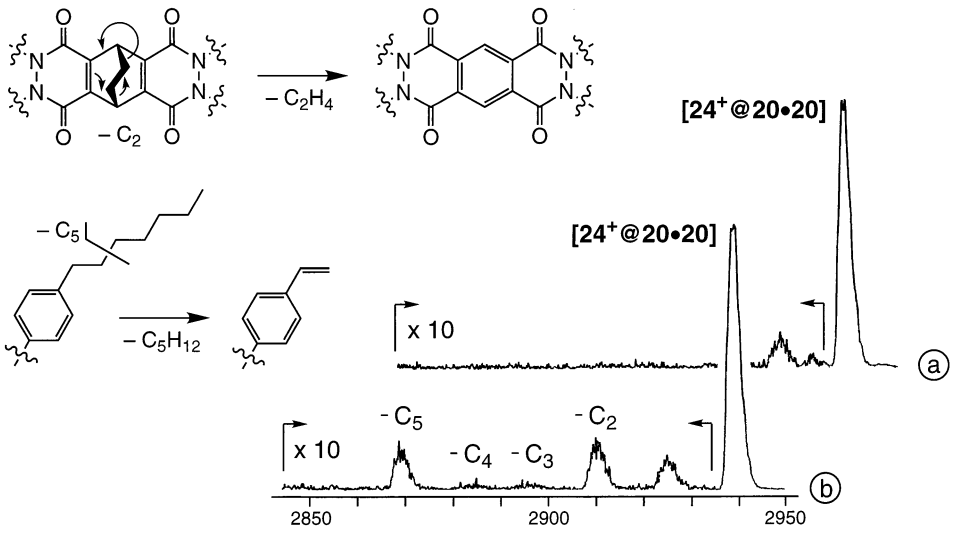
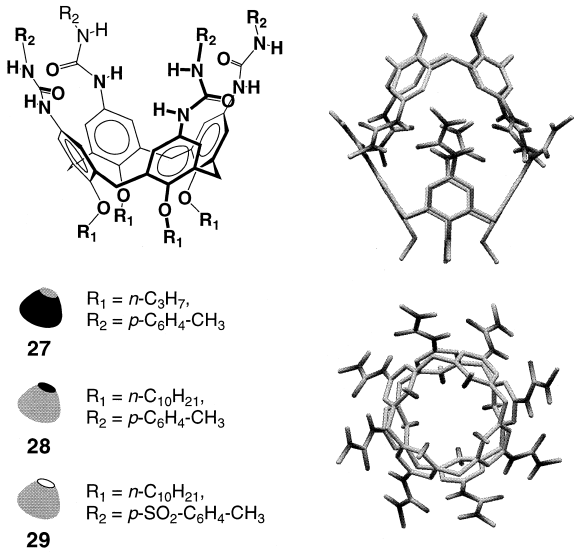


Fig. 2. (a) Detail of the ESI mass spectrum of a $CHCl_3$ solution of **20** and $\mathbf{24}^+BF_4^-$. (b) Same experiment with an additional acceleration voltage of −100 V on the octopole of the mass spectrometer (source-CID experiment). On the left-hand side two processes are shown that are likely to give rise to the observed losses of C_2 and C_5 units from the capsule.

geometric fit of the two monomers is good (e.g. **21** and **22**, both with the same spacer length), the signal for the heterodimer is present in the mass spectrum and even somewhat exceeds the statistically expected intensity (twice of that of the homodimers). Instead, if the two monomers do not fit well (e.g. **20** and **22**, ethylene versus dihydroxy benzene spacers), the heterodimer is by far underrepresented in the spectrum.

Strictly speaking, these experiments confirm the solution-phase structure by using a mass spectrometric probe and NMR experiments are in complete agreement with the mass spectrometry results. However, even if not very likely, the possibility exists that capsules—although intact in solution—rearrange during the electrospray process or after desolvation. If so, one would still expect that the mass spectrometry experiments reflect qualitatively the situation in solution, although the capsular structure would be destroyed in the gas phase. In view of more detailed mass spectrometric studies (see the following), it would therefore be quite advantageous, if the gas-phase ion structure could also be determined. Collision experiments with the "medium softball" **20·20** with $\mathbf{24}^+$ as the guest result in the surprising finding of covalent fragments of the capsule (Fig. 2). For a weakly bound guest, its release from the aggregate would have been expected. However, the presence of signals for losses of C_2 and C_5 units which can be attributed to quite favorable fragmentation processes (Fig. 2), indicates that the energy demand for guest release must be not too far below the activation energy for these two decomposition pathways. Any unspecific structure is expected to bind the cation with a binding energy not greater than a few kilocalories per mole. However, if the capsular structure is still intact in the gas phase, guest release cannot proceed without breaking several hydrogen bonds. This requires an additional amount of energy and increases the barrier for guest release. Consequently, this process becomes less favorable and covalent fragmentation starts to compete.

With these experiments, the capsular structure is unambiguously shown to be intact in the gas phase. At least for special cases like these capsules, mass spectrometry is a valuable tool not only for the measurement of the molecular weight of the capsules, but also for their structural characterization. Several other types of capsules have been characterized similarly. Each of them added some new aspects to the picture, which will be briefly mentioned in the fol-



Scheme 10.

lowing sections without repeating all the experiments that lead to similar results as described previously.

Scheme 10 shows monomers **27**–**29** that are based on a tetraurea calixarene scaffold. The four urea units of each of the two monomers interdigitate and form a capsule with a seam of sixteen hydrogen bonds (Scheme 10). Earlier results with a similar capsule, which has been observed in its MALDI mass spectrum as a protonated dimer without included guest [156], encouraged further investigations with the new ion labeling approach [157,158]. Again, good intensities for dimeric capsules with ionic guests are obtained [Fig. 3(a)–(c)] and the capsular structure could be confirmed by collision experiments. Experiments with mixtures of two different monomers [Fig. 3(d)–(f)] reveal a distinct preference for heterodimer formation from **27** (or **28**) and the sulfonylurea calixarene **29**. In the corresponding mass spectra, no homodimers are observed, whereas a mixture of **27** and **28** with $\mathbf{24}^+$ as the guest gives rise to a mixture close to the statistical distribution. These mass spectrometry results are precisely paralleled by NMR experiments and thus further corroborate the validity of the mass spectrometry approach. Competition experiments with an extended series of quaternary ammonium salts reveal a pronounced dependence of the signal intensity on the size of the guest. Modeling suggests that the best guests fill significantly more space (up to 78%) than neutral guest molecules (~55%). This effect is probably due to cation–π interactions between the charged guest and the aromatic capsule walls which are not present for neutral guests. Larger, dumbbell-shaped aggregates with two and three covalently connected capsules and up to seven noncovalently bound subunits also give detectable signals in their electrospray mass spectra.

Similarly, the tetrameric capsules $\mathbf{30}_4$–$\mathbf{32}_4$ (Scheme 11) could be characterized by mass spectrometry. Quantitative NMR experiments with charged and neutral guests of the same size and shape ($\mathbf{33}^+$, **34**) showed the cation to be bound more strongly than the neutral by more than 4 kcal/mol due to cation–π interactions with the capsule walls [159]. In NMR experiments, the formation of heterotetramers could not be followed due to signal coincidences for the six different species $\mathbf{30}_4$, $\mathbf{30}_3\cdot\mathbf{31}$, $\mathbf{30}_2\cdot\mathbf{31}_2$ (two isomers), $\mathbf{30}\cdot\mathbf{31}_3$, and $\mathbf{30}_4$. When **30** and **31** are mixed with guest $\mathbf{24}^+$ and electrosprayed, signals for the homo- and heterotetramers are observed in the mass spectrum in the statistical 1:4:6:4:1 ratio and confirm the presence of heterotetramers in solution. These results demonstrate the complementarity of the mass spectrometry and NMR methods. Although a quantification of binding constants and cation–π interactions is easily possible with NMR, mass spectrometry is the appropriate method to detect heterotetramerization.

Finally, the "flexiballs" **35·35**–**38·38** (Scheme 12) encapsulate dicationic guests such as $\mathbf{39}^{2+}$ and a series of experiments analogous to those using monocations is possible [160]. Capsule **38·38** surrounds a cavity volume of ~950 Å^3 which is large enough to include typical supramolecular host–guest complexes like the strontium cryptate $\mathbf{40}^{2+}$ opening the field to self-assembling second-sphere inclusion complexes that remind one of the Russian doll Matroshka [161].

One could argue that structure determination of supramolecular architectures by mass spectrometry is limited to special cases and that these results could only be obtained due to the unique features of the capsules. There exist, however, a few other studies

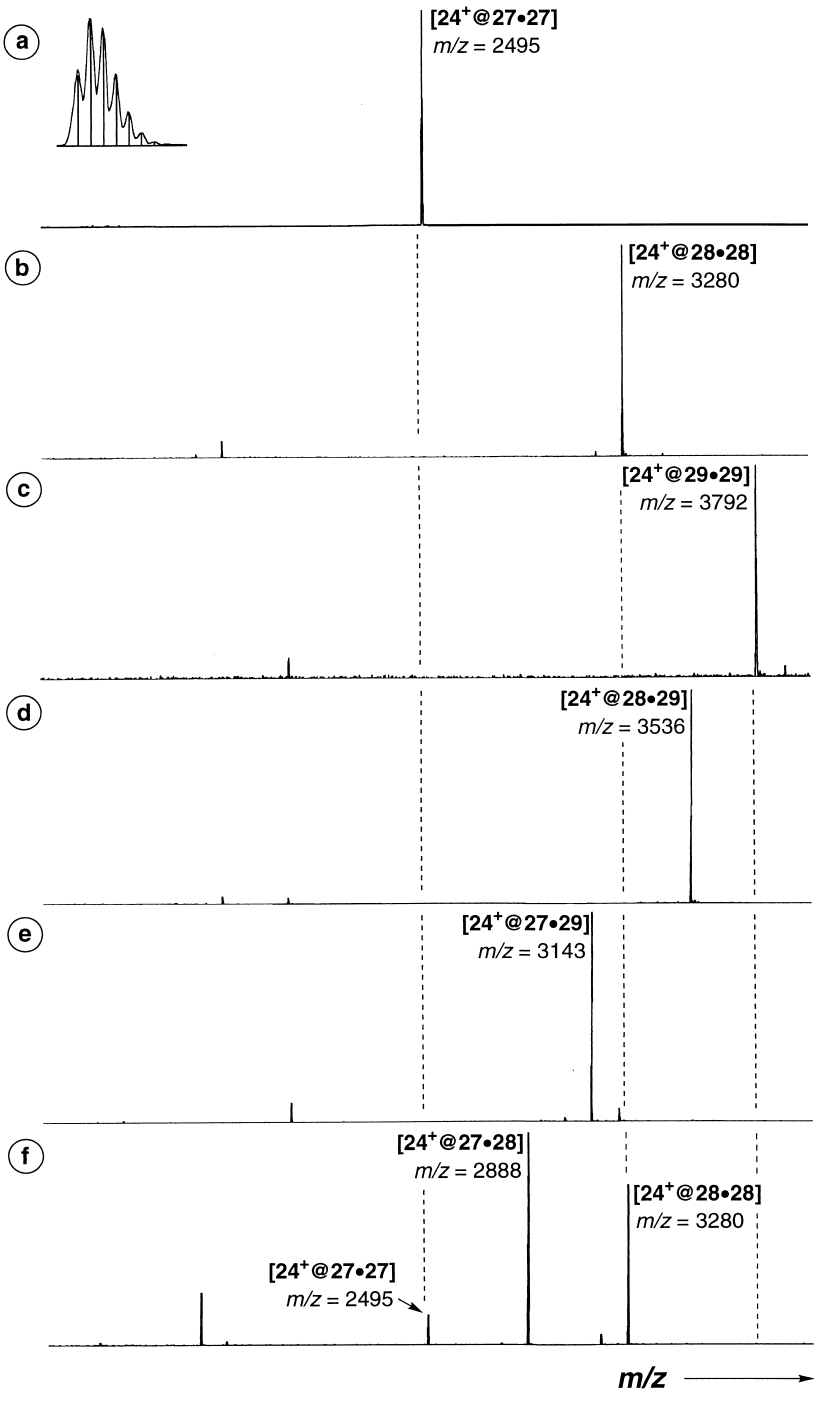
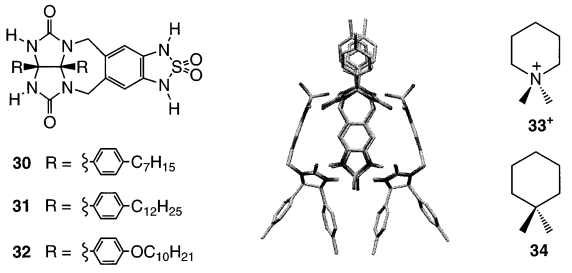


Fig. 3. ESI mass spectra of $CHCl_3$ solutions of (a) **27**, (b) **28**, (c) **29**, and equimolar mixtures of (d) **28** and **29**, (e) **27** and **29**, and (f) **27** and **28** with $\mathbf{24}^+BF_4^-$ as the guest salt. The dashed lines show the positions of the three homodimers [$\mathbf{24}^+$@**27**·**27**], [$\mathbf{24}^+$@**28**·**28**], and [$\mathbf{24}^+$@**29**·**29**] in the ESI spectra (d)–(f).

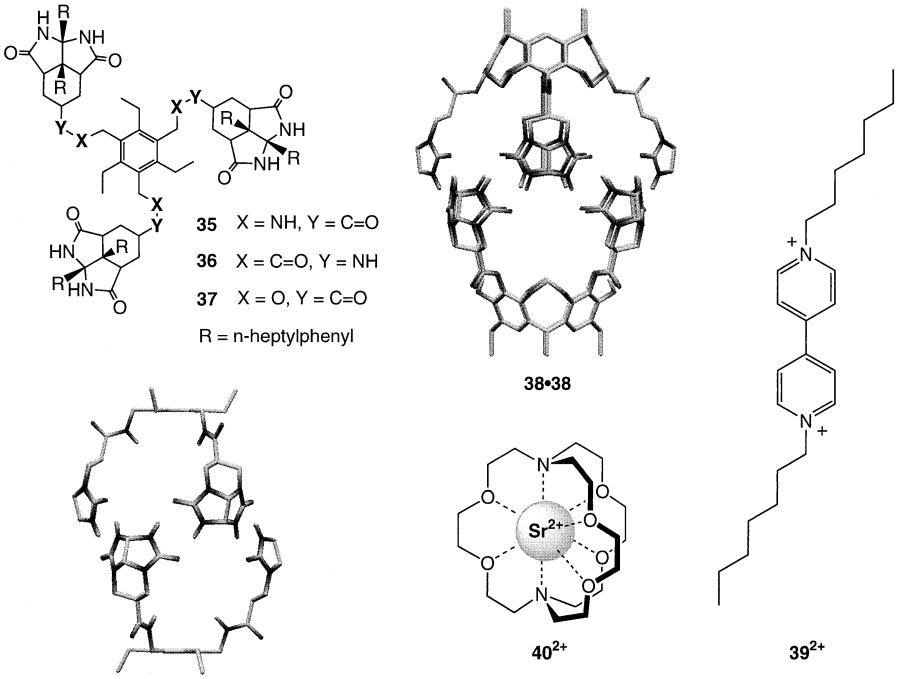


Scheme 11.

that deal with completely different systems and thus underline the importance of mass spectrometry in this area. For example, the complexation site of divalent cations such as Ca^{2+} to cell surface carbohydrates could be determined by collision-induced decomposition (CID) experiments [162]. In another study, the finding of size and shape dependent trends for the binding of amines to α-, β-, and γ-cyclodextrins has been interpreted as evidence that inclusion complexes form rather than unspecific aggregates [163]. These examples suggest that mass spectrometry has a large potential with respect to structure determination of supramolecular aggregates far beyond the few studies mentioned here.

4. Thermochemistry: intrinsic properties and the effects of solvation

Despite several studies that successfully determined quantitative thermochemical solution-phase properties by using a mass spectrometric probe [164–170], several uncertainties remain that may strongly depend on the particular system and might obscure the use of mass spectrometry for these purposes as a general tool. As a general rule, it is relatively safe to compare systems that do not differ much and bear similar structural and energetic features [171]. The larger the differences between two systems become, the unsafer it is to draw quantitative conclusions from their comparison. Some efforts have been made to determine the factors that influence the ion yield in electrospray ionization and a simple mathematical



Scheme 12.

relationship between the solvation energies E_M and the response factor k_M for certain ions has been deduced

$$k_M = C \exp(-0.015\ E_M) \quad (1)$$

where C is a constant that depends on ion charge, ion concentration and instrument parameters) [172].

Nevertheless, the determination of thermodynamic and kinetic data in the gas phase under environment-free conditions, i.e. without the effects of solvents and counterions, promises insight into the intrinsic properties that govern molecular recognition. Comparison of these data to corresponding results obtained in solution can be expected to provide a more profound understanding of the effects of solvation.

A variety of different methods has been developed; some of them provide relative data, e.g. the difference in the binding energies of two different guests to the same host, and some of them are designed to yield absolute data. The binding of alkali metal [173–182], ammonium [183–185], diazonium [186,187], oxonium [188], or tropylium ions [189] to crown ethers and related hosts, such as their linear analogs [190], tripodal oxygen-containing ligands [191], bis-crowned clefts [192–195], and larger systems containing three crown ether subunits [196] has been studied intensely. Some neutral species could be charged by attaching a crown ether and binding a metal cation (mentioned previously) [197–199]. Most of the examples in this section come from these studies and will serve as material for a discussion of the various approaches to a mass spectrometric determination of thermochemical data.

The measurement of intrinsic thermochemical properties does not only provide quantitative data, but can also contribute to a more detailed analysis of qualitative issues. In solution, the binding of a cation to a crown ether is a rather complicated process. Both, the ligand and the cation must be desolvated at least partially. Thus, the solvent and the counterions have a large effect which affects the stability constants as determined in solution. Two problems have been studied intensively [200]. (1) Crown ethers show a distinct size selectivity in cation binding in solution. Does this selectivity exist unchanged in the gas phase as well? [201] If not, how does it change and what are the intrinsic parameters that determine cation binding to crown ethers in the gas phase? (2) Macrocyclic ligands show higher stability constants and greater selectivities as compared to their acyclic analogs due to conformational entropy effects and differences in the solvation energy of cyclic and acyclic ligands. Since the latter effect does not play a role in the solvent-free environment inside a mass spectrometer, both effects could be separated by conducting experiments in the gas phase. This might contribute to a more profound understanding of the macrocyclic effect [202].

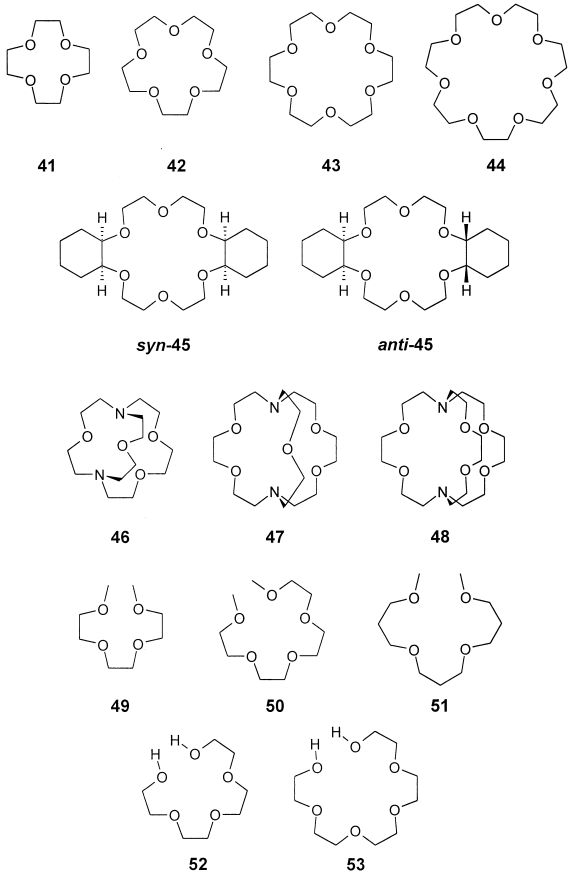
Two different methods have been applied that yield relative binding data: The “kinetic method” [203,204] and the measurement of equilibrium constants for the reaction of a crown ether/metal complex with a neutral crown of different mass. The kinetic method is based on the fragmentation pattern of a complex built from two crown ether ligands C_1 and C_2 attached to a central ion M^+. The measured intensities of the two fragments $C_1{\cdot}M^+$ and $C_2{\cdot}M^+$ reflect the relative fragmentation rates and—assuming that decomplexation and loss of one of the crown ethers from the complex proceeds without substantial barrier—they also reflect the relative binding energies of the two crown ethers in the complex. In order to be able to draw conclusions for crown ether/M^+ complexes instead of the sandwich complexes, binding of the second crown ether ligand is assumed not to have a great influence on the relative binding affinities. The data from such measurements [205–207] allow the construction of affinity ladders (Table 1 and Scheme 13). However, the conversion of these ladders into relative energies is problematic because temperature is ill defined in the highly dilute gas phase. Smaller ions, at least, do not exchange energy, neither by collisions, nor by photon exchange (see the following). Thus, the Boltzmann distribution of internal energy is not valid for such systems and, strictly speaking, it is impossible to determine relative energies. Furthermore, the experiments with the crown ether/M^+ complexes have been performed under CID conditions [205–207]. Although the qualitative rank-

Table 1
Binding selectivity ladders in order of increasing affinity for complexes with crown ethers **41**–**44** and their acyclic analogs **49**–**53** as determined by the kinetic method; values in parentheses give ion binding affinities relative to **41**.

H^+ [a]		Li^+ [b]		Na^+ [b]		K^+ [b]		Rb^+ [b]		Cs^+ [b]		NH_4^+ [a]	
41	(1)	**41**	(1)	**41**	(1)	**41**		**41**	(1)	**41**	(1)	**41**	(1)
49	(2)	**49**	(10)	**49**	(2)	**51**	(2)	**51**	(1)	**51**	(1)	**51**	(4)
42	(20)	**51**	(20)	**51**	(2.2)	**49**	(5)	**49**	(5)	**49**	(10)	**49**	(25)
51	(40)	**52**	(80)	**52**	(20)	**52**	(10)	**52**	(50)	**52**	(40)	**52**	(350)
50	(160)	**43**	(200)	**50**	(200)	**42**	(40)	**42**	(500)	**42**	(250)	**42**	(3 500)
43	(600)	**44**	(200)	**43**	(600)	**53**	(100)	**50**	(1 000)	**50**	(300)	**53**	(15 000)
52	(1 000)	**53**	(600)	**42**	(800)	**50**	(120)	**53**	(2 000)	**53**	(300)	**50**	(17 500)
44	(1 500)	**50**	(800)	**53**	(1 100)	**43**	(1 000)	**43**	(3 000)	**43**	(600)	**43**	(400 000)
53	(5 000)	**42**	(1 200)	**44**	(4 200)	**44**	(3 000)	**44**	(6 000)	**44**	(1 800)	**44**	(400 000)

[a] Taken from [206].
[b] Taken from [205]. Also, see [21,207].

ing of crown ethers for a given ion is likely not to be affected, the quantification is even more problematic.



Scheme 13.

Despite these sources of potential error, the affinity ladders obtained with this approach qualitatively agree well with absolute binding data (Table 5, see the following) and some striking effects have been observed. For example, a comparison of the data for the proton and the ammonium ion reveals much higher relative affinities of the crown ethers to ammonium than to H^+. This effect can be attributed to the ability of ammonium to form multiple hydrogen bonds, probably with a lower entropy of complexation [206]. Most likely also for entropic reasons, the acyclic analogs **49**–**53** (Scheme 13) have lower affinities to alkali metal ions than the crown ethers with the same number of oxygen donors [205]. There exists one study [208] that uses the kinetic method with complexes $M_1^+ \cdot C \cdot M_2X$ of one crown ether C with two different alkali metal ions M_1^+ and M_2^+ and an additional halide ion X^- that compensates the second charge and produces singly positive ions. Upon collision-induced fragmentation, loss of M_1X and M_2X is observed giving rise to $M_2^+ \cdot C$ and $M_1^+ \cdot C$ fragment ions. Again, affinity ladders can be constructed from the signal intensities of these fragments. For example, for 18-crown-6 **43** the ranking follows the order of $Na^+ \geq K^+ > Li^+ > Rb^+ > Cs^+$ which is not quite in line with the absolute binding energies (given in Table 5 and in the following) and theoretical values [209,210]. Conclusions for crown ether/M^+ binding can only be drawn from these experiments, if the assumptions mentioned above are valid and if the

Table 2
Relative binding data (kcal/mol) for alkali metal ion/crown ether complexes as determined by gas-phase equilibrium measurements

M^+ transfer from → to	Li^+	Na^+	K^+	Rb^+	Cs^+
43 → **syn-45**[a]	. . .[b]	−3.9	−1.2	−1.2	−0.8
43 → **anti-45**[a]	−2.0	−1.5	−0.6	−0.4	−0.02
44 → **syn-45**[a]	−3.6	−1.6	−0.2	−1.1	−1.3
44 → **anti-45**[a]	−1.1	0.6	0.6	. . .[c]	. . .[c]
42 → **46**[d]	−4.2	−2.5	−0.7		
43 → **44**[e]	−1.8	−2.2	−0.9	−2.7	<−3.8
43 → **44**[d]	−3.7	−2.9	1.5	2.5	1.9
43 → **45**[d]	−3.0	−2.5	−2.2	−2.4	−2.4
43 → **46**[d]	−4.2	−2.1	−2.6	−3.3	−3.6
44 → **45**[d]	−2.4	−2.3	−2.1	0.5	3.5
44 → **46**[d]	−2.0	−2.4	−3.1	−2.8	−0.2
46 → **47**[d]	0.3	−2.6	−4.0	−2.3	−2.0
46 → **48**[d]	−0.4				
47 → **48**[d]		0.03	−2.7	−2.7	−3.1

[a] Taken from [215]. For the calculation of the reaction free enthalpies from the equilibrium constants, a temperature of $T = 310$ K was assumed.
[b] Equilibrium could not be measured. Only the **syn-45**/Li^+ complexes are observed at longer reaction times.
[c] Equilibrium not observed.
[d] Taken from [214]. For the calculation of the reaction free enthalpies from the equilibrium constants, a temperature of $T = 350$ K was assumed.
[e] Taken from [213]. For the calculation of the reaction free enthalpies from the equilibrium constants, a temperature of $T = 350$ K was assumed.

halide ion does not have any influence on the signal intensities. Since solvation has a strong influence on the ranking [209,211], the halide ion might however have an affect larger than anticipated in that it interacts differently with the two alkali cations in the complex.

Measurements of the gas-phase equilibrium constants [212] in a Fourier transform ion cyclotron resonance (FTICR) instrument overcome some, but not all of the limitations of the kinetic method [213]. In these experiments, a crown ether/M^+ complex is reacted in the gas phase with a second, neutral crown ether and the reaction is followed to equilibrium. In the ion transfer reaction, the two species $M^+ \cdot C_1$ and $M^+ \cdot C_2$ are involved, not the $C_1 \cdot M^+ \cdot C_2$ sandwich complexes. Therefore, the measured equilibrium constants reflect the true differences in the binding affinities of two crown ether ligands. Furthermore, collisional activation is not required and thus does not alter the intensities of the crown ether/M^+ complexes. Nevertheless, the temperature problem still exists and usually energetic data are calculated by assuming a certain temperature (see footnotes in Tables 2 and 3). The binding energy data obtained from these experiments is given in Tables 2 and 3. From these data, the macrobicyclic effect is obvious [214]. Cation transfer

Table 3
Relative binding data (kcal/mol) for RNH_3^+/crown ether complexes as determined by gas-phase equilibrium measurements[a]

RNH_3^+ transfer from → to	R						
	H	Me	*n*-Pr	*i*-Pr	*n*-Bu	*i*-Bu	*t*-Bu
43 → **syn-45**	−1.3	−0.9	−1.0	−0.6	−1.2	−1.2	−0.3
43 → **anti-45**	−0.8	−0.5	−0.5	0.2	−0.2	−0.3	0.4

[a] Taken from [216]. For the calculation of the reaction free enthalpies from the equilibrium constants, a temperature of $T = 310$ K was assumed.

Table 4
Critical energies (kcal/mol) for the dissociation of complexes of ammonium ions with crown ethers **41**–**43** and their linear analogs **49** and **50** as determined by threshold collisional activation measurements in a quadrupole ion trap mass spectrometer[a]

	41	**42**	**43**	**49**	**50**
NH_4^+	32	35	41	32	35
Et_2MeNH^+		30	34	28	29
n-$BuNH_3^+$	37	44	>50	37	43
n-$BuMeNH_2^+$	34	38	41	35	39
$H_2NCH_2CH_2NH_3^+$	35	41	>50	36	39
$(CH_3)_2NCH_2CH_2NH_3^+$		34	39		
$H_2NCH_2CH_2CH_2NH_3^+$	34	39	>50	33	35
$PyridineH^+$	33	36	35	31	34
$2\text{-}PicolineH^+$	31	33	37	31	32
$3\text{-}PicolineH^+$	33	34	39	31	33
c-$C_6H_{11}NH_3^+$	37	42	46	36	40

[a]All values are taken from [221].

from a crown ether to a cryptand with the same number of donor atoms favors the cryptand/M^+ over the corresponding crown ether complex, which can be interpreted in terms of a better preorganization of the three-dimensional array of donors in the cryptand as compared to the more floppy crown ether. A detailed comparison of 18-crown-6 **43** with its cyclohexyl-substituted analogs **syn-** and **anti-45** reveals the importance of polarizability for cation binding [215]. Despite of the greater steric hindrance, **syn-** and **anti-45** bind the alkali cations more strongly than **43**, in particular the smaller ones. A similar effect is observed for ammonium ions (Table 3) [216]. In this study, the experiments—in concert with semiempirical calculations—pointed to a stronger binding of the ammonium ions at the more crowded concave side of **syn-45** with the binding energy to **anti-45** being in-between the binding energies for the convex and the concave side of **syn-45**.

Another advantage of these experiments is that they not only provide relative binding energies, but also readily deliver rate constants for the cation transfer reaction [215]. For example, the transfer of alkali cations from **43** to **syn-** and **anti-45** is quite efficient with rate constants in the order of 20%–90% of the Langevin collision rate depending on the metal ion radius. With increasing cation size the rate constants decrease monotonically. The thermodynamic differences between **43**·M^+ and **syn-** or **anti-45**·M^+ follow the order $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. Thus, the two Li^+ complexes are separated by a larger energy difference than the Cs^+ complexes. According to the Hammond-Polanyi postulate and in good agreement with the reaction efficiencies observed in the experiment, the corresponding barrier height is thus expected to be smaller for the Li^+ transfer than that for the Cs^+ transfer [216].

Similar experiments have been conducted for earth alkali ion binding to crown ethers [217], alkali and alkaline earth binding to calixarenes [218], and the attachment of alkali ions to valinomycin [219]. Finally, the crown ethers have been used to strip alkali ions from hypermetallated biomolecules [220].

In order to obtain absolute values, an elegant and simple method has been developed by using a simple quadrupole ion trap instrument. After their generation, the ions under study are subjected to collisional activation at adjustable kinetic energies. A suitable calibration curve for the linear dependence of the critical energies on the threshold activation voltages can be constructed through processes with known activation energies. Based on this calibration, the critical energies for the fragmentation of crown ether/ammonium ion complexes have been estimated (Table 4) [221]. The error range for these measurements is estimated to be better than ± 6 kcal/mol with a standard deviation of ± 2 kcal/mol. Thus, the values obtained provide a basis for the analysis of trends. For example, the data in Table 4 show a clear dependence of the ammonium ion/crown ether binding energy on

Table 5
Total gas-phase binding energies (kcal/mol) of alkali metal ions to crown ethers **41**–**43**; for comparison, binding energies of smaller, acyclic ethers (DME: dimethyl ether, DXE: dimethoxyethane) and theoretical values are included

	41	**42**	**43**	DME_2	DME_4	DXE	DXE_2
Li^+	90.4[a]			68.2[a]	104.8[a]	58.6[a]	91.6[a]
	85.9[b]			69.4[b]	107.9[b]	61.7[b]	102.4[b]
	91.7[c]	101.4[c]	120.4[c]				
	85.7[i]		−97.5[d]			61.6[i]	102.3[i]
Na^+	60.8[e]	71.3[e]	71.8[e]				
	60.8[f]			38.5[f]	65.8[f]	41.1[f]	71.1[f]
	60.3[c]	78.6[c]	85.6[c]				
	61.7[i]		83.4[d]			42.5[i]	73.9[i]
K^+	45.7[e]	49.3[e]	56.2[e]				
	45.7[g]			28.7[g]	48.8[g]	33.3[g]	56.9[g]
	39.3[c]	53.2[c]	72.0[c]				
	46.9[i]		72.0[d]			31.3[i]	55.0[i]
Rb^+	22.7[e]	27.8[e]	45.9[e]				
	22.7[h]			22.7[h]	33.7[h]	27.5[h]	42.3[h]
	32.9[c]	45.0[c]	61.6[c]				
	39.1[i]		59.2[d]			26.1[i]	35.9[i]
Cs^+	20.6[e]	24.2[e]	40.7[e]				
	20.6[h]			13.6[h]	25.6[h]	23.9[h]	40.0[h]
	28.8[c]	39.7[c]	54.5[c]				
	33.5[i]		49.6[d]			22.3[i]	39.0[i]

[a] Guided-ion beam, [225,226].
[b] Theoretical values (MP2/6-31+G*), [225,226].
[c] Theoretical values (CHARMm force field), [181].
[d] Theoretical values (MP2/6-31+G*), [209].
[e] Guided-ion beam, [211].
[f] Guided-ion beam, [227].
[g] Guided-ion beam MS, [228].
[h] Guided-ion beam MS, [229].
[i] Theoretical values (MP2/6-31+G*), [210].

the number of donor oxygen atoms. Furthermore, the binding energies correlate with differences in the gas-phase basicities of the polyether and ammonium components and their ability to attain favorable linear hydrogen bonds.

Another energy-resolved method uses a similar approach of determining threshold energies with a more elaborate guided ion beam (GIB) mass spectrometer [222–224]. In this instrument, mass selected ions are retarded to a desired kinetic energy and transferred into an octopole which traps the ions radially. The octopole passes through a collision cell containing Xe as the collision gas. The products formed under single collision conditioning are then mass analyzed. By tuning the kinetic energy of the ions, the threshold energy can be determined where a certain fragmentation starts to occur. With the assumption that no substantial barriers exist for fragmentation, the binding energy of alkali ion/crown ether complexes could be determined (Table 5) [211,225–229]. The measured values are in good agreement, although usually somewhat lower than theoretical values at the MP2/6-31+G* level of theory, and the following trends are quite obvious from Table 5. (1) The crown ether/M^+ binding energy decreases with increasing size of the alkali ion. This is probably due to the charge density which is smaller for the larger members of the alkali series. (2) The binding energy increases with the number of available donor oxygen atoms in the crown ether. Most importantly, however, these trends do not reflect the selectivities found in aqueous solution. For example, K^+ is the ion most strongly complexed by 18-crown-6 in water. In contrast, the gas-phase binding energy of

Li^+ and Na^+ is larger than that of K^+. For a more profound understanding of these findings, it is important to consider hydrated ions and their hydration energies [229]. Indeed, for alkali ions surrounded by more than four water molecules, the ranking of their affinities to 18-crown-6 changes in favor of K^+, whereas the gas-phase result remains unaltered for up to four water molecules. As a second effect, the size of the alkali ions comes into play again. Although Na^+ is small enough to be completely wrapped inside the 18-crown-6 cavity, K^+ and larger ions are partially exposed to the solvent. Therefore, for the latter ions water directly interacts with the ion, whereas Na^+ does not have contact with the solvent and therefore is affected less strongly by solvation.

FTICR mass spectrometers allow to trap the ions inside a small cell which is placed in the center of a strong magnetic field. Such an experimental setup is suitable for storing the ions over time and the measurement of rate constants is possible by determining the product signal intensity for a fragmentation reaction of interest after different reaction intervals. If the reaction rates could be derived at different temperatures, an evaluation of the activation parameters at the low-pressure limit would be possible. However, as mentioned previously the Boltzmann distribution is not valid in the high vacuum of a mass spectrometer due to the low number of collisions. The ions do not interchange energy by collisions and thus are not in thermal equilibrium with each other. Consequently, the Arrhenius equation cannot be used to evaluate reaction kinetics. Two methods have been developed in order to circumvent these problems. Instead of collisions, the exchange of infrared photons provides the thermal equilibration of the ions [230,231]. The thermal equilibrium can be reached (1) by excitation with an infrared laser [infrared multiphoton dissociation (IRMPD)] [232] and (2) by photon exchange with the walls of the heated reaction cell inside the mass spectrometer [blackbody infrared radiative dissociation (BIRD)] [233,234]. Due to their large number of vibrational modes, the exchange of photons is much faster than dissociation reactions for large molecules. It is important to note that the temperature of the ions is not known with IRMPD. Nevertheless, Arrhenius kinetics can be applied by using the laser flux density I instead of the temperature T as a measure for the internal energy of the ions. Measurement of a series of rate constants k at different laser flux density I results in a linear correlation of $\ln k$ versus $1/I$ and the slope translates into the activation energy. Since T is not known, the intercept with the ordinate in the modified Arrhenius plot does not correlate with the pre-exponential factor of the Arrhenius equation. Consequently, this factor cannot be determined by these IRMPD experiments. In contrast, T can easily be determined in BIRD experiments just by measuring the temperature of the cell walls, once the ions are in thermal equilibrium with each other and with the walls. Thus, BIRD allows to determine both activation parameters from a conventional Arrhenius plot.

Both methods have been applied to the examination of fragmentation processes of large biomolecules such as proteins. In principle they should be applicable to supramolecular systems as well. An example for an IRMPD study deals with the guest release from the capsule **28·28** containing tetraethyl ammonium as the guest [235]. The gas-phase structure has been shown to be an intact capsule (see above). Before performing the IRMPD experiments, it is important to determine the fragmentation pathway of lowest energy. For this complex, it is the loss of the complete capsule generating uncomplexed tetraethyl ammonium ions. Double-resonance experiments demonstrated the monomer–guest complex not to be an intermediate of this process. Thus, guest release from the capsule proceeds by opening a window without the complete separation of the two capsule monomers. A series of rate constants for this process at different flux densities has been measured and converted into a modified Arrhenius plot. An activation energy of ~16 kcal/mol could be obtained. In view of typical gas-phase cation–π interactions between ammonium cations and benzene derivatives of ~9–10 kcal/mol, the higher barrier for guest release suggests once again that the ion is encapsulated with the barrier due to the energy required to break some of the hydrogen bonds.

As an example for the BIRD method, a study of the activation energies for the dissociation of double-strand oligonucleotide anions will be discussed [236].

A series of complementary and noncomplementary oligonucleotide duplexes, i.e. $(TGCA)_2^{3-}$, $(CCGG)_2^{3-}$, $(AATTAAT)_2^{3-}$, $(CCGGCCG)_2^{3-}$, $A_7{\cdot}T_7^{3-}$, $A_7{\cdot}A_7^{3-}$, $T_7{\cdot}T_7^{3-}$, and $A_7{\cdot}C_7^{3-}$, have been examined with respect to their dissociation kinetics. The activation barrier for separation of the complementary duplexes is significantly higher than that of noncomplementary duplexes and correlates to the dimerization enthalpy determined in solution. These results indicate that the Watson-Crick base pairing through hydrogen bonds is still present in the gas phase, although complete desolvation of the anions might be expected to alter the ion structure significantly. This interpretation is further supported by the finding of intense losses of neutral adenine from $A_7{\cdot}C_7^{3-}$ and $A_7{\cdot}A_7^{3-}$, which do not occur from $A_7{\cdot}T_7^{3-}$. Seemingly, this process is shut down in the complementary duplex by hydrogen bonding of the complementary bases. Molecular dynamics calculations also indicate Watson-Crick base pairing to be intact, although the helical structure is essentially lost in the gas phase. The destruction of the helix is probably caused by the effects of charge repulsion between the anionic sites in the duplex which are increased upon desolvation.

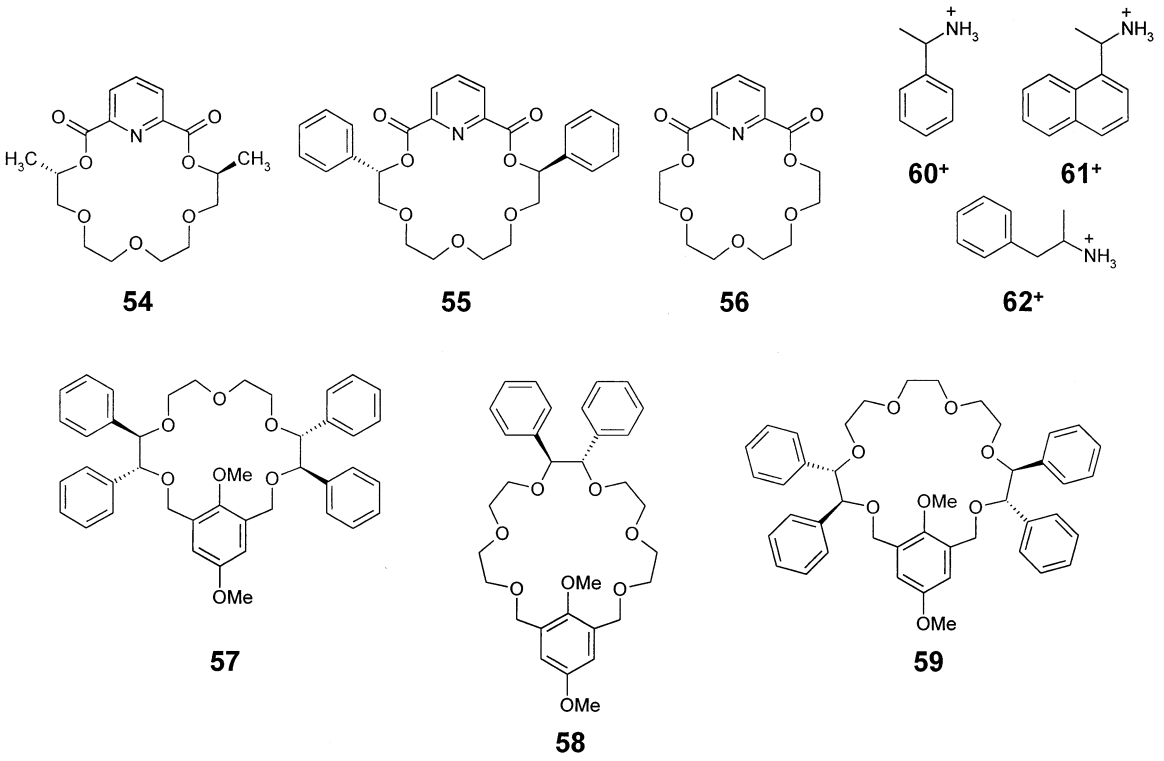
The latter examples underline the importance of energetic data measurements not only for gathering quantitative data, but also for providing a basis for qualitative arguments. The knowledge of barriers and bond dissociation energies might help to examine the structural features of supramolecular aggregates and contribute to other arguments discussed previously.

5. Stereochemistry: detection of chiral recognition by mass spectrometry

In principle, mass spectrometry is not suitable to differentiate enantiomers. Diastereomers can, however, often be distinguished and mass spectrometry has been applied to stereochemical problems in different areas of chemistry [237,238]. In the field of host–guest chemistry [239], several studies deal with cyclodextrin inclusion complexes [240,241] and other carbohydrate complexes [242–244] as well as proton bound tartrate dimers and trimers [245–248]. The largest group of articles is devoted to the examination of complexes of chiral crown ethers with chiral ammonium ions and some illustrative examples from these will be discussed here. Most of the studies applied FAB as the ionization method, some more recent studies also used electrospray ionization.

Two different methods have been developed for assessing the diastereomeric excess of host–guest complexes. Basically, the first one of them studies mixtures of one enantiomer of the host with one enantiomer of the guest and an achiral reference host [249,250]. In a second experiment, the other enantiomer of the guest is mixed with the same chiral and achiral hosts. From the relative peak intensities (RPI) of the two diastereomeric complexes as compared to the reference complex, the diastereoselectivity can be measured. The method has been refined by taking into account the existence of an equilibrium of protonated host ions competing with the host–guest complexes [251]. Scheme 14 shows a few examples for chiral crown ethers (**54**, **55** and **57**–**59**) with **56** as an achiral reference for **54** and **55**. As guests, $\mathbf{60}^+$–$\mathbf{62}^+$ have been complexed to the crown ether hosts and studied by FAB mass spectrometry [249–251].

One of the critical aspects of this approach is that two different experiments have to be performed where the particular instrument conditions must be carefully kept constant in order not to affect the intensity ratios. This problem can be overcome by the enantiomer-labeled guest method [252–255]. It is based on the mass spectrometric examination of one enantiomer of the host with a racemic mixture of the guest. In order to be able to detect both diastereomers separately, one enantiomer of the guest must be isotopically labeled, usually with deuterium. In the same experiment, both complexes are formed and their intensities can be compared directly. However, the stereochemical effect might be altered by isotope effects. This can be probed by control experiments with achiral hosts that are structurally similar to the chiral host used in the study. No effect should be observed in this control experiment. Another way to analyze stereochemical and isotope effects is to perform the same experiment with the second host enantiomer [256]. The product of



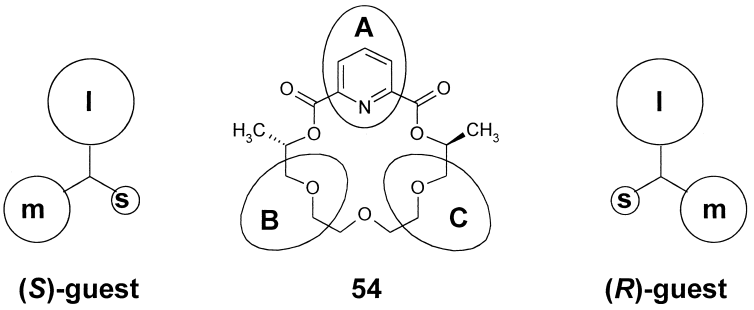
Scheme 14.

the two intensity ratios should equal unity, if no isotope effect is present [257].

The diastereoselectivities observed in the mass spectrometry experiments for some systems differ from those found in solution [251]. Further, some cases have been found, where different ionization methods, e.g. FAB versus ESI [255], gave rise to completely different diastereoselectivities. These findings point to the fact that the ionization procedure might alter the ratios of the species present in solution. Therefore, an approach using gas-phase experiments would be advantageous. Several complexes of chiral ammonium ions with the chiral crown ether **54** have been studied with the cation-transfer equilibrium method outlined above [258,259]. Steric bulk and π–π interactions between the guest and the host contribute to the intrinsic stability difference of the two diasteromeric complexes. For example, **54** forms a complex with **S-61**$^+$ which is more stable than that with **R-61**$^+$ by ~1 kcal/mol [260]. The differences of binding energies to **54** are 0.6 kcal/mol for methylbenzyl ammonium, 0.2 kcal/mol for cyclohexylethyl ammonium, and 0.1 kcal/mol for *sec*-butylammonium. The steric interactions could be explained with a three-point model as depicted in Scheme 15.

6. Mechanistic aspects: ion–molecule reactions and fragmentation processes

In the past, mass spectrometry has provided an extraordinarily powerful tool for the investigation of reaction mechanisms and the intrinsic reactivity of small ions and clusters. Several studies have extended this potential to applications in the field of supramolecular chemistry. In this section, the formation of crown ether/M^+ complexes in the gas phase will be discussed, followed by studies that deal with the collision-induced fragmentations of crown ether/ammonium complexes and the mechanistic pathways for

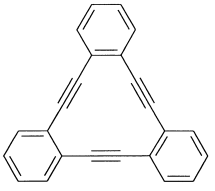


Scheme 15.

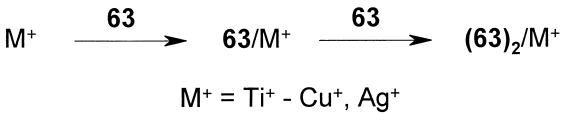
reactions of $Fe(CO)_n^+$ and $Cr(CO)_n^+$ with crown ethers.

If alkali metal ions are reacted in a FTICR mass spectrometer with neutral crown ethers, they form crown/M^+ complexes with high efficiencies of twice the Langevin collision rate or even faster [213,261]. Although in the high vacuum of the instrument no collision partners are present that could remove excess energy from the forming complex fast enough to explain this surprising observation, the complexes are believed to be radiatively stabilized. The attachment efficiencies increase linearly with the cation charge density, which was explained with a charge-induced rearrangement required for a favorable conformation of the crown ether. The efficiency of attachment is higher and depends more strongly on the charge density for the cyclic polyethers than for their acyclic analogs. In a second step, the crown/M^+ complexes react with another crown ether to sandwich-like bis-crown complexes. This reaction depends strongly on the ratio of cation size to cavity size. If the cation is smaller than the cavity, the reaction efficiency for attachment of the second crown ether is unmeasurably low. For larger cations, the efficiency increases by four orders of magnitude from the 1:1 to the 1.25:1 ratio of cation to cavity radii. The dominant role of radiative stabilization of the forming metal-ligand complex has also been observed for completely different systems. Tribenzocyclotriyne **63** (Scheme 16) reacts with transition metal ions to yield **63**/M^+ and $\mathbf{(63)_2}/M^+$ complexes [262,263].

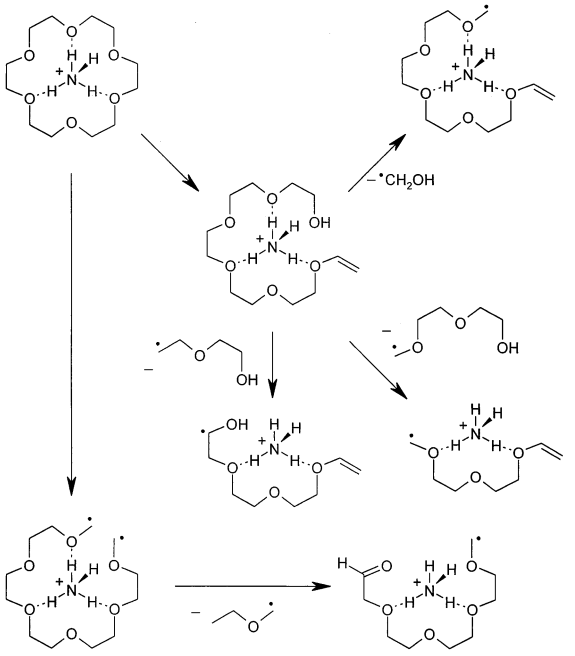
The fragmentations of crown ether/ammonium complexes have been studied by collision-induced decay [264–266]. Those complexes that are weakly bound generate predominantly protonated crowns and ammonium ions as their fragments. In these reactions, the hydrogen bonds are broken and the proton may remain with either the amine or the crown subunit. If high energy collisions are applied, or the binding energy is high, extensive cleavages of covalent bonds is observed to compete with this process. Scheme 17 shows possible mechanistic pathways which are in line with the observed fragments. The CID experiments are not restricted to ammonium/crown complexes, but have also been performed with aromatic diazonium ions attached to the crown ethers [267]. Several intriguing fragmentations have been observed, which involve N_2 loss with concomitant proton transfer to the crown giving rise to an arene/crown complex as well as a hydride transfer from the crown to the N_2 subunit. Finally, the mechanistic



63



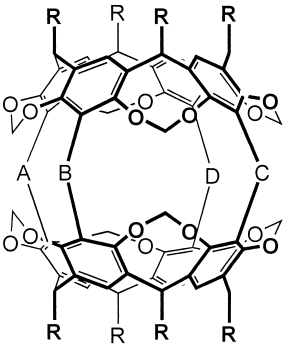
Scheme 16.



Scheme 17.

features of the reaction of bare and ligated $Fe(CO)_n^+$ and $Cr(CO)_n^+$ (n = 1–5) have been studied. For example, C–O insertion of the metal ion is followed by β-hydrogen shifts to form dialdehydes and open-chain ethers [268,269].

Two more studies should be mentioned here. The first one deals with the photodissociation behavior of carcerand/guest complexes [270]. Three different carcerands with two (**64**), three (**65**), and four (**66**) bridging $-CH_2OCH_2-$ groups (Scheme 18) between the two halves of the host have been studied with respect to their flexibility and its influence on the rate for guest release. It has been found that generally larger guests lower the barrier for their escape from the host, probably due to the release of strain. Upon laser desorption, from **64** only sodiated ions are observed without any included guest. This is in line with the larger flexibility and the size of the portals of this host. For **65**, significant signals for the host containing the trapped guests are seen in the mass spectra. The second study reports the use of hydrogen–deuterium exchange reactions in the gas phase for structure elucidation of cyclodextrin inclusion



R = CH_2CH_2Ph

64 A = C = $-CH_2OCH_2-$

65 A = B = C = $-CH_2OCH_2-$

66 A = B = C = D = $-CH_2OCH_2-$

Scheme 18.

complexes [163]. The protonated cyclodextrin exchanges the proton about ten times faster than the cyclodextrin containing an ammonium ion as the guest. This finding points to the formation of inclusion complexes between host and guest rather than unspecific aggregation in line with size arguments discussed previously.

7. Conclusions

The author hopes that in this review the huge potential of mass spectrometric methods for the examination of supramolecular architectures has been demonstrated. So far, the central focus for detailed studies has been on crown ether complexes, but ion generation usually is not an invincible problem anymore. Consequently, the methods that have been applied to crown ether complexes can be used to examine other supramolecular aggregates as well opening a whole new area in mass spectrometry as well as supramolecular chemistry.

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