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Mass spectrometric study of oligourea macrocycles and their anion binding behavior

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Two series, one of tris-urea macrocycles and another of hexakis-urea macrocycles, are examined by (tandem) Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry with respect to their fragmentation patterns and anion binding properties. All macrocycles are based on two different building blocks, one of which is a very rigid xanthene unit and the other one is a more flexible diphenyl ether. The composition and the sequence of these units thus determine their flexibility. During the fragmentation of deprotonated oligourea macrocycles in the gas phase, one urea N–CO bond is cleaved followed by a scrambling reaction within the macrocycle structure. Consequently, fragments are observed that deviate from those that would be expected from the sequence of the subunits. Interesting anion binding properties involve the simultaneous recognition of two chloride anions by one of the hexakis-urea macrocycles, whose flexibility allows this host to form a double-helical structure. Flexibility also determines which of the hexameric receptors bears a high sulfate affinity. The interaction energy between some of the macrocycles and sulfate is high enough to even stabilize the intrinsically unstable sulfate dianion.

Keywords: supramolecular chemistry; hydrogen bonding; anion recognition; urea compounds; macrocycles; gas-phase chemistry; H/D exchange

Introduction

The specific recognition of anions is still challenging.[1–5] Anions have a larger radius compared to the corresponding isoelectronic cations. This leads to weaker electrostatic interactions with their environment. Furthermore, solvation effects have a stronger influence on their host–guest interactions, and intramolecular forces are less directional. In view of the many different anions that are involved in biological functions, anion recognition is nevertheless an important area of research – despite of these problems.

Historically, the field of anion recognition started with cationic host molecules, but meanwhile, many neutral anion receptors have been designed and examined with respect to their selectivities in recognizing a broad variety of anions. A large fraction of these neutral hosts bind anions through hydrogen bonding to urea or thiourea groups.[6–11] The directionality of hydrogen bonds enables the design of receptors with shapes that are complementary to the geometry of specific anions.[1] As urea groups are able to form two strong hydrogen bonds to an appropriate acceptor, a selective recognition of anions can be achieved by incorporating several urea groups into a molecule in a particular spatial orientation. In order to fit the geometry of the anion, the receptor needs to have convergent binding sites.

To produce a receptor that matches the geometry of the planar nitrate ion, the tris-urea compounds **1**–**4** (Scheme 1) with a C_3-symmetrical arrangement of the urea groups were synthesized.[12] To achieve an appropriate preorganization of the urea units, xanthene was used as a rigid spacer. A second, more flexible spacer, a diphenyl ether derivative, was incorporated into some of the receptors in order to allow them to adapt to the anions geometry. Beside all possible cyclic tris-urea derivatives, selected cyclic hexakis-urea analogs **5**–**9** based on the same two building blocks have been prepared.[13] One of these hexakis-urea receptors (**6**) turned out to form an intriguing double-helical structure with two anion binding pockets, in which two chloride ions could bind in close proximity to each other with high selectivity.[14]

Mass spectrometry allows the study of host–guest complexes in a solvent-free environment.[15,16] Since the gaseous complexes are not affected by solvents or counterions, mass spectrometry is a useful tool for investigating the intrinsic host–guest binding properties.[17] Here, we present electrospray ionization-Fourier-transform ion cyclotron resonance (ESI-FTICR) mass spectrometric studies of the anion receptors depicted in Scheme 1. Our experiments aim at the detection of particular anion-binding properties of both series of receptors and will address questions like: Do hexakis-urea receptors **5** and **7**–**9** display similar binding properties as **6** or do their properties depend much on the flexibility of the receptors? Our experiments also include tandem mass spectrometric experiments aiming at unraveling the fragmentation pathways of these macrocycles and their complexes.

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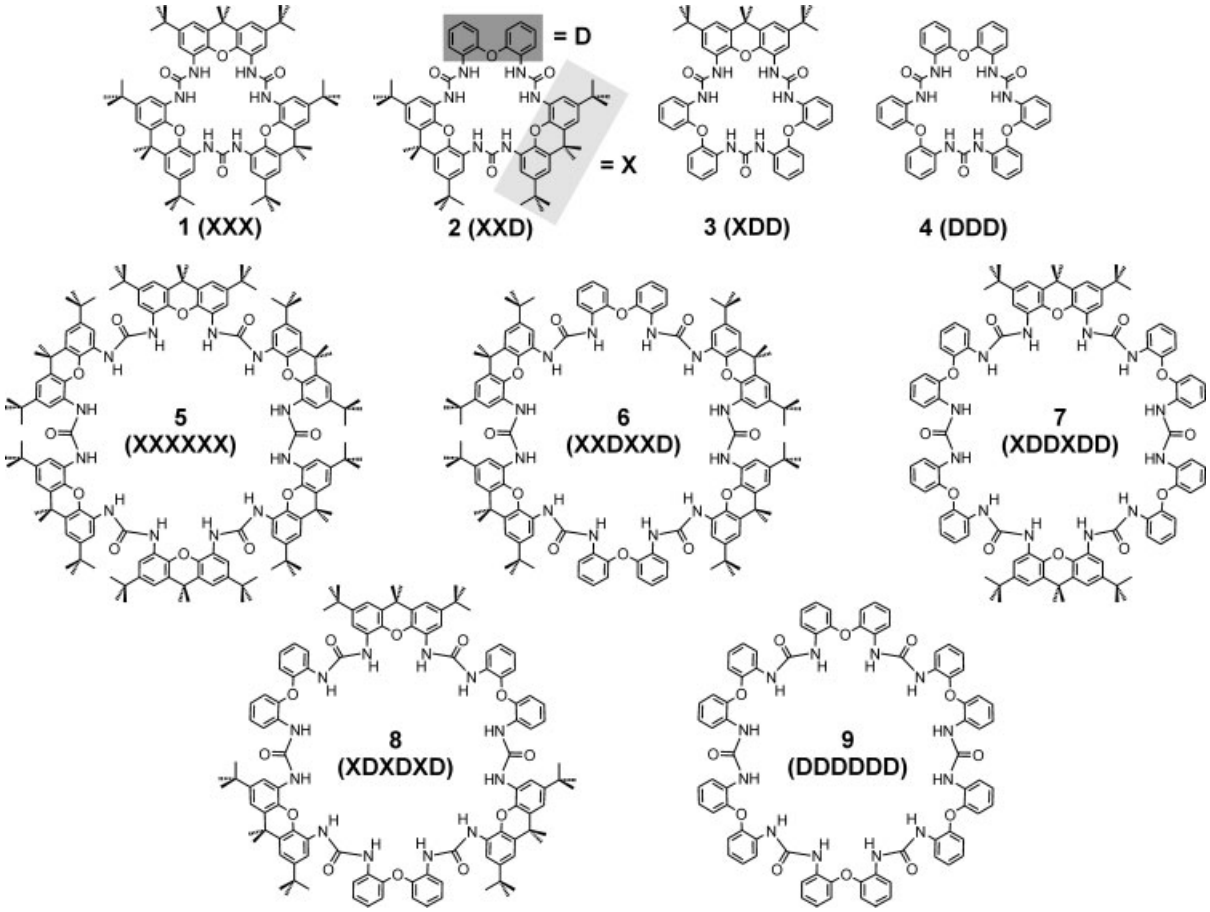


Scheme 1. Cyclic tris-urea macrocycles **1**–**4** and hexakis-urea receptors **5**–**9**. **X** and **D** denote the xanthene and diphenyl ether building blocks.

Experimental

Synthesis and characterization of receptors

The syntheses, purification and characterization of tris-urea receptors **1**–**4**[12] and hexakis-urea macrocycles **5**–**9**[13] have been described in detail earlier.

MS experiments

HPLC-grade spray solvents (tetrahydrofurane (THF), methanol (MeOH), acetonitrile (ACN)) were purchased from LGC Promochem and used as received. Tetrabutylammonium salts of the anions under study were purchased from Acros, and K_2SO_4 from Merck.

ESI mass spectra were recorded on an Ionspec QFT-7 FTICR instrument from Varian Inc., Lake Forest, CA, which is equipped with a 7-T superconducting magnet and a Micromass Z-Spray ESI ion source (Waters Co., Saint-Quentin, France). The spray voltage was set to 3.8 kV. Except if noted otherwise, a 1 : 1 mixture of MeOH and THF served as the spray solvent. The solutions were 10 μM with respect to the concentration of the hosts. Anions were added in equimolar amounts. When the guest salts were insoluble in THF/MeOH, concentrated water solutions were prepared and then added to the solutions of the hosts so that the final water content in the spray solvent was more or less negligible. Analyte solutions were introduced into the ion source with a syringe pump at flow rates of *ca* 1–4 μl/min. The parameters were adjusted as follows: source temperature: 40 °C; temperature of desolvation gas: 40 °C. Other parameters for capillary voltage, extractor cone, and sample cone were optimized for maximum intensities. No nebulizer gas was used for the experiments. The ions were accumulated in the instrument's hexapole for long enough (0.3–2.5 s) to obtain useful signal-to-noise ratios. Next, the ions were introduced into the FTICR analyzer cell, which was operated at pressures below 10^{-9} mbar, and detected by a standard excitation and detection sequence.

For infrared multiphoton dissociation (IRMPD) experiments, the ions of interest were mass-selected in the FTICR cell and activated by a CO_2 laser at a wavelength of 10.6 μm. Sustained off-resonance irradiation collision-induced decay (SORI-CID) experiments were performed by introducing N_2 as collision gas at pressures of $\sim 10^{-8}$ mbar.

For H/D exchange experiments, CH_3OD was used as the reagent. The exchange was not performed in the FTICR cell because the rather low pressures made these reactions inefficient, even when ND_3 was used as the exchange reagent. Instead, a protocol was used in which the exchange was carried out in the accumulation hexapole of the instrument.[18] For this purpose, a sufficient number of ions were accumulated in the hexapole. The entrance quadrupole of the instrument was then set to a voltage prohibiting any new ions to enter the hexapole from the source. Subsequently, the pulsed valve located *ca* 30 cm from the hexapole was opened for the required reaction times to let in the deuteration agent. The ions were then transferred to the ICR cell and analyzed by a standard excitation and detection protocol.

Results and Discussion

Fragmentation pathways of tris-urea macrocycles

In the following sections, the abbreviations D and X are used to denote the constitution of the observed fragments, each bearing an isocyanate group and a deprotonated amine group.

The tris-urea macrocycles **1**–**4** were sprayed from a THF/MeOH (1 : 1) solution in the negative mode. In the ESI-FTICR mass spectra, the signals for complexes of the macrocycles with ubiquitous chloride are the base peaks. Clearly, traces of chloride are sufficient for the formation of the chloride/tris-urea complexes. In addition to the chloride complexes, signals for the deprotonated macrocycles appear in the ESI mass spectra. Depending on the harshness of ionization conditions, the relative intensities of these two signals can be varied over a broad range. In particular, changes of the sample cone voltage induce significant changes of the relative intensities of both ions. Very likely, the anion complex can lose the protonated anion HA to yield the deprotonated macrocycle when ionization conditions are sufficiently hard.

In order to investigate the fragmentation pathways of the deprotonated macrocycles, the $[M - H]^-$ ions were mass-selected and subjected to both IRMPD and SORI-CID experiments. Both fragmentation methods give rise to very similar MS/MS spectra (Fig. 1). The mass differences observed indicate the operation of a fragmentation mechanism, which first generates an isocyanate from the urea unit that was deprotonated (Scheme 2). The simultaneously formed deprotonated amine is highly nucleophilic and thus can intramolecularly attack a second urea unit – if the flexibility of the molecule permits it. Nucleophilic attack at the second urea unit leaves two possibilities for further reaction: (1) An intramolecular transfer of one building block is possible, which in the case of $[\mathbf{1} - H]^-$ (Scheme 2) leads to the same intermediate. We will, however, see below how this process affects the fragments observed for the hexameric analogs. (2) Alternatively, one subunit can dissociate from the complex by cleaving a N–C bond in the second urea moiety. Presumably, a hydrogen-bonded complex is formed, within which a proton transfer can occur. Consequently, both products, i.e. $[\mathbf{X} - H]^-$ and $[\mathbf{XX} - H]^-$, are readily rationalized with this mechanism.

Analogously, mass-selected $[\mathbf{4} - H]^-$ fragments into $[\mathbf{D} - H]^-$ and $[\mathbf{DD} - H]^-$ (Fig. 1(d)). The two **X**/**D** mixed macrocycles $[\mathbf{2} - H]^-$ and $[\mathbf{3} - H]^-$, of course, have different sites at which the deprotonation can take place. Consequently, different fragments are formed such as $[\mathbf{XX} - H]^-$ and $[\mathbf{XD} - H]^-$ from $[\mathbf{2} - H]^-$ and $[\mathbf{DD} - H]^-$ and $[\mathbf{XD} - H]^-$ from $[\mathbf{3} - H]^-$. All these fragments can be explained by invoking the same mechanistic pathways as shown for the simpler case of $[\mathbf{1} - H]^-$.

It is remarkable that no neutral losses of bis-isocyanate fragments are observed at all. This is a strong indication that the fragments observed in the MS/MS spectra are formed through a ring-closing/ring-contracting mechanism. Two alternative mechanisms should be considered: (1) The initial ring cleavage giving rise to the isocyanate in step 1 (Scheme 2) may be followed by a 1,2 elimination within another urea unit. This second step would give rise to an amine and a second isocyanate. However, the urea group in principle can undergo such an elimination reaction in two directions. Consequently, the second isocyanate could be either bound to the $[\mathbf{XX} - H]^-$ fragment or to the leaving neutral fragment, which then would be a bis-isocyanate. This is not observed and, consequently, we can rigorously rule out this first alternative mechanism. (2) A second possibility is an intramolecular proton transfer from a second urea unit to the aryl-NH^- group. This would

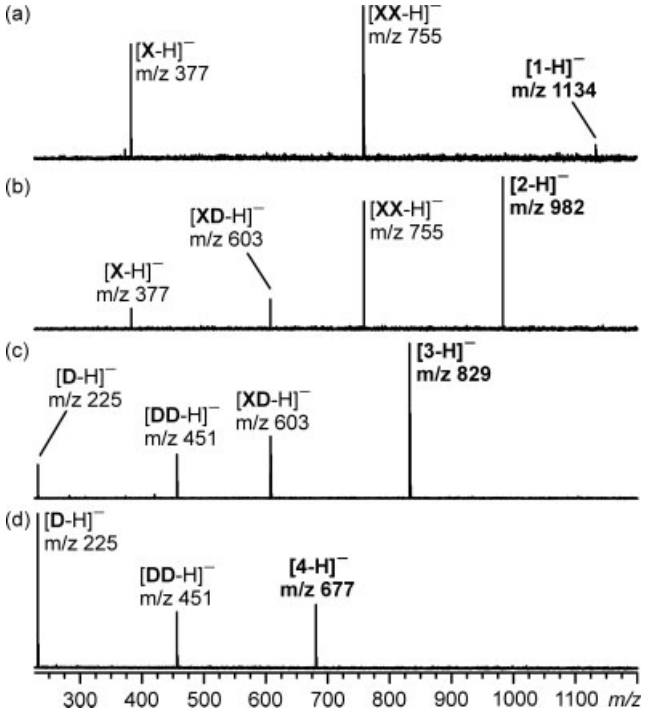
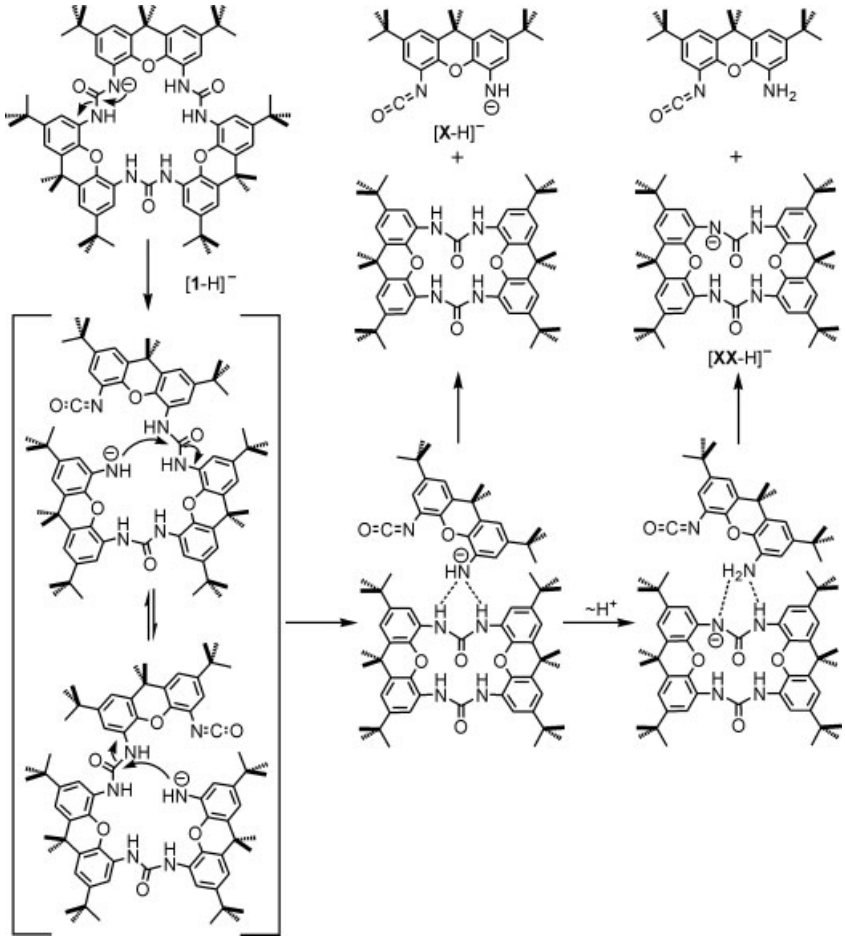


Figure 1. Tandem-ESI-FTICR mass spectra of **1**–**4** (from top to bottom; **X** denotes the xanthene, **D** the diphenylether building blocks). Spectra (a)–(c): IRMPD, (d) SORI-CID as an illustrative example that both types of MS/MS experiment lead to very similar results.

be in line with the expected basicities for these two functional groups. However, again, the deprotonation could occur on each of the two sides of the urea unit and a bis-isocyanate should be among the neutral fragments formed. This is not the case either, and thus the second alternative mechanism can also be ruled out. From these considerations, we conclude the fragmentation to proceed through a ring-closing/ring-contracting nucleophilic attack on the second urea unit.

Complexes of tris-urea macrocycles and anions can quite easily be produced in the ion source just by adding the appropriate tetrabutylammonium salt to the spray solution. In the tandem MS spectra of such mass-selected complex ions, very similar fragmentation reactions are observed (Fig. 2). As expected, the only significant difference is the preceding loss of the protonated anion. This loss generates $[M - H]^-$ ions, which then undergo the same fragmentation processes as discussed above. In the gas phase, anionic hydrogen bonds can have substantial strengths and one might expect weaker covalent bond cleavages to be able to compete with the dissociation of the supramolecular bond, when more than one such ionic hydrogen bond is involved. However, this is not observed here. Two reasons may account for this finding: (1) In order to generate covalent fragments of the macrocycle, two covalent bonds must be broken. This increases the necessary activation energy for fragments derived from covalent cleavages. (2) The anionic guest may template a certain conformation of the macrocycle as well as of its ring-opened intermediate which hamper the intramolecular nucleophilic attack, which was the basis of the mechanism in Scheme 2. It is not completely clear what the real structures of these anion complexes are. At least some anions such as fluoride, acetate, or nitrate are quite basic in the gas phase. They may thus be able to deprotonate the receptor macrocycle and the complex may have the structure of a neutral receptor



Scheme 2. Fragmentation mechanism of [**1** − H]⁻. First an isocyanate is formed, which further fragments into the [**X** − H]⁻ and [**XX** − H]⁻ fragments shown.

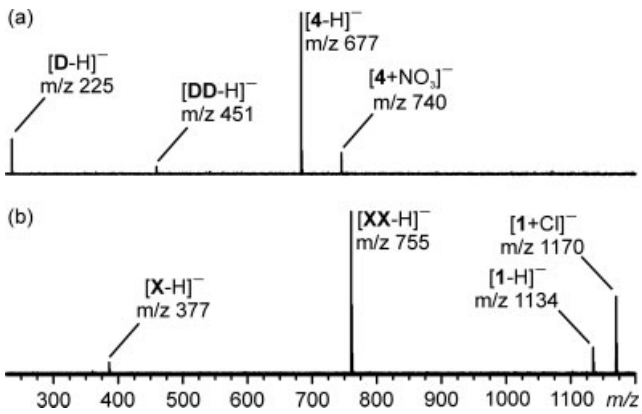


Figure 2. Tandem mass spectra of anion complexes. (a) SORI-CID spectrum of [**4** + NO_3]⁻. (b) IRMPD spectrum of [**1** + Cl]⁻. Again, both methods are represented, each with one example to show the similar results obtained by both.

surrounding an anion, but it may also more accurately be described as a protonated anion being bound to a deprotonated receptor.

Fragmentation of hexakis-urea macrocycles

Figure 3 shows the tandem MS spectra of hexakis-urea macrocycles [**5** − H]⁻ to [**8** − H]⁻. Not surprisingly, [**5** − H]⁻, which is the all-**X** derivative, has the simplest fragmentation pattern. The all-**D** counterpart [**9** − H]⁻ shows a very similar pattern, in which all xanthene units are replaced by diphenyl ethers. In close analogy to the tris-urea macrocycles, only the mono-isocyanate fragments are formed, which indicates that a related mechanism is operative also for the larger macrocycle ions. Consequently, a series of [$\mathbf{X}_n$ − H]⁻ oligomers is observed in the MS/MS spectrum. The MS/MS spectra of **X**/**D** mixed hexamers [**6** − H]⁻ to [**8** − H]⁻, however, show a quite interesting feature. Several fragments that are observed in the fragmentation spectra are *not* consistent with the sequence of **X** and **D** building blocks within the intact macrocycle. Since the sequence is firmly established by the synthetic route used for the preparation of the macrocycles and by the spectroscopic characterization in solution (e.g. their symmetries as obtained from the number of sets of signals observed in the NMR spectra), these fragments indicate the operation of a scrambling process during fragmentation. This finding provides further evidence for the intramolecular nucleophilic attack of the aryl-NH⁻ nitrogen atom on the urea carbonyl group of a second urea unit. This reaction can lead to intramolecular group transfers analogous to that shown in the brackets in Scheme 2. The scrambling thus supports the assumption that fragmentation proceeds through a ring-closure/ring-contraction mechanism as discussed above for the smaller tris-urea macrocycles.

A second aspect is that tetrameric fragments are formed with significantly higher intensity as compared to the pentameric

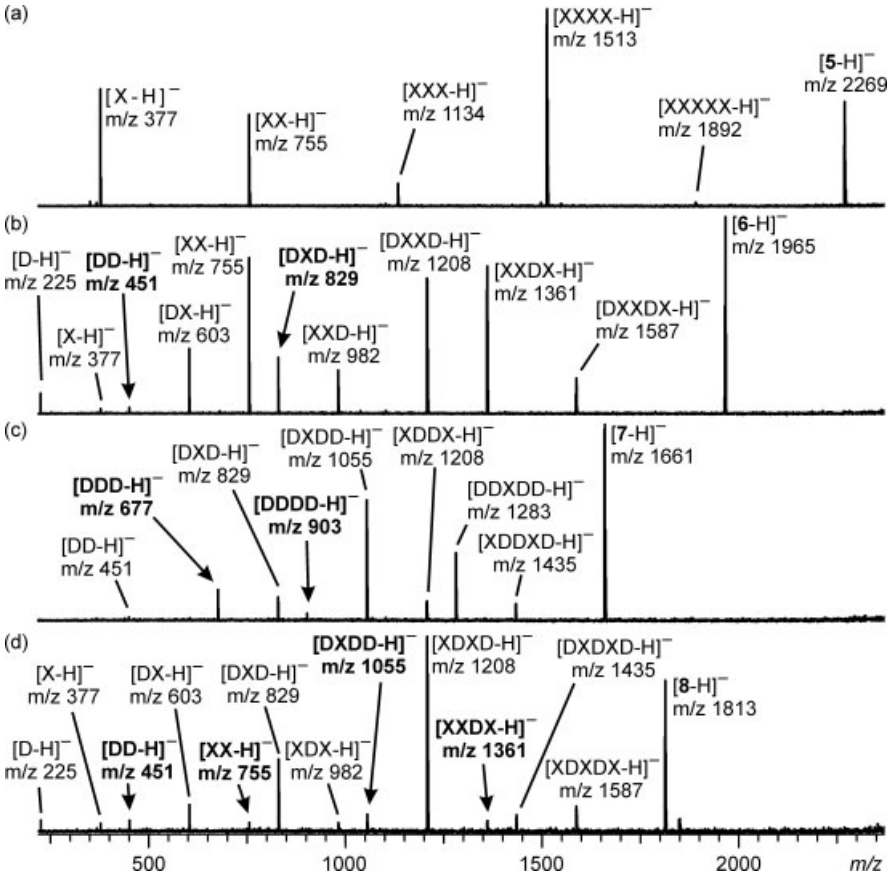
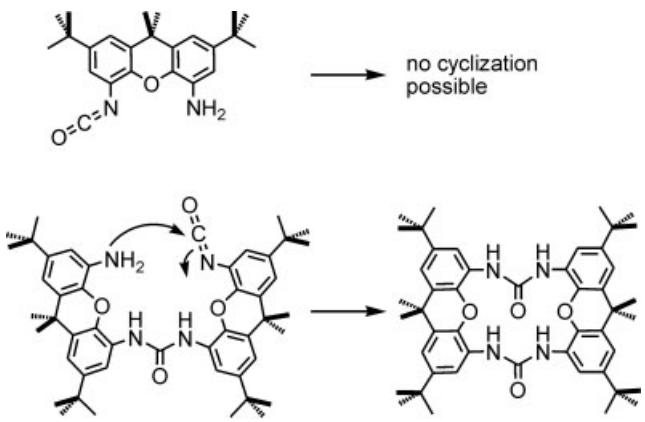


Figure 3. Tandem mass spectra of [**5** − H]⁻ to [**8** − H]⁻ (from top to bottom). The $\mathbf{D}_6$ hexamer [**9** − H]⁻ behaves in close analogy to [**5** − H]⁻ and is therefore not shown. Bold labels and arrows indicate fragments that are not in line with the sequence of **X** and **D** subunits in the corresponding macrocycles (Spectra (a)–(c): IRMPD, (d): SORI–CID.



Scheme 3. A cyclization reaction stabilizing a dimeric neutral fragment resulting from a fragmentation of [**1** − H]⁻ into tetrameric [**XXXX** − H]⁻. Such a reaction is not feasible in the monomeric neutral fragment above which would be produced during the formation of pentameric [**XXXXX** − H]⁻ fragment ions.

fragment ions. This is particularly pronounced in the MS/MS spectrum of [**5** − H]⁻ in which the [**XXXXX** − H]⁻ fragment is hardly visible above the noise, while the [**XXXX** − H]⁻ ion represents the base peak. The other macrocycle ions show a similar behavior though less pronounced. Scheme 3 provides a rationalization for this finding: After cleavage of the hexameric macrocycle, a neutral and an anionic fragment are formed, which likely are bound to each other by hydrogen bonding. The anionic fragment has already undergone ring closure as discussed above. All neutral fragments contain one amino group and one isocyanate. In monomeric units **X**, no ring closure is possible within this neutral fragment. However, dimeric neutrals **XX** are able to undergo an intramolecular ring closure by nucleophilic attack of the amine at the isocyanate carbon atom. This cyclization likely provides a stabilization of the neutral and thus lowers the corresponding exit channel. A similar, but less pronounced, intensity alternation is observed for the trimeric and dimeric product ions. This alternation remains visible when either the collision energies are altered in

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the CID experiments or when the laser flux density is changed in the IRMPD experiments.

H/D exchange experiments with receptors 1, 2, 6, and 7 and their anion complexes

As already mentioned above, tris-urea receptors **1**–**4** bind anions such as chloride, bromide and nitrate. When fluoride is used as the anion, only the deprotonated receptor is observed, likely because bare fluoride is a rather strong base and deprotonates the receptor when the complex is transferred into the gas phase. When 1 equiv. of bromide was added to the receptor, the mass spectra show the bromide complex as well as the complex with background chloride. If the bromide complex is pre-formed by addition of 1 equiv. of bromide to one of the receptors, the addition of 1 equiv. of chloride leads to an instantaneous exchange of the bromide against chloride, which is thus more strongly bound than bromide. We can thus use mass spectrometry to investigate a ranking of anion binding strengths in solution.

In favorable cases, H/D exchange experiments in the gas phase can be used to determine whether exchangeable protons are involved in hydrogen bonding. If so, they are often protected against exchange,[19–22] while those that are not hydrogen-bonded quite rapidly exchange in the gas phase when a deuteration agent is present. Consequently, the H/D exchange reaction may provide a means to determine how many urea protons are involved in anion binding of our macrocycles. This would provide further insight into the structures of the anion complexes under study. H/D experiments were thus performed with the deprotonated receptors **1**, **2**, **6** and **7** and their chloride complexes. The protonated and sodium- or potassium-cationized analogs that are formed in the positive mode were also subjected to an exchange experiment for comparison. Methanol-OD was used as the exchange reagent. After a reaction time of 10 s, the exchange of the urea protons was almost complete for the deprotonated $[\mathbf{1} - H]^-$ and $[\mathbf{2} - H]^-$ as well as the protonated $[\mathbf{1} + H]^+$ and $[\mathbf{2} + H]^+$ macrocycles. However, the corresponding chloride complexes did not show any exchange within the same reaction period. When the exchange experiments are performed with hexakis-urea macrocycles **6** and **7**, the same behavior is observed (Fig. 4). Again, only the $[M - H]^-$ and $[M + H]^+$ ions exchange their urea protons within the reaction times of 10 and 15 s, respectively. No exchange is observed for the monochloride and sulfate complexes $[M + Cl]^-$, the $[\mathbf{6} + SO_4]^{2-}$ and the sodium or potassium adducts.

The fact that deprotonation of one urea group makes the exchange of all NH protons possible can be rationalized by the relay mechanism shown in Scheme 4. Methanol-OD forms a strong anionic hydrogen bond with the deprotonated urea site and a second weaker hydrogen bond with the adjacent urea NH proton. A simple redistribution of electrons through the six-membered transition structure then leads to the exchange which is followed by dissociation of methanol-OH. Similar relay mechanisms have been postulated earlier for the H/D exchange reaction proceeding quickly in protonated ethylene diamine[19] and other molecules.[23] In such a mechanism, the formation of the strong ionic hydrogen bond provides a sufficiently high binding energy, which is available to the complex to overcome the exchange barriers. After this first exchange on the deprotonated urea unit, proton migrations occur between different urea units. They lead to a shift of the deprotonated site and thus finally allow the macrocycle to exchange all urea protons against deuterium.

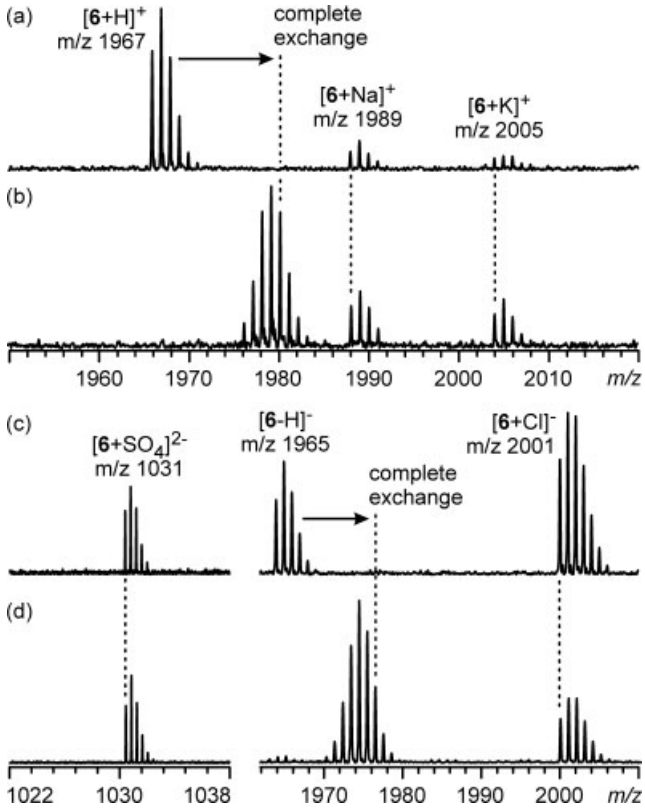
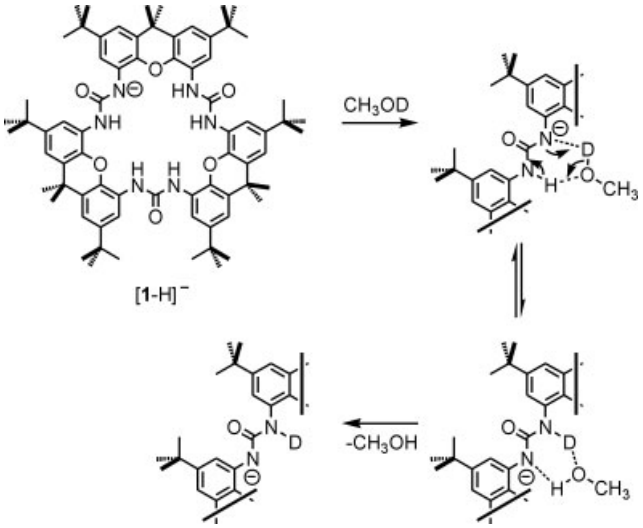


Figure 4. Top: Gas-phase exchange experiment performed with $[\mathbf{6} + H]^+$, $[\mathbf{6} + Na]^+$ and $[\mathbf{6} + K]^+$: (a) without exchange, (b) after 15 s reaction with CH_3OD. Bottom: Exchange experiment performed with $[\mathbf{6} + SO_4]^{2-}$, $[\mathbf{6} - H]^-$ and $[\mathbf{6} + Cl]^-$: (c) no exchange, (d) after 10 s reaction with CH_3OD.

A similar 'relay' or 'flip-flop' mechanism is possible in the protonated macrocycles: X-ray crystal structures and calculated geometries for the tris-urea macrocycles indicate that the urea units do not necessarily exist in their *trans*-configuration only.[12] Structures with one or even two *cis*-configured O=C–NH bonds are accessible. In the *cis*-isomers, similar relay mechanisms are feasible, which lead to the H/D exchange of all protons in the protonated macrocycles.

One question is left now: Why is no proton exchange observed in the chloride and sodium or potassium complexes? Two reasons may account for these findings: (1) On one hand, the absence of any exchange in the chloride complex, for example, might indicate all urea N-H protons to be involved in hydrogen bonding. The anion would thus protect all NHs against isotopic exchange. While this assumption may be credible for the tris-urea macrocycles (the formation of six hydrogen bonds has indeed been observed in a crystal structure of NBu_4^+ $[\mathbf{4} + Cl]^-$[12]), it is certainly not very convincing for **6** or **7** with their 12 urea protons. Furthermore, no exchange is observed in the sodium and potassium adducts. Since the cations are expected to be complexed to urea carbonyl oxygen and thus do not block the NH protons, it remains unclear why no exchange of the free urea NH protons should be accomplished with these ions. Consequently, the absence of H/D exchange products in the experiments conducted with the chloride and cation complexes likely has a different explanation. (2) The second alternative is that two binding sites are needed for the exchange reagent if the exchange is to proceed efficiently. This is the case in the deprotonated macrocycles: The MeOD molecule can form two hydrogen bonds to the deprotonated urea group one of



Scheme 4. A relay mechanism which explains the quite fast exchange of all urea protons in the deprotonated macrocycles **1**–**4**.

which is a strong anionic hydrogen bond.[17] With the protonated macrocycles, the formation of a similarly strong proton bridge is possible. In contrast, the deuteration agent can only form much weaker non-ionic hydrogen bonds or cation bridges, respectively, in the complexes with chloride or sodium. The binding energy of the MeOD is thus lower and, probably, the complex is not able anymore to overcome the deuteration barriers since less internal energy is available. Consequently, no exchange is observed. Unfortunately, no clear information on the number of urea protons involved in the anion binding can thus be gathered through H/D exchange experiments in the gas phase. Nevertheless, the experiments suggest a relay mechanism to be operative during the H/D exchange reaction with the deprotonated and protonated macrocycles, and thus provide at least some more detailed insight into the exchange mechanism.

Special anion binding features of hexakis-urea macrocycles 6 and 7

Hexakis-urea macrocycle **6** is known[14] to form a double-helical structure which possesses two binding pockets. The crystal structure of the $(NBu_4^+)_2$ [**6** + 2Cl]$^{2-}$ complex reveals each of these pockets to bind one chloride through the formation of six hydrogen bonds with three urea units. This special geometry is reflected in the ESI mass spectra of samples containing receptor **6** and 2 equiv. of NBu_4Cl (Fig. 5(a)). Receptor **6** is the only hexakis-urea compound that shows this particular feature. In solution or in the crystal, the counterions compensate for at least some of the charge repulsion between the two chloride anions. In the gas phase, however, no counterions or stabilizing solvent dipoles are available and thus the two anions experience the full charge repulsion. If the distance of the two chloride anions remains unchanged with respect to that found in the crystal structure (d_{Cl-Cl} = 6.029 Å), one would estimate a Coulomb repulsion energy of *ca* 200 kJ/mol for the gas-phase complex. In view of this rather high value, one might expect the structure to change during ionization, but still it is quite remarkable that the host can maintain the binding of both anions even in the gas phase – even more so, if one considers that this dianion represents the base peak in the spectrum.

The small but clearly visible signal for a doubly charged SO_4^{2-} complex at *m/z* 1031 in Fig. 5(a) prompted us to attempt the

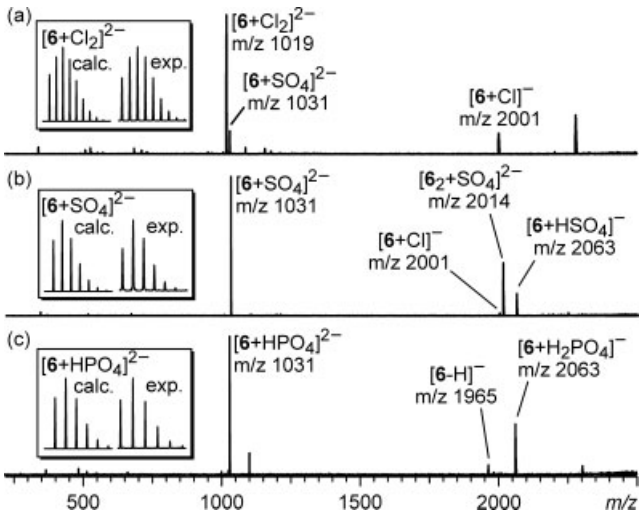


Figure 5. ESI mass spectra of **6** and (a) 2 equiv. NBu_4Cl in THF : MeOH (1 : 1), (b) 1 equiv. K_2SO_4 in THF : MeOH (1 : 1) and (c) 1 equiv. $NBu_4H_2PO_4$ in acetonitrile.

binding of sulfate to the hexameric receptors. Macrocycle **6** again turns out to be special. While **9** does not show any signal for sulfate binding, all other hexakis urea receptors show a signal for [M + SO_4]$^{2-}$. For **5** and **8**, these signals appeared with rather low intensity. Upon the addition of a chloride salt, full exchange of sulfate against one chloride anion was observed. In the spectra of **6** and **7** with K_2SO_4, the [M + SO_4]$^{2-}$ dianions correspond to the base peak (Fig. 5(b)). The addition of chloride has only a minor effect on the appearance of the spectra. From these experiments, we conclude that **6** and **7** bind sulfate even more strongly than chloride in solution. The same trend is observed for dihydrogen phosphate (Fig. 5(c)). Although the details of the complex structures are not known yet, it is clear that the differences in flexibility of the receptors must have an important effect. A too rigid receptor such as **5** is unable to adopt the geometrical arrangements of urea units required for efficient binding. In contrast, very flexible receptors such as **8** and **9**, which bear a large number of **D** instead of **X** building blocks, suffer from an entropic

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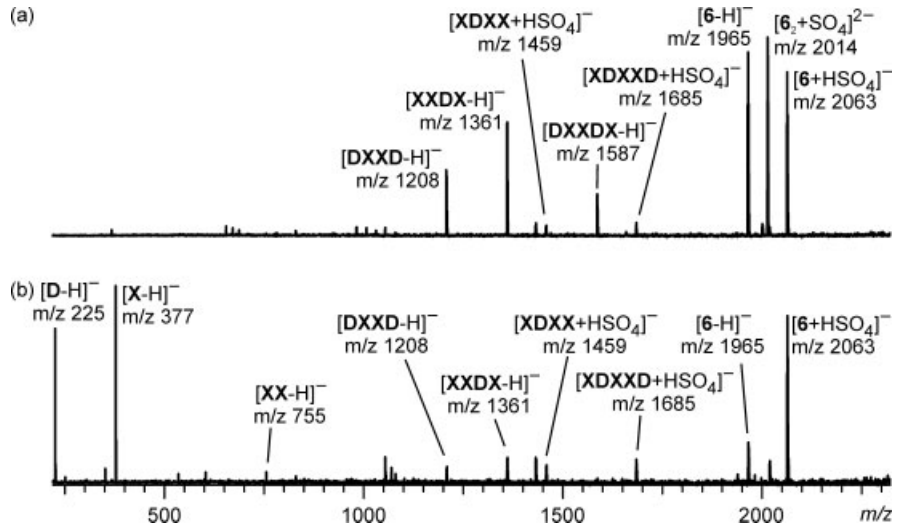


Figure 6. IRMPD spectra of (a) mass-selected $[\mathbf{6}_2 + SO_4]^{2-}$ and (b) mass-selected $[\mathbf{6} + HSO_4]^-$.

penalty when the receptor conformation is fixed upon anion binding.

The binding of sulfate is particular with respect to the stability issues related to small dianions. It is well known that bare sulfate dianions are unstable in the gas phase. They undergo a quick electron autodetachment to yield the singly charged $SO_4^{-\bullet}$ anion radical. Theory predicts this anion radical to be more stable than the dianion by *ca* 130–160 kJ/mol, depending on the level of calculation used.[24] Experimental work suggests sulfate to become stable as a dianion when solvated with at least three water molecules forming a total of six hydrogen bonds.[25,26] The hexakis-urea macrocycles **6** and **7** must therefore bind sulfate with a similar binding energy. Two other possibilities should be taken into account: (1) In the gas phase, the sulfate dianion might be sufficiently basic to deprotonate one urea unit. In this case, however, we would deal with an HSO_4^- guest anion binding to a negatively charged receptor molecule. Again, the binding energy must be quite high in order to overcome charge repulsion. (2) The sulfate might also be nucleophilic enough to open the receptor macrocycle at one of the urea units leaving one charge on each end of the chain. However, if such a structure is formed, one would certainly expect that sulfate-bearing fragments are formed in CID experiments; but this is not observed. Instead, a rather clean fragmentation into HSO_4^- and $[\mathbf{6} - H]^-$ or $[\mathbf{7} - H]^-$ is found. We therefore conclude the macrocycle to retain its cyclic structure.

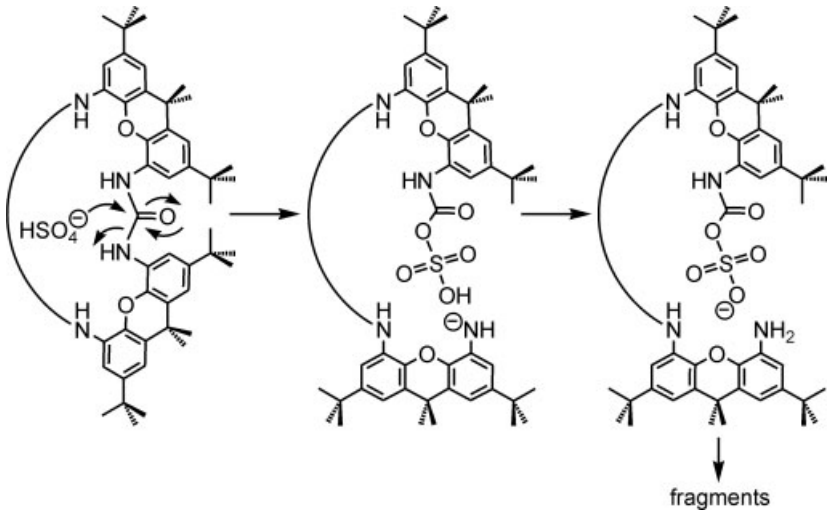
Sulfate also forms dimer–anion complexes $[\mathbf{6}_2 + SO_4]^{2-}$ that appear in the ESI mass spectrum at *m/z* 2014. When these dimer dianions are subjected to a fragmentation experiment (Fig. 6(a)), they fragment as expected and yield $[\mathbf{6} - H]^-$ at *m/z* 1965 and $[\mathbf{6} + HSO_4]^-$ at *m/z* 2063. Consequently, charge repulsion drives the fragmentation into two singly charged particles through a deprotonation of one of the receptors by the sulfate dianion. The subsequent fragmentations, which have been confirmed independently by mass-selecting and fragmenting the $[\mathbf{6} + HSO_4]^-$ ion directly (Fig. 6(b)), are quite surprising; indeed, fragments appear that contain the hydrogen sulfate moiety, i.e. $[\mathbf{XDXXD} + HSO_4]^-$ at *m/z* 1685 and $[\mathbf{XDXX} + HSO_4]^-$ at *m/z* 1459. This marked contrast in the fragmentation between $[\mathbf{6} + HSO_4]^-$ and $[\mathbf{6} + SO_4]^{2-}$ can easily be explained. In the fragmentation of the dianion, charge repulsion favors the deprotonation/charge separation exit channel. Even if the nucleophilicity of sulfate dianions is high in the gas phase, this exit channel is more favorable due to the repulsive Coulomb forces. For $[\mathbf{6} + HSO_4]^-$, however, no such forces play a role and no such favorable exit channel is available. Instead, this ion can react through a nucleophilic attack of the hydrogen sulfate anion at one of the urea units as shown in Scheme 5. A structure forms that corresponds to a mixed anhydride of a carbamic acid and sulfuric acid. A final proton transfer to the imide concludes this rearrangement. Upon further activation, the open-chain hexamer fragments within one of the other urea moieties yielding *inter alia* the two sulfurylated fragments observed.

Conclusions

The present study provides insight into the fragmentation patterns and anion binding capabilities of tris- and hexakis-urea macrocycles **1**–**9**. The fragmentation mechanisms are interesting because a group transfer reaction precedes the fragmentation, which leads to the scrambling of the xanthene and diphenyl ether (**X** and **D**) building blocks in the receptors. In the MS/MS spectra, fragment ions are thus observed that do not correspond to the sequence of these building blocks.

H/D exchange experiments were performed in the gas phase in order to determine the number of hydrogen bonds involved in anion binding. Unfortunately, however, this goal could not be achieved. Instead, these experiments provided insight into the exchange mechanisms in agreement with earlier literature reports on other sample molecules and functional groups.

While the anion binding behavior of the tris-urea receptors is rather simple, the hexakis-urea analogs show several highly interesting aspects. (1) Two chloride anions are bound to macrocycle **6** in close proximity, while such a binding mode has not been observed for the other receptors. Receptor **6** is thus able to overcome charge repulsion effects quite efficiently. (2) Macrocycle **7** and even more so **6** bind sulfate dianions quite strongly and thus prevent the electron autodetachment which would otherwise be expected. (3) In the fragmentation of the hydrogen sulfate complex of **6**, covalent fragments are detected, which still contain the HSO_4^- anion. It is rather unlikely that the noncovalent interaction between both molecules is stronger than the covalent bonds – in particular, if one takes into account that two covalent bonds must



Scheme 5. Generation of sulfate-containing fragments from [**6** + HSO_4]$^-$.

be broken in order to release a fragment. Consequently, this observation points to a covalent, open-chain adduct of HSO_4^- and the macrocycle which forms at least as a fragmentation intermediate.

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