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Cooperative Effects

Cooperative Binding of Divalent Diamides by *N*-Alkyl Ammonium Resorcinarene Chlorides

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Abstract: *N*-Alkyl ammonium resorcinarene chlorides, stabilized by an intricate array of hydrogen bonds leading to a cavitand-like structure, bind amides. The molecular recognition occurs through intermolecular hydrogen bonds between the carbonyl oxygen and the amide hydrogen of the guests and the cation–anion circular hydrogen-bonded seam of the hosts, as well as through CH···π interactions. The *N*-alkyl ammonium resorcinarene chlorides cooperatively bind a series of di-acetamides of varying spacer lengths ranging from three to seven carbons. Titration data fit either a 1:1 or 2:1 binding isotherm depending on the spacer lengths. Considering all the guests possess similar binding motifs, the first binding constants were similar (K_1: $10^2\ M^{-1}$) for each host. The second binding constant was found to depend on the upper rim substituent of the host and the spacer length of the guests, with the optimum binding observed with the six-carbon spacer (K_2: $10^3\ M^{-2}$). Short spacer lengths increase steric hindrance, whereas longer spacer lengths increase flexibility thus reducing cooperativity. The host with the rigid cyclohexyl upper rim showed stronger binding than the host with flexible benzyl arms. The cooperative binding of these divalent guests was studied in solution through ^{1}H NMR titration studies and supplemented by diffusion-ordered spectroscopy (DOSY), X-ray crystallography, and mass spectrometry.

Introduction

High affinity and/or selectivity of substrate recognition by synthetic receptors is an increasing area of research with applications in biology, such as the recognition of specific amino-acid side chains on a protein.[1] Exploiting cooperativity to achieve such recognition is one of many design options. Cooperativity arises from the interplay of two or more interactions behaving differently from expectations based on the properties of the individual interactions acting in isolation.[2–4] This phenomenon is usually observed in multivalent processes in which the coupling of interactions can lead to positive or negative cooperativity, depending on whether one process favors or disfavors another. Cooperativity is vital in systems chemistry because it can lead to collective properties that are absent in the individual molecular components, and this phenomenon constitutes one of the most important properties of molecular systems in biology.[2,3,5] Positive cooperativity occurs when the binding of one substrate leads to higher affinity of subsequent substrates, and is a common phenomenon in substrate recognition by enzymes.[6] Cooperative effects are known to play an important role in the binding of ion pairs by heteroditopic receptors.[7–11]

N-Alkyl ammonium resorcinarene halides, obtained from the ring opening of tetrabenzoxazines in the presence of mineral acids heated at reflux, are stabilized by a strong circular hydrogen-bonded cation–anion seam ($(\cdots NR'R''H_2^+\cdots X^-\cdots NR'R''H_2^+\cdots X^-\cdots)_2$).[12,13] These organic salts are usually referred to as hydrogen bond analogues of covalent cavitands due to their conformational and cavity size similarities.[12,13] These large organic salts are versatile receptors that can bind a variety of neutral guests through several weak interactions utilizing the electron-rich resorcinarene cavity and the hydrogen-bonded cation–anion seam.[13,14]

Amides possess both hydrogen-bond-donating and accepting groups, making them suitable guests for these receptors.[14] This functional group is commonly employed in various technological applications.[15] In biology, the amide or peptide bond is the principal structural element in proteins and is essential to a vast number of drugs, with the iconic penicillin being a good example.[16] Because the use of amides is very broad, the quest for receptors that can suitably bind them is an interesting and a continuously developing area of research.[15] Recent results from our group show that *N*-methyl-substituted amides possessing hydrogen-bond-donating –NH and accepting –C=O groups are particularly good guests for the resorcinarene salt receptors.[14] Together with strong hydrogen bonds, these receptors can also bind amides through sev-

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eral weak CH···π interactions.[14] The positive results from the binding of mono-amides prompted the investigation of cooperativity in the binding of a series of divalent homoditopic diamides of varying spacer lengths. A similar phenomenon has been reported, in which negatively charged homoditopic guests with aromatic end groups template dimeric assembly in the presence of positively charged calixarenes in aqueous media.[17]

In the study described herein, we set out to investigate the cooperative binding of diamides **4–7** by the *N*-alkyl ammonium resorcinarene chlorides **1–3**. In the process, diacetamides **4–7** of varying spacer lengths, ranging from three to seven carbons, were synthesized and used as homoditopic guests. Cooperative binding of these diamides by **1–3** was investigated in solution through a series of ^{1}H NMR titration studies and supported by diffusion-ordered NMR spectroscopy (DOSY NMR), single-crystal X-ray diffraction analyses, and electrospray ionization mass spectrometry (ESI-MS) in the gas phase.

Results and Discussion

N-Alkyl ammonium resorcinarene chlorides **1–3** (Figure 1), with cyclohexyl and benzyl substituents, were synthesized according to reported procedures.[12,13] The $-CH_2$ of the benzyl groups introduces a degree of flexibility to the upper rim of

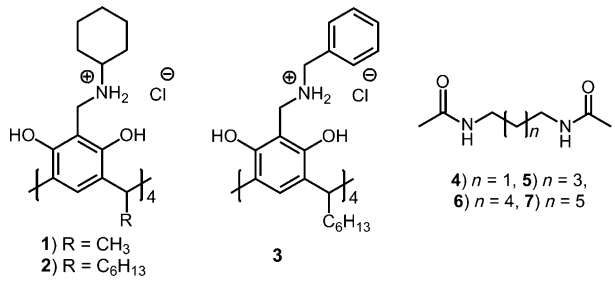

Figure 1. *N*-Alkyl ammonium resorcinarene chlorides **1–3** and the diamide **4–7** guests.

the receptors because of the additional rotatable bond compared with cyclohexyl. The strong circular hydrogen-bonded cation–anion seam, formed between the spherical chloride anions and the ammonium cations, results in an extended interior cavity suitable for the recognition of a variety of guest molecules.[13,14,18] The resorcinarene receptors **1–3** are C_{4v} symmetric in solution as observed from their ^{1}H NMR spectra. We recently showed that these types of receptors could bind a variety of small neutral amides such as *N*-alkyl and *N*-aryl acetamides possessing hydrogen-bond-donating and accepting groups.[14] From the previous results, the idea of the possible cooperative binding of diamides was initiated and subsequently, a series of *N*-alkyl diacetamides **4–7** were synthesized through the Schotten–Baumann reaction in which an acid chloride reacts with amines under basic conditions.[19,21] The length of the spacer between the –NH groups of the diamides varies from three to seven carbons (Figure 1).

Solution studies

The binding of the mono-acetamides by analogues of the receptors show 1:1 binding stoichiometry, as determined by Job plot experiments.[22,23] Based on this knowledge, one would expect the receptors to bind both ends of the diamides in a similar 1:1 binding stoichiometry, hence resulting in an overall 2:1 host–guest binding stoichiometry. Job plot experiments involving the receptor **3** with the guest **4** showed a 1:1 stoichiometry as the predominant species, whereas it was 2:1 with guests **5–7** (see the Supporting Information, Figure S5). The determination of the stoichiometry of complex systems other than 1:1 can be a limitation of the Job method,[24] therefore the binding stoichiometry could be supported by DOSY NMR spectroscopy, titration data, and analysis by X-ray crystallography.[24]

Diffusion-ordered NMR spectroscopy

Diffusion can be used to determine intermolecular interactions in solution because the diffusion coefficient of a molecular species under specific conditions (e.g., concentration, solvent, temperature, etc.) depends on its molecular weight, size, and shape.[25,26] These receptors are known to encapsulate solvent molecules within their cavities,[13,14] as shown from the X-ray structure of the cyclohexyl analogue **2** obtained from a chloroform/diethyl ether mixture. A 1:2 host–guest complex with two chloroform molecules in the cavity was found.[14] Weak interactions such as $N(NH_2^+)$–H···Cl($CHCl_3$), C–H···Cl($CHCl_3$), and C($CHCl_3$)–H···Cl^- hold the chloroform in the cavity of the receptor.[14] The inclusion of the $CDCl_3$ in the cavity of **3** was also supported by its diffusion coefficient of 1.615×10^{-5} cm^2 s^{-1} (pure $CDCl_3 = 2.226\times10^{-5}$ cm^2 s^{-1}).[27]

Our recent study with the *N*-propyl analogue showed these large organic salt compounds to aggregate into dimeric assembly in chloroform.[14] The diffusion coefficient of receptor **3** (30 mM) in $CDCl_3$ at 303 K was $(0.378\pm0.03)\times10^{-5}$ cm^2 s^{-1} (Figure 2, Table 1). Reported diffusion coefficients of dimeric long chain resorcinarenes in chloroform were around 0.35×10^{-5} cm^2 s^{-1}.[28] Based on the DOSY experiments (Figure 2, Table 1, and the Supporting Information, Figures S6–S9) in chloroform, the receptor **3** exists as a dimer, presumably interacting through electrostatics (salt aggregation), van der Waals, and/or hydrogen bond interactions. Methanol is known to compete with hydrogen bonds and thus disrupts assemblies held together by hydrogen bonds in solution.[28] To confirm the host is a dimer in solution, 10 and greater than 2000 equivalents of CD_3OD were added to the receptor and the DOSY was measured under the same conditions. The diffusion coefficient of the receptor after the addition of 10 equivalents of CD_3OD was $(0.510\pm0.07)\times10^{-5}$ and $(0.593\pm0.12)\times10^{-5}$ cm^2 s^{-1} after more than 2000 equivalents, clearly showing the presence of smaller species, that is, the monomeric receptor **3**. Because CD_3OD can only disrupt a complex or aggregate, the diffusion coefficient in chloroform thus suggests a solvent-filled dimeric aggregate that degrades into monomers in the presence of CD_3OD (Table 1). DOSY experiments for guests

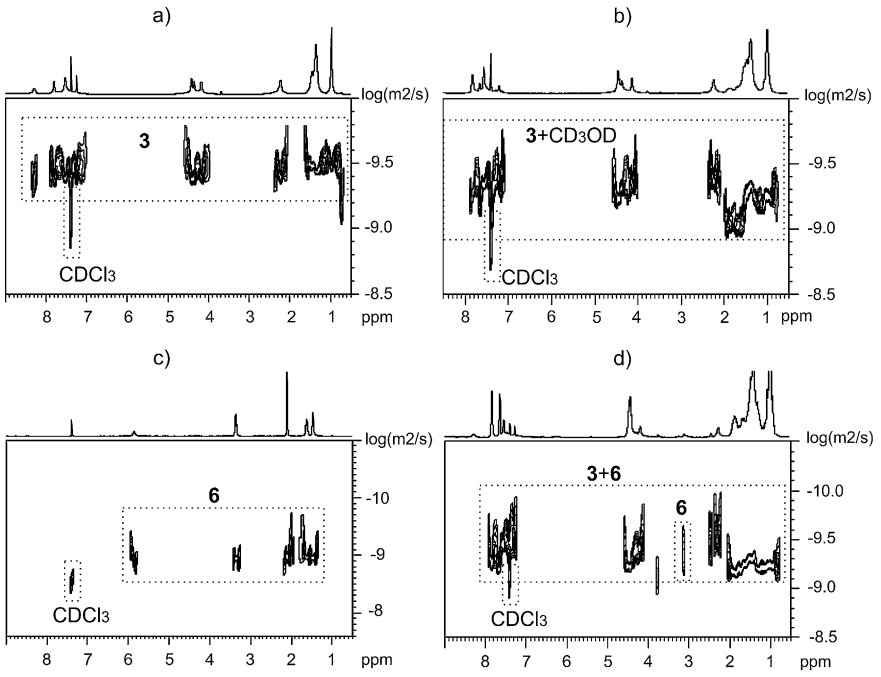

Figure 2. 2D DOSY NMR spectra (in $CDCl_3$ at 303 K) of a 30 mM sample of: a) **3**, b) **3** + ten equivalents of CD_3OD, c) **6**, and d) the host–guest assembly **3** + **6**, showing the chemical species present in the sample. Chemical shifts [ppm] are shown on the *x* axis and the diffusion coefficients [$\log m^2 s^{-1}$] on the *y* axis of the 2D plot.

Table 1. Average diffusion coefficients *D* [$\times 10^{-5}$ $cm^2 s^{-1}$] of the guests **4**–**7** and host **3** in different combinations in $CDCl_3$[a] at 300 K. The molar ratios of the host–guest mixtures were 2:1.

	D, Guest	*D*, Host		*D*, Guest	*D*, Host
3	–	0.378 ± 0.03	**3**/CD_3OD (10 equiv)	–	0.510 ± 0.07
			3/CD_3OD (> 2000 equiv)	–	0.593 ± 0.12
4	1.272 ± 0.01	–	**3**/**4**	0.699 ± 0.30	0.403 ± 0.02
5	1.113 ± 0.09	–	**3**/**5**	0.814 ± 0.06	0.412 ± 0.01
6	1.102 ± 0.04	–	**3**/**6**	0.411 ± 0.10	0.455 ± 0.09
			3/**6**:CD_3OD (10 equiv)	0.571 ± 0.21	0.575 ± 0.17
7	1.078 ± 0.04	–	**3**/**7**	0.748 ± 0.05	0.448 ± 0.06

[a] Diffusion coefficients of $CDCl_3$ ranges $1.615–2.032 \times 10^{-5}$ $cm^2 s^{-1}$ for mixtures containing the hosts, and $2.174–3.069 \times 10^{-5}$ $cm^2 s^{-1}$ for mixtures containing only the guests. Diffusion coefficient of CD_3OD is $\approx 2.1 \times 10^{-5}$ $cm^2 s^{-1}$.[27]

4–**7** and 2:1 host–guest mixtures were analyzed under the same conditions (Figure 2, Table 1, the Supporting Information, Figures S6–S9). The changes in the diffusion coefficients of the complexed guests from the free species to values nearer those of the hosts are an indication of a host–guest complex in solution. Taking the 2:1 host–guest mixture of **3** and **6** as an example, the diffusion coefficient of the host **3** was $(0.455 \pm 0.09) \times 10^{-5}$ and $(0.411 \pm 0.10) \times 10^{-5}$ $cm^2 s^{-1}$ for guest **6**. This shows a clear encapsulation of **6** and a subsequent host–guest assembly closer to the host aggregate seen in pure $CDCl_3$. Ten equivalents of CD_3OD were added to this sample and the DOSY results show diffusion coefficients of the host **3** to be $(0.575 \pm 0.17) \times 10^{-}$ and $(0.571 \pm 0.21) \times 10^{-5}$ $cm^2 s^{-1}$ for guest **6**. This indicates that CD_3OD breaks up the dimeric assembly into smaller species. The diffusion coefficient of **6** still indicates an assembly larger than the free guest **6** ($1.102 \pm 0.04 \times 10^{-5}$ $cm^2 s^{-1}$).

Considering that the exchange is fast on the NMR timescale, we conclude a dimeric assembly disassembles in the presence of methanol into smaller assemblies consisting of the pure monomers and 1:1 monomeric complexes in a dynamic mixture. The diffusion coefficients from host **3** and guests **4**–**7** are presented in Table 1. From these results, the diffusion coefficient for the six-carbon spacer **6** is closest to the host **3** suggesting a best-fit assembly. The DOSY experiments thus confirm a dynamic mixture consisting of 1:1 and 2:1 host–guest assemblies in solution.

^{1}H NMR Titration

Cooperativity was investigated through a series of ^{1}H NMR titration experiments between the hosts **2**/**3** and the diamides **4**–**7** in $CDCl_3$ at 303 K. In the experiments, an increasing amount of each diamide was added to a solution of either of the resorcinarene hosts **2** and **3**. At each stage, 0.2 equivalents of the guests were added, up to three equivalents. From then, larger amounts were then added to a maximum of six equivalents. Complexation-induced shielding of the ^{1}H NMR resonances corresponding to the guest protons was observed (Figure 3 and 4; see also the Supporting Information, Figures S10–S15). The observed shifts result from the shielding effects of the aromatic rings of the bowl-shaped host cavity upon addition of the guests. The guest exchange is fast on the NMR timescale. Changes to the proton resonances of the $-COCH_3$, $-NH$, $-NCH_2$, and $-CH_2$ signals of the guests, as well as the $-OH$ and $-RR'NH_2^+$ signals of the hosts, were observed. These changes are attributed to hydrogen bonds and CH···π interactions formed upon complexation.

The $-NH_2$ and $-OH$ signals play a key role in stabilizing the host structure and are thus affected when the diamide sits in the cavity of the hosts. The most intense spectral changes were observed for signals from the methyl protons of the diamide guests. Taking into consideration the shielding effect of the aromatic rings of the resorcinarene skeleton, the highly shielded methyl protons indicate that the methyl group of the

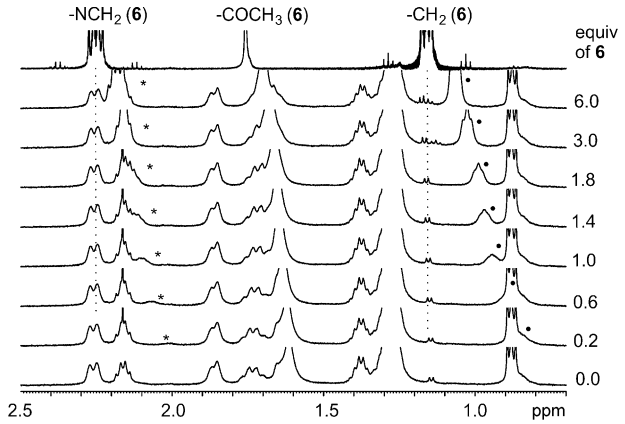

Figure 3. Selected region of the ^{1}H NMR spectra observed upon the titration of the diamide guest **6** to the *N*-cyclohexyl ammonium resorcinarene chloride host **2** in $CDCl_3$ at 303 K. Stars and black dots show the changes in the $-NCH_2$ and $-COCH_3$ signals, respectively.

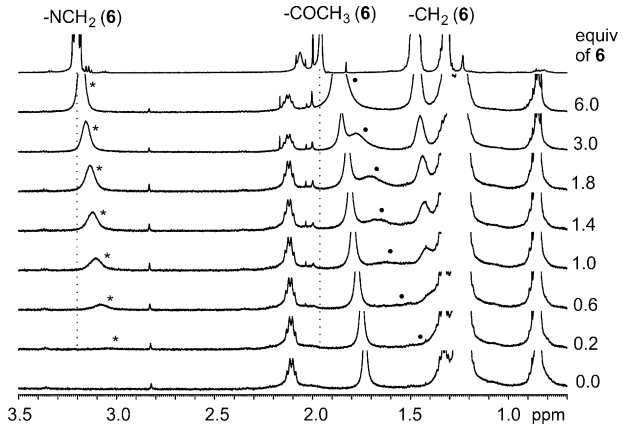

Figure 4. Selected region of the ^{1}H NMR spectra observed upon the titration of the diamide guest **6** to the *N*-benzyl ammonium resorcinarene chloride host **3** in $CDCl_3$ at 303 K. Stars and black dots show the changes in the $-NCH_2$ and $-COCH_3$ signals, respectively.

guest [$CH_3C{=}O$] sits deep in the cavity of the host (Figure 3 and 4; see also the Supporting Information, Figures S10–S15).

Thus, the signal changes of the methyl protons are the most suitable to follow in calculating the binding constant of the guests. However, due to severe overlap between $\delta = 1$–3 ppm, these signals could not be reliably followed in all cases. For consistency, the -NCH_2 signals of the guests that lie above the cation–anion belt and thus do not strongly feel the anisotropy of the resorcinarene phenyl rings were reliably monitored and the binding constants were obtained from the fit of the non-linear least squares titration curve of the respective titration data (Figure 3 and the Supporting Information). The titration data for guest **4** fit a 1:1 host–guest binding isotherm, whereas the data for the longer spacer containing guests **5**–**7** best fit a 2:1 host–guest binding isotherm. Binding constants (see the Supporting Information, Figures S16–S23) for the complexes were determined by using the winEQNMR2 computer program[29] and are presented in Table 2. K_1 and K_2 are defined in the Supporting Information.

Table 2. Binding constants K_1 [M^{-1}] and K_2 [M^{-1}] for hosts **2**/**3** with guests **4**–**7**.[a]

	K_1	K_2	$\alpha = (4K_2/K_1)$[b]
4·2	469 ± 60	–	–
4·3	238 ± 21	–	–
5·2	370 ± 31	3407 ± 310	36.83
5·3	267 ± 18	2059 ± 202	30.84
6·2	423 ± 31	4820 ± 273	45.57
6·3	222 ± 40	3990 ± 616	71.89
7·2	482 ± 28	4245 ± 264	35.22
7·3	144 ± 33	3314 ± 438	92.05

[a] Obtained from monitoring the guest $-NCH_2$ signals in $CDCl_3$ at 303 K. [b] α represents the interaction parameter used to describe cooperativity.

The upper rim substituent of the host has a direct effect on the binding affinity towards the guests. The first binding constants were generally higher for the resorcinarene host **2** over **3** (e.g., **6**@$\mathbf{2}_2$ K_1: 423 M^{-1} and **6**@$\mathbf{3}_2$ K_1: 222 M^{-1}, Table 2). The more rigid cyclohexyl groups of host **2** provide a more fixed cavity, whereas the benzyl groups of **3** introduce a degree of flexibility, which lowers the binding affinity. The first binding constants K_1 were generally similar for each host. The length of the spacers between the two binding sites of the guests had a significant effect on the overall binding as seen from the second binding constants K_2. The guest **4** was too short and could only fit into the cavity of one host. For the longer guests **5**–**7**, positive cooperativity was indicated by monitoring the second binding constant K_2, which was larger than K_1 in all cases.

The interaction parameter α (used to describe cooperativity and determined by $\alpha = 4K_2/K_1$, see the Supporting Information) was large for guests **5**–**7**.[3,4] The highest K_2 was observed with the six-carbon spacer diamide **6** (**6**@$\mathbf{2}_2$: K_2: 4820 M^{-1}, $\alpha = 45.57$ for host **2**; **6**@$\mathbf{3}_2$: K_2: 3990 M^{-1}, $\alpha = 71.89$ for host **3**, Table 2). This result shows the six-carbon diamide **6** is of optimum length and fits perfectly in the cavity of the two receptors at both ends. This result also compliments the DOSY measurement between the receptor **3** and guest **6** (Figure 2, Table 1). Strain and repulsion are responsible for the lower binding of the shorter spacer guest **5**, whereas more flexibility can explain the lower binding of the longer spacer guest **7** (Table 2). The second binding constant showed a chain length dependent binding strength.

In all cases, the effect of the upper rim substituent of the hosts influences the binding. Whereas the cyclohexyl groups are rigid, the phenyl rings of the benzyl groups are known to be oriented parallel to the plane of the hydrogen bond seam $(\cdots H\text{-}(R')N^+(R'')\text{-}H\cdots X^-\cdots H\text{-}(R')N^+(R'')\text{-}H\cdots X^-)_2$ thus introducing a degree of flexibility on the upper rim of the host **3**.[30] Higher binding constants were observed for the rigid host **2** over the more flexible host **3**, but for the longer spacer guests **6** and **7**, cooperativity was generally higher for the resorcinarene host **3** over host **2** (Table 2). This implies that it is more favorable for the second host to bind the other end of the guest after the first binding process has occurred.

Solid state analyses

To further study these systems in the solid state, single crystals suitable for X-ray analysis were obtained from a 1:4 mixture of the resorcinarene host **1** and guest **6** in $CHCl_3$. The structure clearly shows the 2:1 (**6**@$\mathbf{1}_2$) host–guest assembly in the solid state. Similar to the situation with the resorcinarene and monoamide system,[14] each binding motif (CH_3CONH–) of diamide **6** is located in the center of the cavity of each resorcinarene host in the plane of the cation–anion belt, with the amide nitrogen atoms involved in N(amide)–H···Cl^- hydrogen-bonding interactions (Figure 5). Simultaneously, the amide methyl

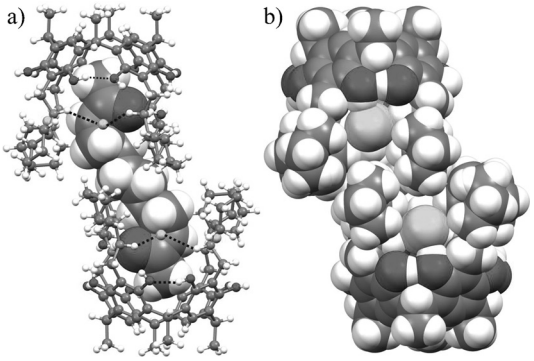

Figure 5. X-ray structure of the host–guest dimeric 2:1 capsule **6**@$\mathbf{1}_2$; a) ball and stick representation with the guest in CPK mode; b) CPK representation of the capsule.

group hydrogen atoms participate in C–H···π interactions with the interior cavity of the host. The $-NCH_2$ of the guest is located above the cation–anion belt, far from the phenyl rings of the resorcinarene skeleton and thus explain the smaller shifts of these protons in solution. In addition, one Cl^- anion is excluded from each resorcinarene host and this position is occupied by a water molecule with a O–H(water)···O=C(carbonyl) hydrogen bond. The missing negative charge is accounted for by the deprotonation of one of the ammonium groups on the resorcinarene salts. Interestingly, the conformation of the methyl carbonyl groups is gauche to the 1,6-hexanediaminyl chain. This conformation efficiently involves the van der Waals interactions of the cyclohexyl groups such that the dimerization energy of the resorcinarene pair is minimized to the largest extent (for a more detailed description, see the Supporting Information).

Gas phase analyses

The host–guest assemblies were investigated in the gas phase through a series of ESI-MS analyses.[31,32] A $CHCl_3$/acetonitrile mixture was used as the spray solvent in which *N*-alkyl ammonium resorcinarene chlorides can be ionized in the gas phase.[13,14] These salts are held together by several weak interactions, emphasized by the many species seen in the gas phase. The loss of multiple hydrogen chlorides (HCl) from the hosts is a common phenomenon with these large organic salts. Even at very soft ionization parameters, it is often rare that these salts can survive the high vacuum of a mass spectrometer and be seen fully intact.

A mixture of the hosts **2**/**3** and amide guests **4**–**7** in the positive-ion mode resulted in 1:1 host–guest complexes. In the spectrum containing host **2**, in the positive-ion mode, progressive loss of HCl resulted in signals corresponding to $[\mathbf{2}-2\,HCl+H]^+$ ($m/z=1341$), $[\mathbf{2}-3\,HCl+H]^+$ ($m/z=1305$), and $[\mathbf{2}-4\,HCl+H]^+$ ($m/z=1269$) (Figure 6a). Similarly, a spectrum

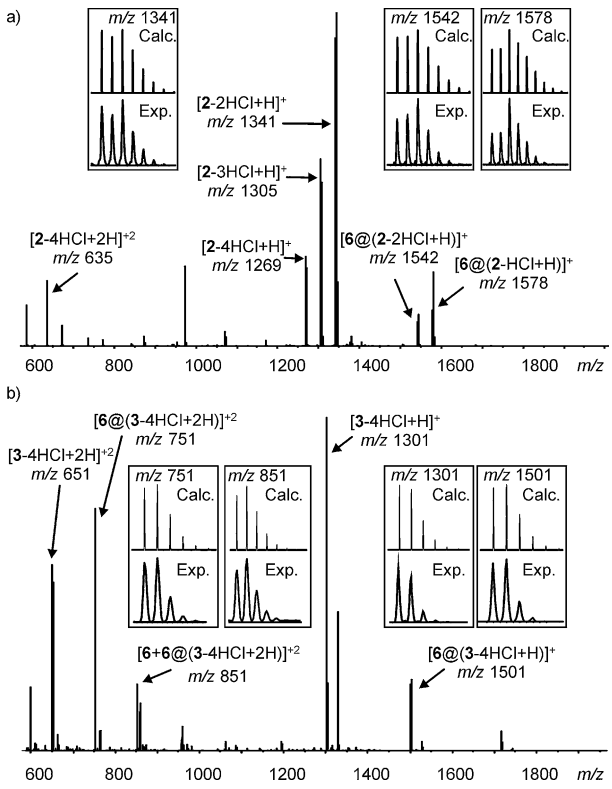

Figure 6. ESI mass spectrum showing several signals corresponding to 1:1 monomeric complexes: a) mixture of host **2** and diamide **6**; b) mixture of host **3** and diamide **6**. Inset: experimental and calculated isotope patterns of selected signals.

containing resorcinarene host **3** in the positive ion mode shows progressive loss of HCl, resulting in signals corresponding to $[\mathbf{3}-3\,HCl+H]^+$ ($m/z=1337$), $[\mathbf{3}-4\,HCl+H]^+$ ($m/z=1301$), and $[\mathbf{3}-4\,HCl+2H]^{+2}$ ($m/z=651$) (Figure 6b). Taking a mixture of host **2** and the diamide **6** as an example, signals corresponding to 1:1 host–guest complexes, such as $[\mathbf{6}@(\mathbf{2}-HCl+H)]^+$ ($m/z=1578$), $[\mathbf{6}@(\mathbf{2}-2\,HCl+H)]^+$ ($m/z=1542$), and $[\mathbf{6}@(\mathbf{2}-4\,HCl+2\,H)]^{2+}$ ($m/z=635$) were observed (Figure 6a). Similarly, the mass spectrum of a mixture of host **3** and the diamide **6** show signals corresponding to 1:1 host–guest complexes $[\mathbf{6}@(\mathbf{3}-4\,HCl+H)]^+$ ($m/z=1501$) and $[\mathbf{6}@(\mathbf{3}-4\,HCl+2\,H)]^{2+}$ ($m/z=751$) (Figure 6b). In this sample, a signal corresponding to 1:2 host–guest assembly $[\mathbf{6}+\mathbf{6}@(\mathbf{3}-4\,HCl+2\,H)]^{2+}$ ($m/z=$ 851) was also observed, which can be attributed to aggregation of the diamides. The isotope patterns obtained by experiment agree with those simulated on

the basis of natural abundances. Other minor signals were observed in the mass spectra, assumed to either be unspecific assemblies or fragmentation adducts. Samples containing combinations of the resorcinarene hosts **2**/**3** and the other diamide guests **4**–**7** were measured and analyzed with results showing similar patterns as observed in Figure 6 (see the Supporting Information, Figures S24–29).

Conclusion

The present work reports the cooperative binding of diacetamides **4**–**7** (homoditopic guests) by monovalent *N*-alkyl ammonium resorcinarene chloride **1**–**3** receptors. Job plots, ^{1}H NMR titration, and DOSY NMR experiments revealed 2:1 complexes for the longer spacer guests **5**–**7**. The ^{1}H NMR titration studies reveal that the lengths of the carbon-chain spacer have a profound effect on the binding stability and stoichiometry, with a statistical distribution based on the length of the spacer. The optimum binding was observed with the six-carbon spacer between the amides confirming the best-fit scenario of the host. The three-carbon spacer was too short and led to 1:1 binding. The second binding constants K_2 for the longer guests were larger than K_1 in all cases, confirming a positively cooperative binding process. A 2:1 binding stoichiometry was unambiguously confirmed in the solid state by X-ray single-crystal determination, which shows a Cl^- anion excluded from the aggregation. Despite the tendency for this host to subsequently lose hydrogen chloride, the observation of the 1:1 host–guest complexes in the gas phase shows the complexes to be stable enough to resist the high vacuum of the mass spectrometer. Higher assemblies, such as $[\mathbf{6}+\mathbf{6}@(\mathbf{3}-4\,HCl+2\,H)]^{+2}$ ($m/z=$ 851), resulting from diacetamide aggregation were also observed in some cases. These results show that the *N*-alkyl ammonium resorcinarene chloride hosts (i.e., a hydrogen-bonded analogue of a covalent cavitand) possess a cavity robust enough to cooperatively bind diacetamides. One intriguing aspect of this work is the fact that a receptor held together by multiple weak interactions can utilize the same weak interactions to cooperatively bind guest molecules. This work provides a useful addition to the library of host–guest assemblies held together by weak interactions.

Experimental Section

Materials

The resorcinarene hosts **1**–**3** and the diamide guests were synthesized according to reported procedures.[12–14,19–21] Experimental details for the synthesis and characterization data of resorcinarene hosts and guests are presented in the Supporting Information. DOSY measurements were performed on a Bruker Avance 400 MHz spectrometer with a specialized inner thin filament NMR tube to minimize convection. Titration experiments were carried out on a Bruker Avance DRX 500 MHz and 400 MHz spectrometers. The mass spectrometric experiments were performed on a micromass LCT ESI-TOF instrument equipped with a Z geometry ion source and a QSTAR Elite ESI-Q-TOF mass spectrometer equipped with an API 200 TurboIonSpray ESI source from AB Sciex (former MDS Sciex) in Concord, Ontario (Canada). Details on the DOSY, NMR titrations, and mass spectrometric studies are presented in the Supporting Information. For X-ray crystallographic analysis, the data was collected at 123 K on an Agilent Super-Nova Diffractometer using mirror-monochromatized $Cu_{K\alpha}$ ($\lambda=1.54184$ Å) radiation. All details about data collection and reduction, as well as structure solution and refinement are given in Supporting Information. CCDC-1039550 for $\mathbf{6}@\mathbf{1}_2$ contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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