primary ammonium ions and the two cavity-based receptors leads to an unprecedented specificity for primary ammonium ions over secondary, tertiary and quaternary ones. These binding properties were exploited for the selective liquid-liquid extraction of primary ammonium salts from water and for the selective recognition of lysine containing peptides, opening new perspectives in the field of peptide sensing.

**Keywords:** Hexahomotrioxacalixarenes – Ammonium ions – Supramolecular chemistry – Host-guest systems – Molecular recognition.

## Introduction

The primary amino group and its protonated ammonium form are major functional groups in chemistry and biology. Many biomolecules such as neurotransmitters (dopamine, serotonin, etc.), polyamines (spermine, spermidine, etc.), amino acids, peptides and proteins contain one or multiple primary amino groups. Primary amines are also important industrial products despite their reported toxicity for the environment and aquatic organisms.[1] The development of novel strategies to efficiently sense or separate primary amines and ammonium ions is thus a field of major importance.[2] Since the seminal work of Pedersen in 1967,[3] considerable efforts have been undertaken to design molecular receptors that exhibit high affinity and selectivity for ammonium ions.[4] It is well known that primary ammonium ions, $RNH_3^+$, form stable complexes with 18-crown-6 (18C6) and its derivatives,[5] while secondary ammonium ions prefer larger crown ethers.[6] This selectivity has been rationalized in part by the good H-bonding complementarity between the $D_{3d}$ symmetrical complexing conformation of 18C6 and the $C_{3v}$ symmetry of the primary ammonium group $NH_3^+$.

An appealing strategy for the design of ammonium ion receptors consists in using cavity-based systems such as calixarenes,[7] resorcinarenes,[8] hemicryptophanes,[9] or pillararenes.[10] Indeed, all

these cavitands provide an electron π-rich pocket that can participate in the stabilization of the ammonium-receptor complex through cation–π and CH-π interactions, while ensuring a size and shape cavity-based selectivity.[11] These artificial cavity-based receptors are notably studied for the binding and recognition of *N*-methylated lysine, paving the way for applications in the biological field.[12] $C_{6v}$ or $C_{3v}$ symmetrical cavitands are of particular interest for primary ammonium groups as, similarly to 18C6 derivatives, they can offer a H-bonding acceptor site displaying a high complementarity. Examples of $C_{3v}$ symmetrical calix[6]arenes displaying a good selectivity for primary ammonium ions have been reported,[13] however, due to the flattened cone conformation of the receptor framework, these systems are limited to the binding of small or linear guests.

In this context, hexahomotrioxacalix[3]arenes, which have mostly been studied for the complexation of metal cations,[14] are particularly attractive preorganized molecular platforms that combine a 18C3 moiety to a polyaromatic cavity (inset Figure 1).[15] It has been observed that, when locked in their cone conformation,[16] they can bind primary ammonium ions in organic solvents,[17] including biogenic ones.[18] Homooxacalix[3]arene-based ditopic receptors for the simultaneous binding of primary ammonium ions and either a metal ion or an anion have also been developed.[19] In 2002, a chromogenic homooxacalix[3]arene-based colorimetric sensor for amines was reported to also detect secondary amines but with a slight selectivity for primary amines.[20] However, the system was not blocked in the cone conformation and the amines were more likely recognized outside of the polyaromatic cavity.

No systematic study of the selectivity of homooxacalix[3]arenes toward primary vs. secondary, tertiary and quaternary ammonium ions has to date been reported and the mode of recognition of primary ammonium ions has also not yet been thoroughly looked at. The host-guest complex is sometimes represented with the organic cation threading through the 18C3 macrocycle in order to interact with H-bond donor groups present on the phenoxy units, despite the fact that

it is classically accepted that a 18-membered macrocycle is too small to let a molecule thread through it.[21]

With the aim of contributing to the development of molecular sensors for biogenic ammonium ions and peptides,[22] we have undertaken an intensive study of the selectivity and binding mode of homooxacalix[3]arenes. Herein, we report on a systematic study, by NMR and via *in silico* modelling, of the binding properties of two known homooxacalix[3]arene-based receptors **1**[23] and **2**[24] (Figure 1) toward a wide variety of ammonium ions in different solvents, including protic ones. While both receptors are blocked in the desired cone conformation, capped receptor **2** is conformationally more preorganized and an enhancement of its binding properties was thus a priori expected.[25]

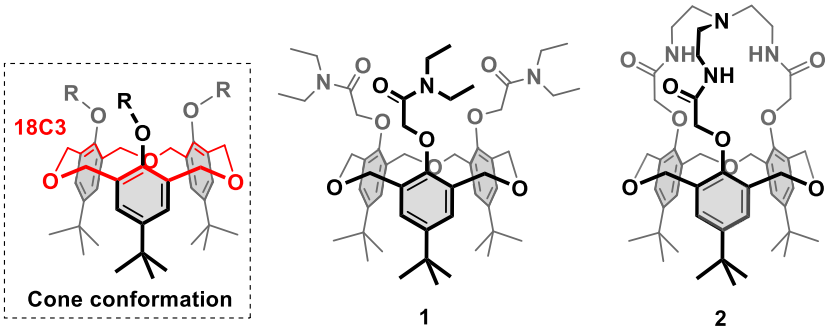


**Figure 1.** Structures of hosts **1** and **2**. Inset: general structure of a cone hexahomotrioxacalix[3]arene showing the 18C3 moiety.

## Results and Discussion

**Preliminary binding study between host 1 and hexylammonium.** The ability of homooxacalix[3]arene **1** to bind primary ammonium ions in a protic environment was evaluated by $^1$H NMR spectroscopy through the addition of $HexNH_3^+Pic^-$ to the host dissolved in mixtures of $CDCl_3/CD_3OD$ of different ratios (from 1:0 to 1:2) (Table 1). In all cases, a slow exchange on the NMR chemical shift timescale was observed and the intra-cavity complexation of the ammonium ion, evidenced by the presence of high-field signals (Figure 2a vs. 2b for the $^1$H

NMR spectra obtained in 1:0.25 $CDCl_3/CD_3OD$; assignments achieved through COSY and HSQC experiments).[26] In pure $CDCl_3$, the complex **1⊃HexNH$_3^+$** was obtained quantitatively upon the addition of 1 equiv. of the guest, indicating an association constant that is too high to be determined accurately by NMR (log $K$ >5).[26] Very interestingly, even in the highly competitive environment of a 1:1 ratio of $CDCl_3/CD_3OD$, a large affinity constant was observed (log $K$ = 3.6 ± 0.1). Binding was however much weaker (log $K$ < 1) in a 1:2 ratio of $CDCl_3/CD_3OD$. The C$H_3$C$H_2$ protons of the amide groups of host **1** are essentially not affected upon complexation, indicating that these groups are not involved in the recognition of the guest. In contrast, a downfield shift of the Ar*H* and *t*Bu protons is observed (see Figures 2a and 2b), as well as a significant splitting of the signal of the ArC$H_2$ protons into two well-separated sets of signals (a doublet at 5.43 ppm for the axial protons and another doublet at 4.29 ppm for the equatorial protons). This is characteristic of a homooxacalix[3]arene displaying a rigid more open cone conformation upon complexation with the ethereal oxygen atoms directed toward the interior of the cavity (see structure displayed in Figure 2b).[24a] This conformation allows for three-point [N-H···O] hydrogen bonding between the ether oxygen atoms of the host and the $NH_3^+$ protons of the ammonium ion. The complexation induced shifts (CISs) of the included hexylammonium ion show that the protons in the α position (i.e. C$H_2NH_3^+$) are located at the centre of the polyaromatic cavity ($CIS_{CH2NH3+}$ = -2.69 ppm, inset Figure 4). Consistent with this result, NOESY experiments showed the spatial proximity of the protons in the β position with Ar*H* and *t*Bu protons of the receptor.[26]

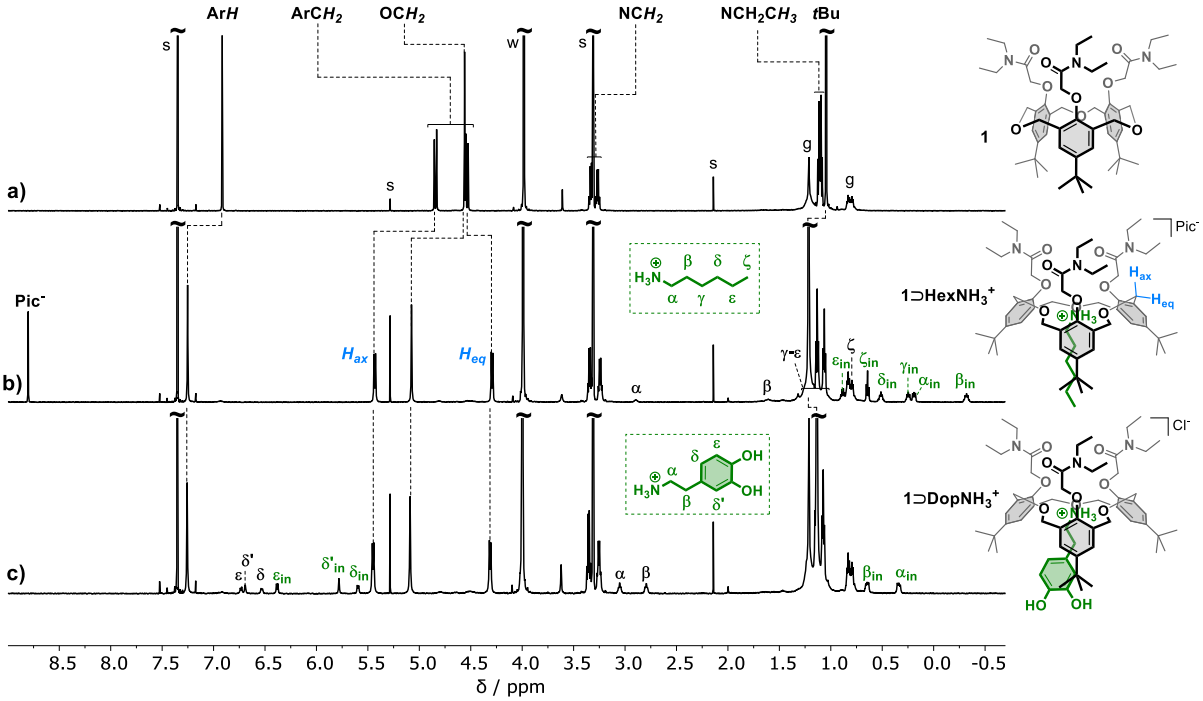


**Figure 2.** $^{1}$H NMR spectra (298 K, 600 MHz) in a 1:0.25 $CDCl_3/CD_3OD$ solution of a) **1**, b) **1** + 1.2 equiv. of $HexNH_3^+Pic^-$ and c) **1** + 1.6 equiv. of dopamine hydrochloride. s: residual solvents; w: residual water; g: grease.

**Table 1.** Association constants of host **1** for $HexNH_3^+Pic^-$ measured by $^{1}$H NMR spectroscopy at 298 K in mixtures of $CDCl_3/CD_3OD$ of different ratios; [**1**] = $10^{-3}$ M.

| Ratio of $CDCl_3/CD_3OD$ | 1:0 | 1:0.25 | 1:1 | 1:2 |
|---|---|---|---|---|
| log *K* | >5 | 4.7 ± 0.2 | 3.6 ± 0.1 | <1 |

The recognition mode for $HexNH_3^+$ was further investigated *in silico* by molecular mechanics conformational analysis (Figure 3). Both the position of the ammonium ion and the conformation adopted by host **1** are consistent with the data obtained by NMR spectroscopy. The host displays an open cone conformation with the oxygen atoms of the ether bridges pointing toward the heart of the cavity. The bound ammonium ion is deeply inserted into the receptor, with only the last two carbon atoms of its alkyl chain protruding from the polyaromatic cavity. The $NH_3^+$ group is located at the center of the ethereal macrocycle with its nitrogen atom nearly in the plane defined by the three oxygen atoms of the macrocycle (distances from the $O_3$ plane = 0.36 Å and from the corresponding centroid = 0.37 Å, see inset Figure 3). The

ammonium head is positioned so that it can establish three H-bonds with the ether bridges (N-O distances < 3.06 Å, 149.4° < N-H-O angles < 164.7°) (Figure 3b). All these data show a remarkable three-point H-bonding complementarity, similarly to what is generally observed with 18C6 derivatives.[27] Indeed, as suggested by Trueblood with 18C6, the main factor governing the geometry of the interaction with primary ammonium ions is the depth of penetration of the $NH_3^+$ group into the macrocycle.[28] Besides the H-bond interactions with the ethereal macrocycle, additional stabilizing interactions are also observed: (i) all the phenoxy oxygen atoms are at a very short distance from the ammonium center (N-OAr distances < 2.86 Å) suggesting ion-dipole interactions, (ii) one of the phenoxy oxygen atoms (O4) is involved in a three-centered H-bond[29] with the ammonium head and (iii) the polyaromatic cavity allows π-cationic (N-Ar distances < 4.06 Å) and CH-π interactions. In other words, the environment provided by the homooxacalix[3]arene skeleton greatly contributes to the stabilization of the cationic guest.

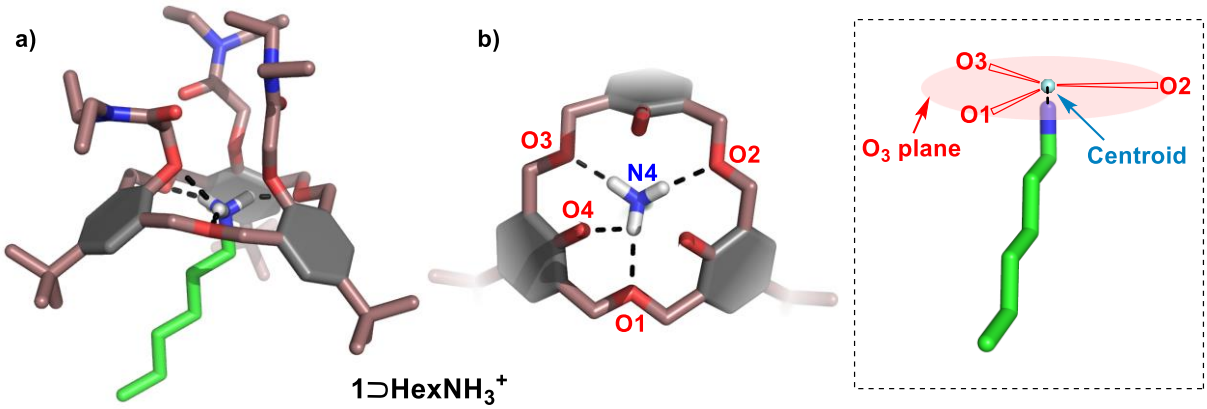


**Figure 3.** Energy minimized structure of the complex **1⊃HexNH$_3^+$** (stick representation). a) Side view; b) Truncated top view highlighting the recognition mode. H-bonds are indicated by dashed lines. Inset: schematic representation of the three ethereal oxygen atoms centroid. Selected distances (Å): 0.36 ($O_3$ plane-N4), 0.37 (centroid-N4), 2.76 (O4-N4), 2.84 (O3-N4), 2.89 (O2-N4), 3.06 (O1-N4). Selected angles (°): 114.1 (N4-H-O4), 149.4 (N4-H-O2), 155.7 (N4-H-O1) and 164.7 (N4-H-O3). With the exception of the polar H, all the hydrogen atoms of **1** and of $HexNH_3^+$ are omitted for clarity.

**Binding of various primary ammonium ions by host 1.** The binding of linear ammonium ions $RNH_3^+X^-$ ($X^-$ = $Cl^-$ or $Pic^-$; R = methyl-, propyl-, *n*-butyl-, hexyl or dodecyl) in $CDCl_3/CD_3OD$ (1:0.25) was investigated in order to evaluate the influence of the chain length on the recognition properties of host **1**. In all cases, addition of the ammonium salt led to the formation of the corresponding inclusion complex **1⊃RNH$_3^+$** (Figure 4).[26] Similar CISs were observed for all the host-guest complexes, indicating a similar recognition mode (i.e. an open cone conformation for host **1** and an inclusion of the ammonium ion in the cavity with the ammonium head at the level of the ethereal macrocycle). Except for $MeNH_3^+$, large association constants were determined for the different guests, indicating that the chain length does not influence the affinity (Table 2, entries 1-5). For $MeNH_3^+$, the association constant is one order of magnitude lower (i.e. log $K$ = 3.7 ± 0.3), which can be explained by the fact that this cation is too small to fill correctly the cavity.

The interaction of the host with $NH_4^+Pic^-$ was also studied and, in this case, the small ammonium ion is most likely bound at the level of the amido arms as attested by the change of the chemical shift of the NC*H*$_2$ protons of the host and the absence of significant shifts for the cavity protons.[26]

The binding of $PrNH_3^+$, *n*-$BuNH_3^+$ and $HexNH_3^+$ added as the chloride salt was compared to the results obtained with the picrate salt. Despite the very different coordinating properties of these two counter anions, almost no difference was observed at the level of the NMR spectra and of the binding affinities (Table 2, entries 2-4). This can be explained by the fact that the anions are well solvated in protic environments and their influence in the host-guest process is thus negligible.

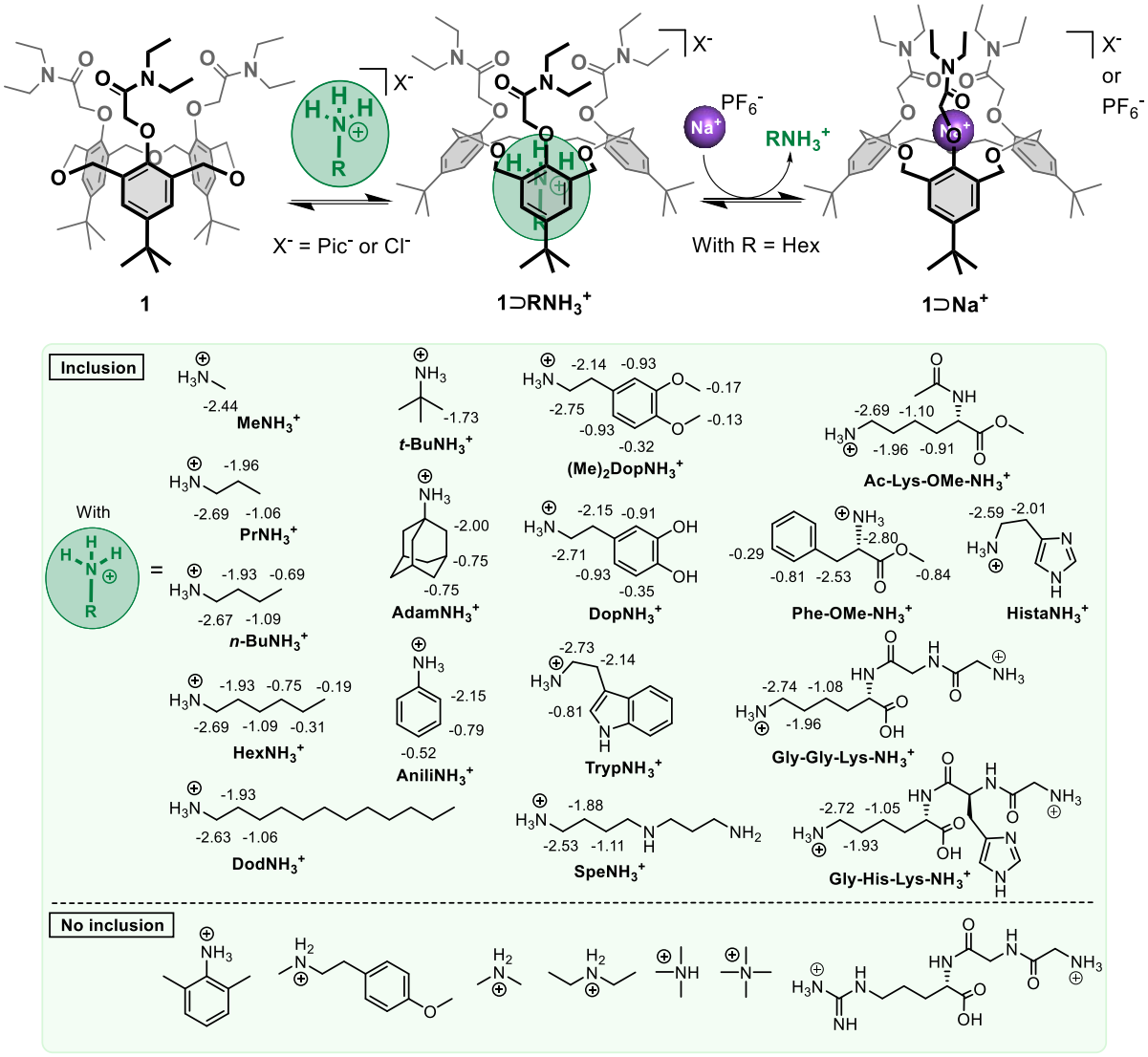


**Figure 4.** Host-guest properties of host **1** toward ammonium ions and $Na^+$. Inset: CISs determined by $^1H$ NMR spectroscopy in 1:0.25 $CDCl_3/CD_3OD$.

The affinity of receptor **1** toward sterically hindered primary ammonium ions (i.e. *t*-butylammonium, 1-adamantylammonium and anilinium salts) was also evaluated (Figure 4). In all cases, the CISs for both the host and the guest protons indicate a mode of recognition similar to that of the linear guests.[26] The affinity for the bulky *t*-$BuNH_3^+$ and $AdamNH_3^+$ was found to be only slightly weaker than that of the linear ammonium ions (Table 2, entries 6 and 7 *vs.* entries 2-5) and a log $K$ = 3.5 was measured for the anilinium ion, $AniliNH_3^+$. This highlights the remarkable ability of host **1** to bind a large variety of primary ammonium ions. No trace of

inclusion of the *o*,*o*'-disubstituted 2,6-dimethylanilinium ion was observed by NMR spectroscopy,[26] which can be explained by the steric clash between the two methyl groups of the cationic guest and the aromatic walls of the cavity.

**Table 2.** Association constants for hosts **1**, **2** and **2.H**$^+$ with ammonium ions (Pic$^-$ and/or Cl$^-$ salts) measured by $^1$H NMR spectroscopy in 1:0.25 $CDCl_3/CD_3OD$ at 298 K; [**1**] = [**2**] = $10^{-3}$ M.

| | | log *K* | | | |
|---|---|---|---|---|---|
| Entry | Guest[a] | Host **1** | | Host **2** | Host **2.H**$^+$ |
| | | Pic$^-$ salt | Cl$^-$ salt | | |
| 1 | $MeNH_3^+$ | 3.7 ± 0.3 | - | - | - |
| 2 | $PrNH_3^+$ | 4.7 ± 0.2 | 4.4 ± 0.1 | 1.7 ± 0.2 (Pic$^-$) | 2.8 ± 0.1 (Pic$^-$) |
| 3 | *n*-$BuNH_3^+$ | 4.6 ± 0.1 | 4.5 ± 0.2 | - | - |
| 4 | $HexNH_3^+$ | 4.7 ± 0.2 | 4.5 ± 0.2 | 2.0 ± 0.1 (Pic$^-$) | 2.8 ± 0.1(Pic$^-$) |
| 5 | $DodNH_3^+$ | - | 4.6 ± 0.1 | - | - |
| 6 | *t*-$BuNH_3^+$ | 4.1 ± 0.2 | - | 1.5 ± 0.1 (Pic$^-$) | 2.2 ± 0.2 (Pic$^-$) |
| 7 | $AdamNH_3^+$ | - | 4.2 ± 0.1 | - | - |
| 8 | $AniliNH_3^+$ | - | 3.5 ± 0.1 | - | - |
| 9 | $(Me)_2DopNH_3^+$ | 4.8 ± 0.2 | - | 1.8 ± 0.1 (Pic$^-$) | 2.9 ± 0.1 (Pic$^-$) |
| 10 | $DopNH_3^+$ | - | 4.2 ± 0.1 | 1.6 ± 0.1 (Cl$^-$) | 2.6 ± 0.1 (Cl$^-$) |
| 11 | $TrypNH_3^+$ | 4.5 ± 0.1 | - | - | - |
| 12 | Ac-Lys-OMe-$NH_3^+$ | - | 3.8 ± 0.1 | - | - |
| 13 | Phe-OMe-$NH_3^+$ | - | 2.8 ± 0.1 | - | - |
| 14 | $HistaNH_3^+$ | - | 2.7 ± 0.3 | - | - |
| 15 | $SpeNH_3^+$ | 3.3 ± 0.1 | - | - | - |
| 16 | Gly-Gly-Lys-$NH_3^+$ | 3.1 ± 0.1 | - | - | - |
| 17 | Gly-His-Lys-$NH_3^+$ | 2.7 ± 0.1 | - | - | - |

[a] Ammonium ion used as its Pic$^-$ and/or Cl$^-$ salt.

In a further series of experiments, we evaluated the complexation of biologically relevant amines under their protonated ammonium form. Neurotransmitters such as dopamine (and its dimethylated derivative $(Me)_2DopNH_3^+$) and histamine, amino acids derivatives as well as tryptamine and spermidine were studied in 1:0.25 $CDCl_3/CD_3OD$ (Figure 4). All these guests

led to the formation of endo-complexes according to the recognition process described above.[26] As a representative example, the $^1$H NMR spectrum of the complex **1⊃DopNH$_3^+$** obtained upon the addition of 1.6 equiv. of dopamine hydrochloride to host **1** is given in Figure 2c. If compared to the $^1$H NMR spectrum of **1⊃HexNH$_3^+$** (Figure 2b), the quasi-identical chemical shifts for the host protons show that **1** adopts a similar open-cone conformation in both cases. The association constants (log *K*) for all these guests range from 2.7 to 4.8 (Table 2, entries 9-15), with the most encumbered guests at the level of the ammonium head and the most polar ones giving rise to the weaker affinity constants. The cationic side chain of the lysine derivative Ac-Lys-OMe-$NH_3^+$ is more strongly bound than the encumbered α-ammonium group of the phenylalanine derivative Phe-OMe-$NH_3^+$ (Table 2, entries 12 vs. 13).

No binding of the diprotonated form of histamine was observed but, upon gradual addition of triethylamine (this base cannot be complexed in the cavity, *vide infra*), the complexation of the monoprotonated form Hista$NH_3^+$ was clearly observed.[26] Spermidine was not bound in its trisamine form, but upon the gradual addition of picric acid, $^1$H signals corresponding to the endo-complex **1⊃SpeNH$_3^+$** were observed. After the addition of more than 1 equiv. of acid, the NMR signals of the complex were still observed but with a lower intensity.[26] The lower, or absence of, affinity for the polyprotonated form of histamine and spermidine can be explained by their good solvation in the protic environment from which it becomes difficult to extract them.

The ability of host **1** to bind more complex guests such as tripeptides was also evaluated in 1:0.25 $CDCl_3$/$CD_3OD$. Three tripeptides were investigated, two containing a lysine residue (Gly-Gly-Lys and Gly-His-Lys) and a third without a lysine residue (i.e. Gly-Gly-Arg). Solutions of host **1** in the presence of a small excess of the tripeptides were prepared. The peptides were not easily soluble in the chosen solvent mixture but became more soluble upon the progressive addition of PicH (up to 25 equiv.). In the case of the Gly-Gly-Arg tripeptide the

receptor signals remained unaffected even with 8 equiv. of added PicH (Figure 5a). In contrast, for the peptides bearing a lysine residue, new high-field signals corresponding to the host-guest complexes **1⊃Gly-Gly-Lys-$NH_3^+$** (Figure 5b) and **1⊃Gly-His-Lys-$NH_3^+$** were clearly observed and increased with the concentration of acid.[26] Very interestingly, only one new NMR signature was detected over the course of the titrations with these two tripeptides and the chemical shifts of the high-field signals below 0 ppm were quasi-identical to those observed when using the bis-protected lysine derivative (Ac-Lys-OMe-$NH_3^+$) (Figure 5b vs 5c). These data are consistent with the selective binding of these tripeptides at the level of the primary ammonium group in the side chain of the lysine residue and not at the level of the N-terminal group of the peptides nor at the level of the guanidinium group of an arginine residue. This result illustrates a remarkable shape selectivity and opens the door to the selective recognition of peptides bearing lysine residues.

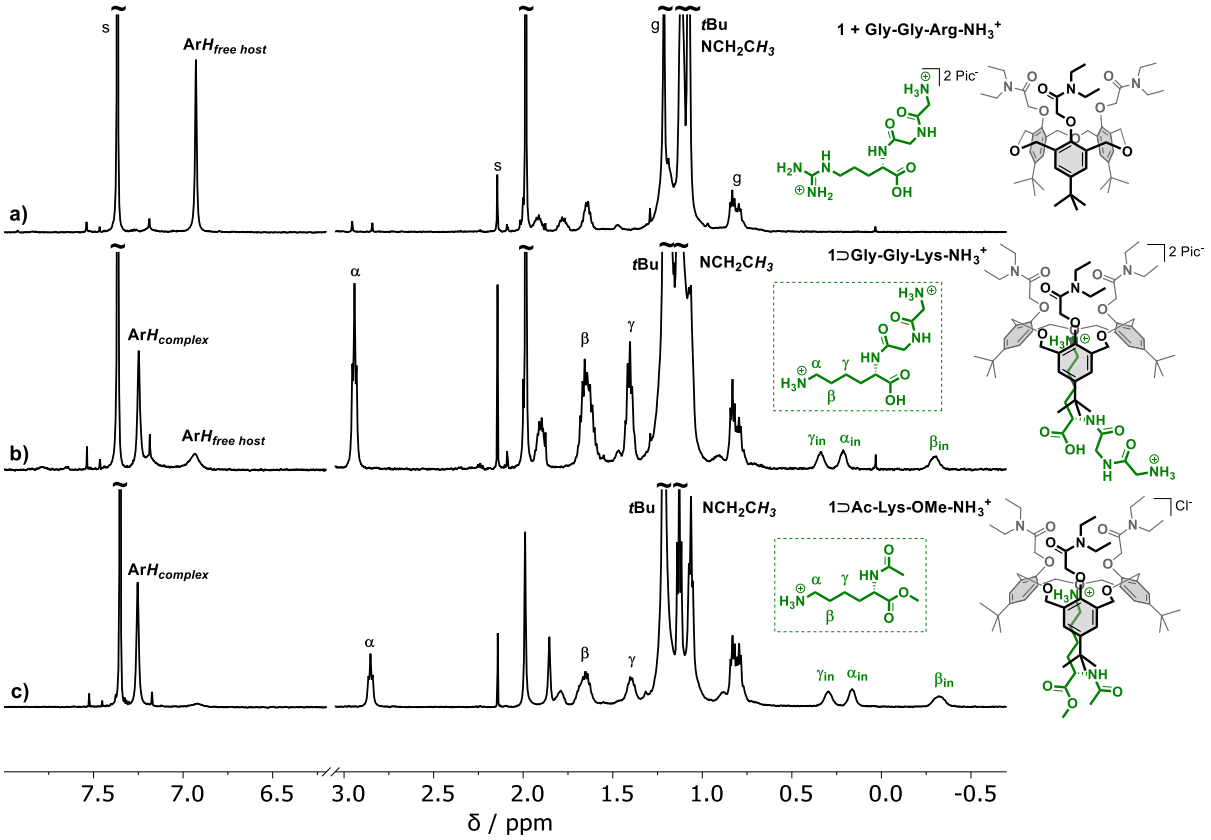


**Figure 5.** High field and aromatic region of the $^1$H NMR spectra (298 K, 600 MHz, 1:0.25 $CDCl_3/CD_3OD$) of a) **1 + Gly-Gly-Arg-$NH_3^+$** obtained through the addition of the neutral guest (~1.5 eq. solubilized) and ~8 eq. of

picric acid, b) **1⊃Gly-Gly-Lys-NH$_3^+$** obtained through the addition of ~4.5 eq. of the neutral guest and ~14 eq. of picric acid and c) **1⊃Ac-Lys-OMe-NH$_3^+$** obtained through the addition of 2.4 equiv. of the ammonium chloride salt. s: residual solvents; g: grease.

*In silico* studies by molecular mechanics conformational analysis of several host-guest complexes **1⊃RNH$_3^+$** were performed,[26] some of the resulting energy minimized structures are displayed in Figure 6. In all cases, a recognition mode similar to the one observed for HexNH$_3^+$ was obtained, with three H-bonding interactions with the ether bridges. A further H-bond with one of the phenoxy oxygen atoms is also observed, except for the guests that are sterically hindered in the α position of the ammonium group (AdamNH$_3^+$, *t*-BuNH$_3^+$ and AniliNH$_3^+$). The ammonium head of the guests is buried in the cavity with a $O_3$ plane-N (guest) distance lower than 0.70 Å in all cases, similarly to what is observed with 18C6 (Table 3). Furthermore, the average N-O and N-OAr distances are between 2.78 and 3.13 Å, suitable for the formation of H-bonds.

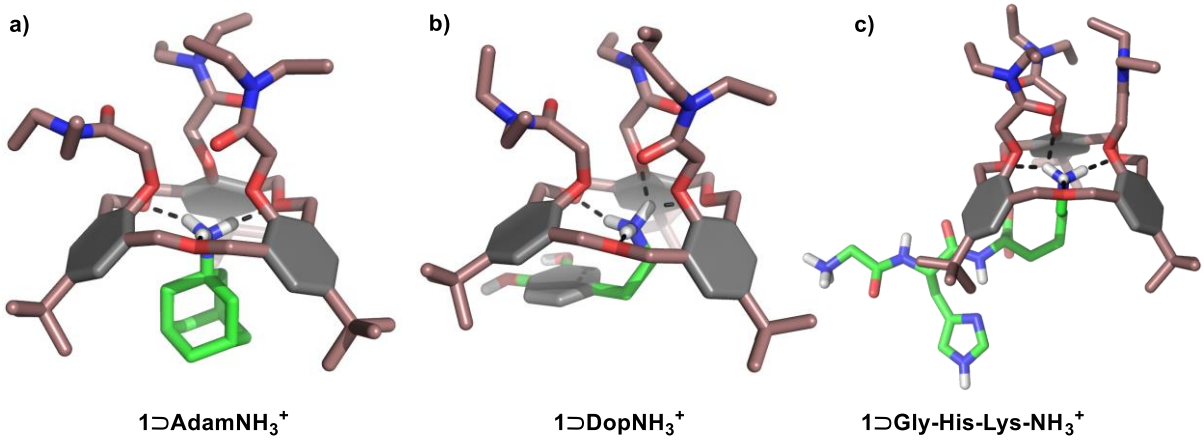


**Figure 6.** Energy minimized structures of complexes a) **1⊃AdamNH$_3^+$**, b) **1⊃DopNH$_3^+$** and c) **1⊃Gly-His-Lys-NH$_3^+$** (stick representation). H-bonds are indicated by dashed lines. With the exception of the polar H, all the hydrogen atoms are omitted for clarity.

**Table 3.** Various distances for hosts **1**, **2** and **2.H**$^+$ with ammonium ions determined from the energy minimized structures and for 18C6 with $MeNH_3^+$ determined from a X-ray structure.[28]

| Guest | Distance $O_3$ plane-N(guest) (Å) (**Host**) | Average N-O distance (Å) (**Host**) | Average N-OAr distance (Å) (**Host**) |
|---|---|---|---|
| $MeNH_3^+$ | 0.33 (**1**), 0.64[a] (**18C6**) | 2.93 (**1**) | 2.78 (**1**) |
| $HexNH_3^+$ | 0.36 (**1**) | 2.93 (**1**) | 2.79 (**1**) |
| $AdamNH_3^+$ | 0.68 (**1**) | 3.00 (**1**) | 2.96 (**1**) |
| *t*-$BuNH_3^+$ | 0.70 (**1**), 0.93 (**2**), 1.00 (**2.H**$^+$) | 3.01 (**1**), 3.07 (**2**), 3.09 (**2.H**$^+$) | 2.90 (**1**), 3.26 (**2**), 3.35 (**2.H**$^+$) |
| $AniliNH_3^+$ | 0.58 (**1**) | 2.94 (**1**) | 2.85 (**1**) |
| $DopNH_3^+$ | 0.57 (**1**) | 2.97 (**1**) | 2.89 (**1**) |
| Ac-Lys-OMe-$NH_3^+$ | 0.50 (**1**) | 2.94 (**1**) | 2.82 (**1**) |
| Phe-OMe-$NH_3^+$ | 0.61 (**1**) | 2.97 (**1**) | 2.90 (**1**) |
| Gly-His-Lys-$NH_3^+$ | 0.35 (**1**) | 2.96 (**1**) | 3.01 (**1**) |
| $Me_2NH_2^+$ | 1.22 (**1**) | 3.38 (**1**) | 3.54 (**1**) |
| $Et_2NH_2^+$ | 1.35 (**1**) | 3.43 (**1**) | 3.66 (**1**) |
| $Na^+$ | 1.53[b] (**1**) | - | - |

[a] See ref 28.

[b] Complexed in *exo*, i.e. at the level of the amido groups.

**Binding of primary ammonium ions by capped host 2.** For comparison purposes, the ability of the capped receptor **2** to bind various primary ammonium ions ($PrNH_3^+$, $HexNH_3^+$, *t*-$BuNH_3^+$ $(Me)_2DopNH_3^+$ and $DopNH_3^+$) was also evaluated by NMR spectroscopy in 1:0.25 $CDCl_3/CD_3OD$ (Figure 7). In all cases, the appearance of a new NMR signature characteristic of the host-guest complex **2⊃RNH$_3^+$** was clearly observed.[26] The CISs are comparable to those observed with host **1**, indicating a similar mode of recognition (inset Figure 7). This result confirms that the phenoxy substituents of homooxacalix[3]arenes **1** and **2** are not involved in the recognition of the primary ammonium ions that are accommodated in the cavity. It is noteworthy that the competing protonation of the tertiary amino group of the trenamide cap was not observed even in presence of a large excess of ammonium ions (i.e. no downfield shift of

the C$H_2$N protons of the cap), denoting a weak basicity for this group. Even the addition of ~50 equiv. of trifluoroacetic acid was not sufficient for the complete protonation of the cap, which was achieved only in presence of *ca.* 2 equiv. of triflic acid (TfOH).[26] Surprisingly, the binding constants with host **2** and **2.H**$^+$ are respectively three and two orders of magnitude lower in comparison to those with host **1** (Table 2).

In order to rationalize the lower binding properties of host **2**, molecular mechanics conformational analyses of complexes (**2** and **2.H**$^+$)⊃***t*-BuNH$_3^+$ were performed and the results compared to those undertaken with host **1**.[26] To have an idea of the flattening of the cavities in the presence of a guest, the angles between the three planes containing the different aromatic rings of the receptor were measured.[26] The homooxacalix[3]arene polyaromatic cavity is less flattened, and thus less open, in the case of the capped systems **2** and **2.H**$^+$ (mean angles of 110.1°, 105.0° and 103.1° for complexes (**1**, **2** and **2.H**$^+$)⊃***t*-BuNH$_3^+$, respectively), suggesting that the rigid trenamide cap prevents the polyaromatic cavity from adopting an optimal open cone conformation for the deep insertion of ammonium ions (see Table 3 in the case of *t*-BuNH$_3^+$).

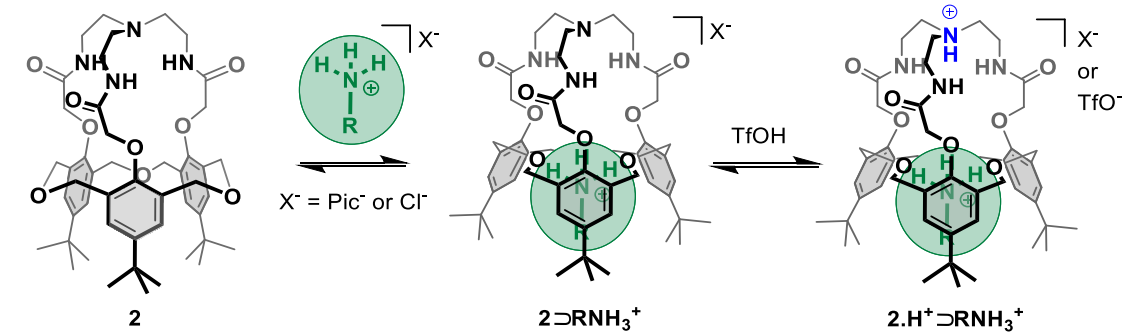

**Figure 7.** Host-guest properties of capped receptor **2** toward ammonium ions. Inset: CISs determined by $^1$H NMR spectroscopy in 1:0.25 $CDCl_3/CD_3OD$.

**A remarkable specificity for primary ammonium ions.** The binding of secondary, tertiary and quaternary ammonium ions by hosts **1** and **2** was also investigated by $^1$H NMR spectroscopy in 1:0.25 $CDCl_3/CD_3OD$ (Figures 4 and 7). Di-, tri- and tetramethylammonium as well as diethylammonium and *N*-methyl-2-(4-methoxyphenyl)-ethylammonium were tested. In all cases, the $^1$H NMR spectra of hosts **1** and **2** did not change significantly, even in the presence of an excess of these guests, and the intracavity inclusion of these ammonium ions was not observed.[26] This was also the case in pure $CDCl_3$.[26] This highlights that hosts **1** and **2** are specific to primary ammonium ions, even in a protic environment where primary ammonium ions are more solvated than secondary and tertiary ones, and consequently more difficult to extract from the solution. With the secondary, tertiary and quaternary ammonium ions, the three-point [N-H···O] binding motif is obviously not possible, and this weaker complementarity may explain in part the specificity for primary ammonium ions.

To gain further insight into the understanding of this specificity, the structures of complexes **1**⊃(**$Me_2NH_2^+$** and **$Et_2NH_2^+$**) were investigated *in silico*.[26] The calculations show that these secondary ammonium ions are less buried in the cavity compared to primary ammonium ions; even the very bulky primary ammonium $AdamNH_3^+$ is more deeply confined into the polyaromatic cavity of **1** than $Me_2NH_2^+$ or $Et_2NH_2^+$ (Table 3). The nitrogen atom of the secondary and tertiary ammonium ions is too far from the $O_3$ plane of the 18C3 for H-bonding or ion-dipole interactions with the phenoxy oxygen atoms (Table 3). To allow the positioning of the ammonium at a H-bond distance of the 18C3 macrocycle, one of the aromatic ring would have to be expelled from the cavity, leading to a distorted cone conformation for the homooxacalix[3]arene.

**Competitive binding and extraction studies.** Receptor **1** is known to bind $Na^+$ strongly (log $K > 10^7$ in 1:1 THF/$CHCl_3$)[23a] at the level of its ionophoric binding site composed of nine oxygen atoms (Figure 4). With the aim of using host **1** for the extraction of ammonium ions from complex aqueous mixtures such as biological media, we decided to study if ammonium ions could be bound in the presence of $Na^+$. Progressive addition of $NaPF_6$ to a 1:0.25 $CDCl_3/CD_3OD$ solution of **1⊃HexNH$_3^+$** led to the appearance of the signals corresponding to the complex **1⊃Na$^+$** and the disappearance of those characteristic of the ammonium ion in the receptor cavity (Figure 4).[26] This result can be rationalized by the fact that both cations compete for the ethereal macrocycle, even if they are bound on opposite sides of the crown ether. A strong repulsive charge-charge interaction would thus result from the simultaneous binding of both cations (a distance of *ca.* 1.9 Å between $Na^+$ and the nitrogen of the ammonium group was estimated from modeling studies).[26] The relative affinity $K_{Na+/HexNH3+}$ was estimated to be greater than $10^2$, and the sodium ion can thus be seen as an effector with a negative allosteric control on the recognition of organic cations by **1**.

The ability of host **1** to extract ammonium salts from water was then investigated. Biphasic mixtures of **1** in $CDCl_3$ and either $HexNH_3^+Cl^-$ or $AdamNH_3^+Cl^-$ (10 equiv.) in $D_2O$ were vigorously stirred for 10 min. In both cases, NMR signals corresponding to the complex **1⊃RNH$_3^+$** were observed in the organic phase.[26] No signals were observed for the free guest indicating that all the ammonium cations extracted from the aqueous phase are complexed. It is noteworthy that no extraction was observed when host **1** was not present in the chloroform phase. Very interestingly, in the presence of NaCl (10 equiv.) in the aqueous phase containing the chloride ammonium salt, only the signals of the complexes **1⊃RNH$_3^+$** were detected besides those of free host **1**.[26] The difference in the binding mode of primary ammonium ions compared to $Na^+$ as well as the higher hydrophilicity of $Na^+$ can explain why **1** is able to selectively extract primary ammonium ions from an aqueous phase into chloroform.

## Conclusion

The host-guest properties of two $C_{3v}$ symmetrical cone-homooxacalix[3]arene-based receptors (i.e. **1** and **2**) toward ammonium ions were investigated through NMR and *in silico* studies. The studies show that both receptors are able to bind various primary ammonium ions in a protic environment. They adopt an open cone conformation with the oxygen atoms of the ethereal macrocycle directed toward the cavity interior. The bound ammonium ion is deeply inserted into the polyaromatic cavity with its the $NH_3^+$ group nearly in the $O_3$ plane defined by the 18C3 moiety. Our results confirm that the 18C3 macrocycle is too small to let a molecule thread through it, precluding H-bonding interactions between the guest ammonium group and the amido groups of the ArO substituents on the receptor. Besides the three-point H-bonding network, which is classically observed in the case of complexes between 18C6 derivatives and ammonium ions, the phenoxy oxygen atoms (ArO) as well as the polyaromatic pocket of hosts **1** and **2** also contribute to the stabilization of the guest. Surprisingly, lower affinities were observed with the capped receptor **2**, which is rationalized by the fact that the rigid trenamide cap restricts the openness of the polyaromatic cavity, preventing it from adopting an optimal open cone conformation for the deep insertion of ammonium ions.

Similarly to what has been described with 18C6 derivatives,[28] the key point for an optimal complexation of ammonium ions by homooxacalix[3]arenes is the degree of insertion of the $NH_3^+$ group into the ethereal macrocycle: the shorter the distance between the nitrogen atom of the ammonium ion and the $O_3$ plane defined by the oxygen atoms of the macrocycle, the higher the affinity.

As the access to the binding 18C3 unit is sterically controlled by the polyaromatic corridor, primary ammonium ions that are encumbered in close proximity to their $NH_3^+$ group present lower affinities. In the case of tripeptides, the amino side chain of a lysine residue is thus more

strongly bound than the *N*-terminal group. Host **1** is also able to discriminate between the guanidinium group of an arginine residue and the primary ammonium group of the lysine side chain. Such a remarkable shape selectivity for the lysine residue could find many applications in the biomedical field.

Even more remarkably, hosts **1** and **2** are reluctant to bind secondary, tertiary and quaternary ammonium ions even in pure chloroform. Indeed, all these cations cannot be accommodated deeply enough into the cavity to be stabilized. To our knowledge, this is the first time that a specificity for primary ammonium ions over secondary, tertiary and quaternary ammonium ions is evidenced with homooxacalix[3]arenes and, more generally, with cavitands. Finally, while **1** is known to strongly bind $Na^+$, the selective liquid-liquid extraction of ammonium ions from an aqueous solution containing an excess of $Na^+$ was observed, opening interesting perspectives in the separation or detection of ammonium ions.

All these results strongly differ from what is generally observed with cavitands such as calixarenes, resorcinarenes, hemicryptophanes, pillararenes, etc.[4] These systems lack the $C_{3v}$ ethereal moiety and are thus mostly disposed to accommodate quaternary ammonium or pyridinium ions in their electron rich cavity. In the case of water-soluble systems based for example on CDs or cucurbiturils, the selectivity is mostly governed by the hydrophobic effect and not on the degree of substitution of the ammonium group. All in all, cone-homooxacalix[3]arenes represent a unique class of molecular receptors whose potential in the field of ammonium recognition has been underestimated. Future work in our laboratories is directed toward the elaboration of homooxacalix[3]arene-based systems for the selective binding and detection of lysine-containing peptides in water.

**Experimental Section**

**General Information.** $^{1}$H NMR spectra were recorded on either a 600 or a 400 MHz spectrometer and the $^{13}$C NMR spectra were recorded on the 400 MHz spectrometer. NMR parameters (acquisition time, recycling times and signal accumulation) were chosen to ensure that quantitative data could be obtained from signal integration in the $^{1}$H NMR spectra. The NMR spectra were recorded at 298 K unless otherwise stated. Chemical shifts are expressed in ppm. Traces of residual solvents were used as internal standards. $CDCl_3$ was filtered through a short basic alumina column to remove traces of DCl. The $^{1}$H NMR signals were assigned on the basis of 2D NMR analyses (COSY, HSQC). Compounds **1**[23] and **2**[24] were prepared as previously described.

**Complex 1⊃HexNH$_3^+$**: 1.2 equiv. of $HexNH_3^+Pic^-$ were added to host **1** in 1:0.25 $CDCl_3/CD_3OD$. $^{1}$H NMR (600 MHz, 298 K): $\delta$ (ppm) 7.25 (s, 6H, Ar*H*), 5.43 (d, 6H, *J* = 8.4 Hz, ArC$H_{ax}$), 5.08 (s, 6H, OC$H_2$), 4.29 (d, 6H, *J* = 8.4 Hz, ArC$H_{eq}$), 3.34 (q, 6H, *J* = 7.2 Hz, NC$H_2$), 3.24 (q, 6H, *J* = 7.2 Hz, NC$H_2$), 1.22 (s, 27H, *t*Bu), 1.13 (t, 9H, *J* = 7.2 Hz, $NCH_2CH_3$), 1.06 (t, 9H, *J* = 7.2 Hz, $NCH_2CH_3$), 0.88 (m, 2H, $CH_2CH_3^{guest}$), 0.64 (t, 3H, *J* = 7.8 Hz, $CH_3^{guest}$), 0.51 (m, 2H, $CH_2CH_2CH_3^{guest}$), 0.25 (m, 2H, $CH_2CH_2CH_2NH_3^{+guest}$), 0.19 (t, 2H, *J* = 8.4 Hz, $CH_2NH_3^{+guest}$), -0.32 (m, 2H, $CH_2CH_2NH_3^{+guest}$). $^{13}$C{$^{1}$H} NMR (100MHz, 298 K): $\delta$ (ppm) 167.9, 162.8, 156.0, 148.3, 142.2, 130.4, 130.0, 126.7, 72.3, 71.4, 40.8, 40.4, 39.6, 34.5, 31.4, 30.4, 29.8, 25.5, 24.2, 22.3, 14.2, 13.7, 13.1. ESI-MS (+): m/z calcd for $C_{60}H_{97}N_4O_9^+$ $[M+HexNH_3]^+$ 1017.7, found 1017.5.

**NMR Titration Experiments.** All experiments were prepared following a similar protocol. A known volume (~600 μL) of a solution of known concentration of the host (~$10^{-3}$ M) was placed in an NMR tube, and the $^{1}$H NMR spectrum recorded either on a 400 or a 600 MHz spectrometer. Aliquots of a stock solution of the guest were successively added, and the $^{1}$H NMR spectrum recorded after each addition. In general, aliquots were added until no changes in the host signals were observed. In all cases, two sets of signals were observed over the course

of the titration, indicating slow host–guest exchanges on the $^{1}$H NMR chemical shift timescale. Association constants (log *K*) were determined via integration of the signals of the different species (host, guest and complex). The association constants were determined as the mean value of the constants calculated based on different spectra and with the integration of different signals. The error was then estimated as the difference between this mean value with the smallest and largest association constants determined.

**Procedure for the liquid-liquid extraction of ammonium salts $RNH_3^+$ by host 1.** Biphasic mixtures of either $HexNH_3^+Cl^-$ or $AdamNH_3^+Cl^-$ (40 mM) in $D_2O$ (150µL) and host **1** (1 mM) in $CDCl_3$ (600µL) were vigorously stirred at room temperature for 10 min. The extraction of the ammonium salts $RNH_3^+$ was evaluated by $^{1}$H NMR spectroscopy through the detection of the signals corresponding to the endo-complex **1⊃RNH$_3^+$** in the organic phase.

**Molecular Modeling.** Monte Carlo multiple minimum (MCMM)[30] conformational searches (100 steps per torsion angle, maximum 1000 steps in total) were performed in Schrödinger Release 2019-1, using the OPLS3e or the MMFFs force field[31] with $CHCl_3$ as selected solvent in Maestro MacroModel (version 11.9.011).

## Supporting information

NMR spectra ($^{1}$H, $^{13}$C, COSY, HSQC and NOESY) and energy minimized structures of the host-guest complexes are given in the supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.


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