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Host-Guest Chemistry of Truncated Tetrahedral Imine Cages with Ammonium Ions

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Dedicated to Professor *Jean-Marie Lehn* on the occasion of his 80th birthday.

Three shape-persistent [4+4] imine cages with truncated tetrahedral geometry with different window sizes were studied as hosts for the encapsulation of tetra-*n*-alkylammonium salts of various bulkiness. In various solvents the cages behave differently. For instance, in dichloromethane the cage with smallest window size takes up NEt_4^+ but not NMe_4^+, which is in contrast to the two cages with larger windows hosting both ions. To find out the reason for this, kinetic experiments were carried out to determine the velocity of uptake but also to deduce the activation barriers for these processes. To support the experimental results, calculations for the guest uptakes have been performed by molecular mechanics' simulations. Finally, the complexation of pharmaceutical interested compounds, such as acetylcholine, muscarine or denatonium have been determined by NMR experiments.

1. Introduction

Supramolecular chemistry as we know it today goes back to the findings of Pedersen of crown ethers and their selective binding of alkali metal cations depending on ring size.[1] Inspired by these findings, Jean-Marie Lehn and coworkers designed three-dimensional congeners of crown-ethers; the macrobicyclic cryptands, accompanied by a significant increase of association constants and selectivities towards the alkaline metals.[2] Later, larger host molecules or supramolecular capsules were developed to accommodate larger guests or molecular cations to generate fundamental knowledge or mimic biochemical recognition events.[3] Still, more 50 years after the seminal papers of Pedersen were published, cation binding recognition events are still appealing, e.g. to template dynamically formed ortho-ester[4] or as stabilized reaction intermediates within the confined space of cages or capsules to accelerate chemical reactions.[5] The larger the host molecules are, the more difficult their synthesis get.[6] Often, multiple steps are required resulting in low overall yields.[7] By the introduction of dynamic covalent chemistry (DCC),[8] shape-persistent organic cages become more readily available in a few steps, often with high yields in the multiple bond forming reaction to the cages due to the reversible nature of the bond formation.[7b,9] A large number of various cage sizes and geometries have meanwhile been realized by DCC,[9b,10] such as tetrahedra,[11] prisms,[12] cubes,[13] adamantoids,[14] and others.[15] Even larger cages with diameters of three and more nanometers were reported.[16]

Besides the gain to fundamental understanding of cage formations,[17] one of the main aspects was the investigation of gas sorption by porous organic cages.[9b,13c,18] Despite early investigations of binding guest molecules inside the cavities of shape-persistent organic cages,[15a] there has not too much been done in this respect in recent years,[19] which is in contrast to the large number and variety of studied host-guest complexes based on e.g. hydrogen bonding capsules[20] or coordination cages.[21] For instance, Cooper et al. used smaller tetrahedral imine cages with narrow windows to selectively separate isomeric mixtures of alkylated benzenes,[22] or more recently, to separate H_2 from D_2.[23] The same cages were used as stationary phases on columns to separate various analyte mixtures.

Here we present our studies of host-guest binding of ammonium ions by shape-persistent [4+4] imine cages with a truncated tetrahedral geometry.[24] The three investigated [4+4] imine cages are structurally related and differ mainly in the window sizes, which are adjusted by various long substituents on the used 1,3,5-triformylbenzene.[24]

2. Results and Discussion

The truncated [4+4] imine cages were synthesized by reacting the conformationally fixed triethyltriamine **1**[25] with the corre-

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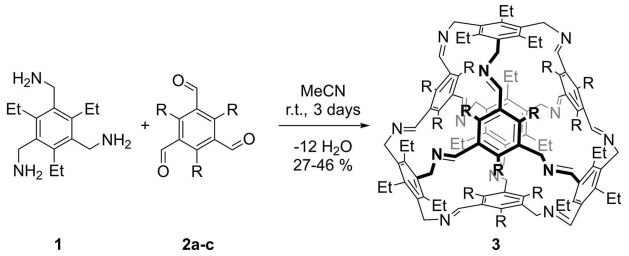
sponding trialdehydes **2a–c** in a 1:1 stoichiometry in acetonitrile at room temperature (Scheme 1).[24] Here the missing link, cage **3-Me** was synthesized and isolated in 37% yield, which is in between the prior reported yields of 27% (**3-H**) and 46% (**3-Et**).[24]

3-Me was fully characterized by NMR spectroscopy and MALDI MS ($m/z = 1599.0816$ $[M+H]^+$). By DOSY experiments in CD_2Cl_2 ($T = 298$ K) a diffusion coefficient of $D = 6.6 \cdot 10^{-10}$ $m^2 s^{-1}$ was measured, corresponding to a solvodynamic radius of $r_s = 0.8$ nm. These values are between the one of **3-H** ($D = 6.9 \cdot 10^{-10}$ $m^2 s^{-1}$, $r_s = 0.8$ nm) and **3-Et** ($D = 4.5 \cdot 10^{-10}$ $m^2 s^{-1}$, $r_s = 1.2$ nm) and fits to the estimated molecular dimension ($d = 1.5$ nm) according to the data from single crystal X-ray diffraction (Figure 1b). Single crystals of cage **3-Me** were grown from dichloromethane (Figure 1). The compound crystallizes in the orthorhombic space group A_{ma2} ($Z = 4$) forming channels between the cage molecules with diameters of 9 Å ×11 Å, respectively (Figure 1c). The outer diameter of cage **3-Me** is with 1.5 nm nearly the same as found for cages **3-H** (1.6 nm) and **3-Et** (1.6 nm).[24] It is worth mentioning that in contrast to the structures of cages **3-H** and **3-Et** the imine bonds are found to exist in various conformations (Figure 1a). Some are nearly orthogonal to the aromatic π-planes with the imine protons pointing inside the cavity and other imine units are nearly coplanar to the aromatic ring, stabilized by conjugation. The space filling model of **3-Me** cage reveal a relative closed character (Figure 1b) with narrow windows for molecules accessing the inner cavity. To estimate the volume and window sizes of the cages **3-H**, **3-Me** and **3-Et** as potential hosts in solution, the preferred conformations and corresponding cavity volumes of these three cages were determined by DFT calculations (B3LYP, 6-31G) with DCM as solvent (Figure 2a,b). The cross-sections of the window sizes (distances between the atom centers of two closest carbon atoms) of the three cages decrease with the bulkiness of the substituents of the former trialdehyde linker (**3-Et**: 7.1·3.4 Å = 24.1 Å^2; **3-Me**: 7.1·4.0 Å = 28.4 Å^2 and for **3-H**: 7.2·6.9 Å = 49.7 Å^2). The corresponding calculated cavity volumes (for a probe radius of 1.4 Å) follow the same trend (**3-H**: 337 Å^3, **3-Me**: 253 Å^3 and **3-Et**: 218 Å^3).



Scheme 1. [4+4]-condensation of trimethylamine **1** and trisaldehydes **2a–c**. R = H, Me, Et.

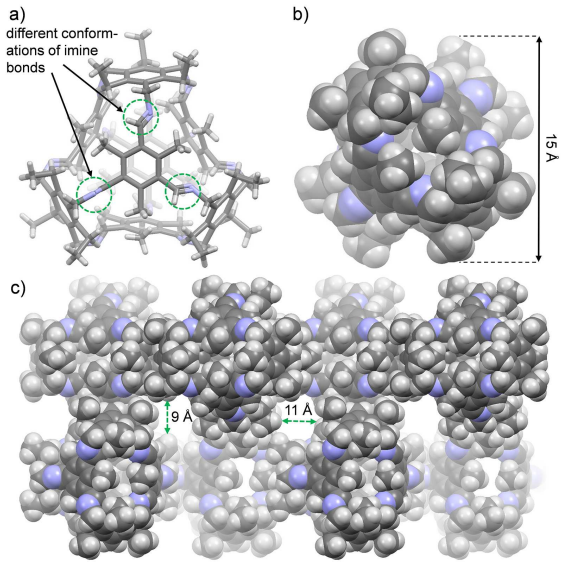


Figure 1. Single-crystal structure of **3-Me**. a) Capped stick model. b) Space-filling model. c) Space-filling model of the packing along the crystallographic *b*-axis (1x1x2 unit cell).

Because we were interested in the uptake of ammonium ions, we also estimated the volumes of the homologous series of tetra-*n*-alkyl-ammonium ions (Figure 2c), which is between 95 Å^3 for the smallest guest NMe_4^+ and 304 Å^3 for the biggest guest NBu_4^+. Correlating the volumes of the host cavities with those of the ammonium ions as potential guests, the occupancies were estimated (Table 1).[26] According to Rebek's "55% rule",[27] it is expected that cage **3-H** should be able to take up NMe_4^+, NEt_4^+ and even NPr_4^+ but not NBu_4^+. Cage **3-Me** with a smaller cavity volume should be able to host NMe_4^+ and NEt_4^+ but not the two larger ones and **3-Et** should take up the smallest cation NMe_4^+ and maybe is able to host the next larger NEt_4^+. For the latter the estimated occupancy is with 75% borderline according to Rebek's rule.[27a]

We started the complexation experiments with **3-H** as host and NEt_4^+ as guest in CD_2Cl_2 as solvent. As counter ion, the weekly coordinating anion BF_4^- was chosen. After 18 hours the mixture was analyzed by ^{1}H NMR spectroscopy (Figure 3b). To our delight the formed host guest complex shows a separate set of signals in the ^{1}H NMR spectrum, with the resonances of one equivalent of encapsulated guest. The signal for the CH_2-group of the encapsulated guest is shifted up-field by $\Delta\delta = -2.5$ ppm from $\delta = 3.24$ to 0.74 ppm, whilst the resonance of the CH_3-group is shifted by $\Delta\delta = -1.99$ ppm from $\delta = 1.32$ to −0.67 ppm (Figure 3c).

Furthermore, DOSY NMR experiments confirm that encapsulated tetra-*n*-alkyl-ammonium ions diffuse at the same rate as the host cages with a diffusion coefficient of $D = 7.1 \cdot 10^{-10}$ $m^2 s^{-1}$ ($r_s = 0.7$ nm, see Supporting Information). After

Table 1. Calculated occupancies of the space in the cavity by ammonium guests.

guest	occupancy [%][a]		
	3-H	**3-Me**	**3-Et**
NMe_4^+	28	38	44
NEt_4^+	48	64	75
NPr_4^+	70	93	108
NBu_4^+	90	120	139

[a] occupancy = V_{guest}/V_{cavity}

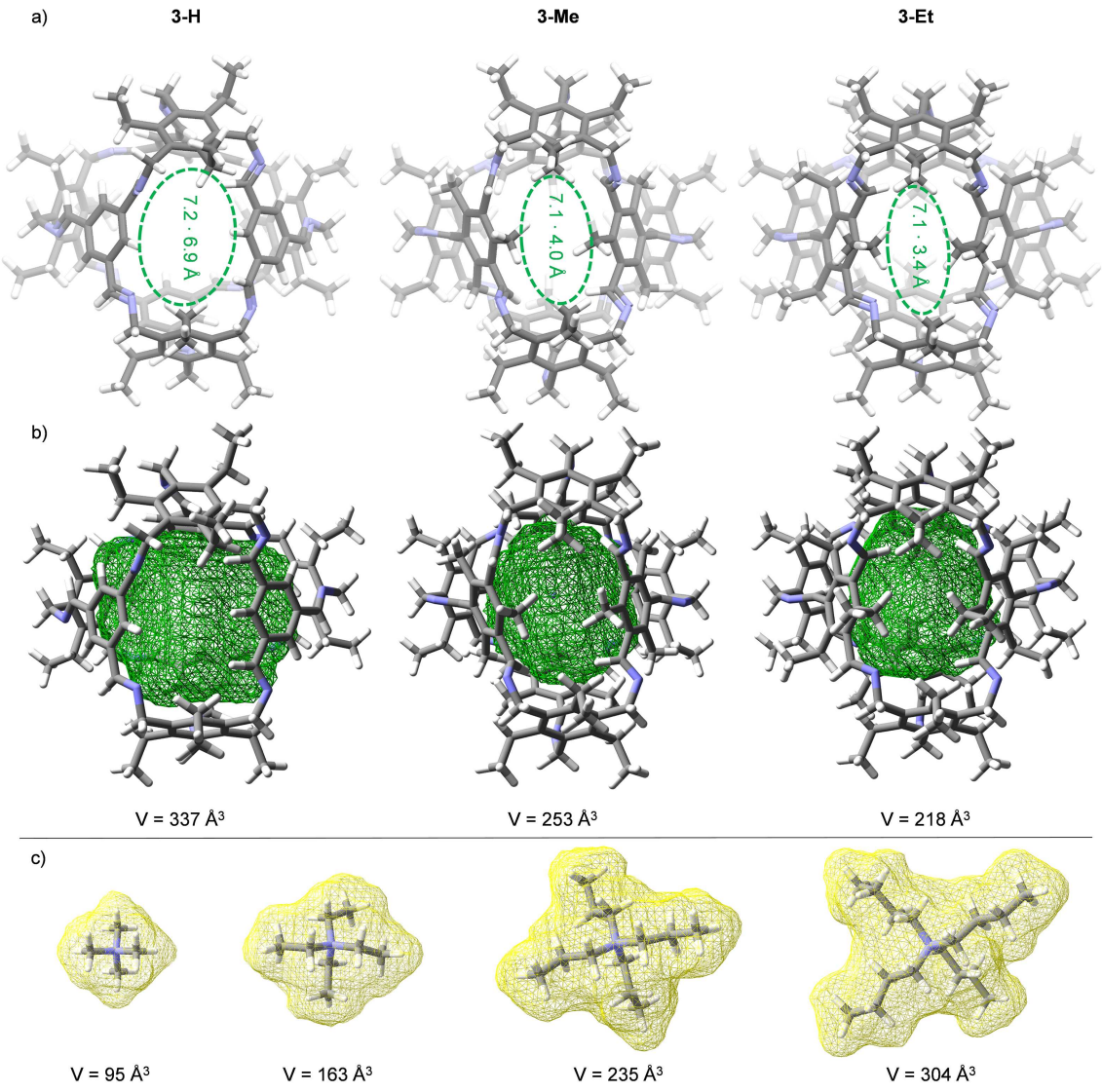


Figure 2. DFT calculated structures of the three cages **3-H**, **3-Me** and **3-Et** in DCM and the guests NMe_4^+, NEt_4^+, NPr_4^+ and NBu_4^+. a) Window size; b) Illustration of the cavity volume computed with SwissPDBViewer.[28] c) Volume of the guests.

another 48 hours no further change of integral ratios was observed, suggesting that the system is in the thermodynamic equilibrium. Due to the slow exchange rate, compared to the NMR timescale, the association constant can be calculated by considering the mass balance law (for details, see Supporting Information). Equilibrium concentrations are taken by integration of characteristic signals of the host-guest complex and those of free host and guest.[29]

For $\mathbf{NEt_4^+}\subset$**3-H** an association constant of $K_a = 2.4 \cdot 10^3\ M^{-1}$ was determined. As mentioned above, by comparison of the relative integrals of bound guest to bound host, a stoichiometry of 1:1 was obtained. This ratio was confirmed by MALDI-TOF MS experiments (see Figure 4), were the singly charged ion was found ($\mathbf{NEt_4^+}\subset$**3-H** ($m/z = 1560.1794$; calc. for $C_{104}H_{128}N_{13}^+ = 1560.0448$). Next we investigated the complexation behavior for smaller and larger ammonium ions. NMe_4^+ is bound inside the cavity, but the association constant drops by two orders of magnitude to $K_a = 1.9 \cdot 10^1\ M^{-1}$. For NPr_4^+ a higher association constant ($K_a = 1.9 \cdot 10^3\ M^{-1}$) was found, with a comparable value as NEt_4^+ and for NBu_4^+ no binding was detected. Again, by MALDI-TOF MS experiments only the singly charged ions were found ($\mathbf{NMe_4^+}\subset$**3-H**; $m/z = 1504.1229$; calc. for $C_{100}H_{120}N_{13}^+ = 1503.9822$) and $\mathbf{NPr_4^+}\subset$**3-H** ($m/z = 1616.2149$; calc. for $C_{108}H_{136}N_{13}^+ = 1616.1074$) suggesting a 1:1 host-to-guest ratio.

The next potential host compound that was studied was cage **3-Me** with narrower windows. It is worth mentioning that in comparison to free **3-H**, the free **3-Me** shows a strong peak broadening in the ^1H NMR spectrum when CD_2Cl_2 is used as solvent. Most likely this is due to slow solvent exchange on the NMR timescale. As soon as the cavity of the cage is blocked by a guest, sharp signals are observed again (see Supporting Information). As expected, **3-Me** binds NMe_4^+ ($K_a = 4.7 \cdot 10^1\ M^{-1}$) and NEt_4^+ ($K_a > 1 \cdot 10^5\ M^{-1}$).[29] The larger guests NPr_4^+ and NBu_4^+ do not fit any more. In contrast to the other two cages,

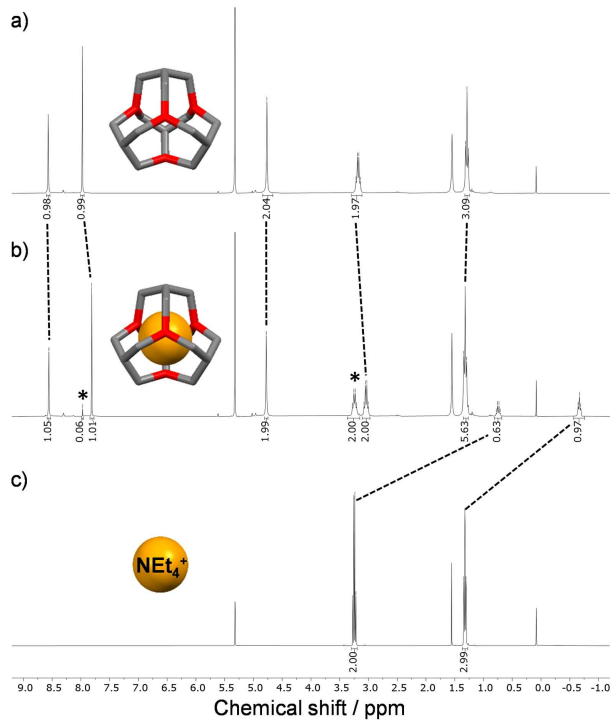


Figure 3. Representative ^{1}H NMR spectra (CD_2Cl_2, 300 MHz) of the host guest experiments. a) **3-H**. b) Mixture of NEt_4BF_4 (3 eq.) and **3-H**, the shift of the signals for the host-guest complex are highlighted with dotted lines. * residues of free **3-H** and of free NEt_4BF_4. c) NEt_4BF_4 without host.

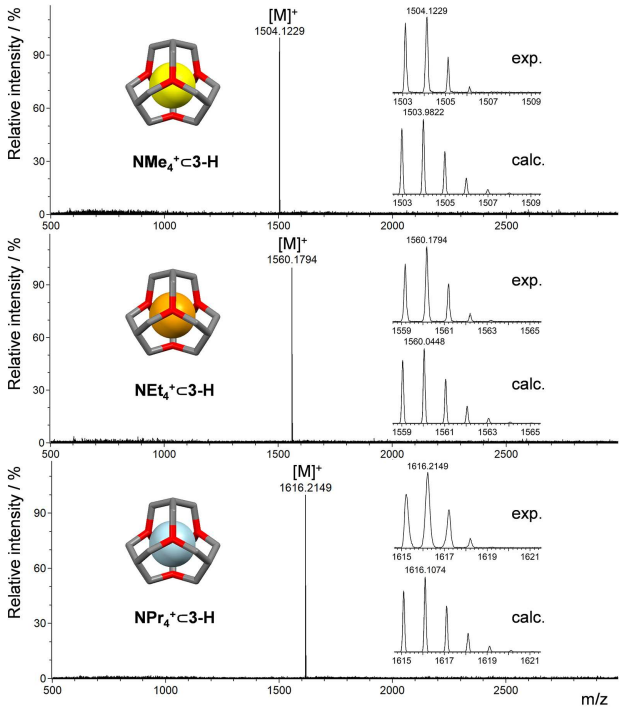


Figure 4. MALDI-TOF MS experiments of NMe_4^+⊂**3-H**, NEt_4^+⊂**3-H** and NPr_4^+⊂**3-H**. Inlets: Comparison of calculated and measured *m/z*-value.

cage **3-Et** behaved a little bit differently than intuitively expected: It only takes up NEt_4^+ ($K_a > 1 \cdot 10^5\ M^{-1}$) but not the smaller NMe_4^+.

We studied the complexation behavior in other, less polar solvents (THF-d_8, toluene-d_8 and $CDCl_3$) and only for **3-H** host-guest complexation was observed. In THF-d_8 **3-H** binds the whole series slightly stronger than in DCM (Figure 5). For NMe_4^+ an association constant of $K_a = 2.1 \cdot 10^1\ M^{-1}$ was determined and for guests NEt_4^+ and NPr_4^+ again, the association constants are beyond $K_a > 1 \cdot 10^5\ M^{-1}$. Most interestingly, in this solvent, even NBu_4^+ is picked up with a relatively large association constant of $K_a = 2.1 \cdot 10^3\ M^{-1}$.

It is worth mentioning that the terminal protons of the propyl chains of NPr_4^+ are less up-field shifted than the β-protons of the chains (see Supporting Information), suggesting that the chains are "folded" in a manner that the resonance of the β-protons is more influenced by the aromatic "wall" of the cages, which is in line with observations made before e.g. for capsules.[30] The same effect, even more pronounced was detected with the butyl chains of NBu_4^+ accompanied by a significant peak broadening of the encapsulated guest signals. Rebek and co-workers described in their work, that packing coefficients higher than 65% lead to an artificial freezing of the guest in the cage, which is responsible for the peak

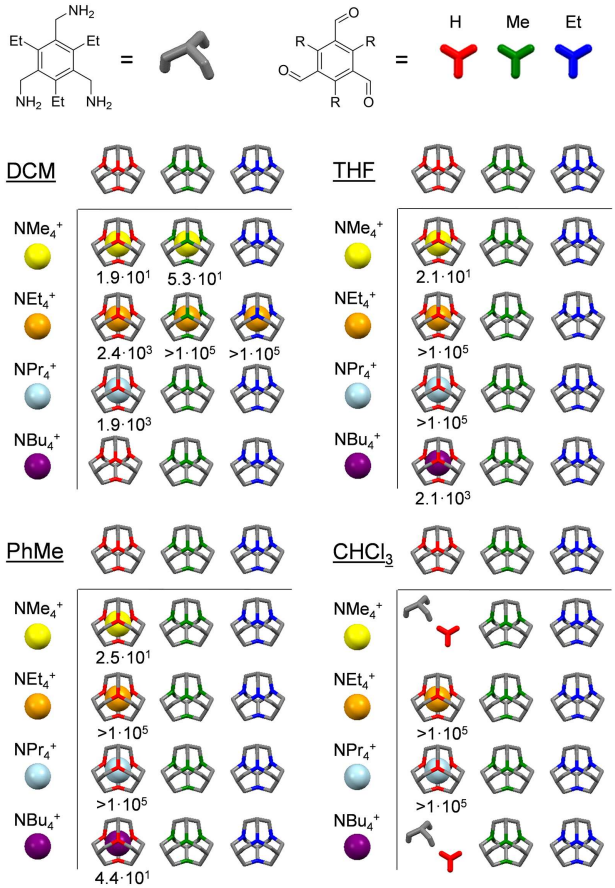


Figure 5. Schematic summary of the size selectivity with association constants K_a [M^{-1}] for the encapsulation in different solvents (for experimental details and standard deviations, see Supporting Information).

broadening.[27a] This is in agreement with our observations, indicating a restricted movement of the cations in the cavity compared with the freedom it has in the solvent.

In toluene-d_8 basically the same trend is observed, although the bare ammonium salts are of low solubility herein. Again, even NBu_4^+ is complexed with $K_a = 4.4 \cdot 10^1\ M^{-1}$. The binding of NBu_4^+ in these two solvents seem to be contradictive to the above discussed calculated occupancies in combination with Rebek's rule (see Table 1).[27a] However, from the complex **NPr_4^+⊂3-H** we got a single crystal structure by X-ray diffraction showing that the cavity is expandable in volume (458 Å^3) (Figure 6a). Taking this volume now to calculate the occupancy for NBu_4^+, one clearly is with 66% below the limit according to Rebek's rule.[27a] The **(NPr_4^+·toluene⊂3-H)BF_4** complex crystallizes in the monoclinic space group $P_{21/c}$ ($Z = 4$), with five molecules toluene outside the cage and one inside. The additional toluene molecule inside the cavity further stabilizes the guest by cation-π interaction in a distance of 4.4 Å (Figure 6, b. Distance measured from π-plane of aromatic ring to positively charged nitrogen). All alkyl chains of the guest point towards the windows. The counter ion BF_4^- is located outside the cage cavity.

In $CDCl_3$, both NEt_4^+ and NPr_4^+ were bound with significantly larger association constants ($K_a > 1 \cdot 10^5\ M^{-1}$) than in DCM. From previous work we know that cage **3-H** is not stable in $CHCl_3$ and decomposes by time, most likely due to traces of hydrochloric acid.[24] So it is in the case of NMe_4^+ and NBu_4^+ and decomposition is faster than complexation, making any assumption of association constants impossible.

Contrary, in the case of NEt_4^+ and NPr_4^+ the decomposition is significantly lowered due to a stabilizing effect, which reminds one to e.g. the tobacco mosaic virus, keeping its tubular form only with the RNA encapsulated.[31] The reaction rate of the decomposition could be slowed down by two orders of magnitude from $k_{dec} = 1.6 \cdot 10^{-5}\ s^{-1}$ (free **3-H**) to $k_{dec} = 1.3 \cdot 10^{-6}\ s^{-1}$ (**NEt_4^+⊂3-H**) and $k_{dec} = 3.3 \cdot 10^{-7}\ s^{-1}$ for **NPr_4^+⊂3-H** (Figure 7).

Comparisons of the host-guest complexes with the ammonium salts by ^{19}F-NMR spectroscopy showed no significant shifted peak for the BF_4-counteranion, like it was found in other works.[32] Furthermore, by ^{1}H-^{19}F HOESY experiments no coupling of fluorine with any of the cage protons was found (see Supporting Information), suggesting that the anion is not bound inside the cavities. This is in agreement with the obtained crystal structure of the **(NPr_4^+·toluene⊂3-H)BF_4** complex (see discussion above).

To further investigate the influence of the counter ion on the binding in **3-H**, **3-Me** and **3-Et**, tetra-*n*-alkyl-ammonium iodides were studied in DCM. In contrast to the before used BF_4^- salts the association constants dropped for all complexes (Table 2). NMe_4^+ is bound about three times less with cages **3-H** and **3-Me** ($K_a = 0.7 \cdot 10^1\ M^{-1}$ and $1.5 \cdot 10^1\ M^{-1}$), when the stronger coordinating iodide is present.[33] As observed before, cage **3-Et** with the narrowest windows does not take up NMe_4^+ at all. NEt_4^+ is complexed by all three cages with significantly smaller association constants dropping several orders of magnitude, clearly revealing that separation of solvent-shared ion pairs[34] is negatively contributing to the overall Coulomb

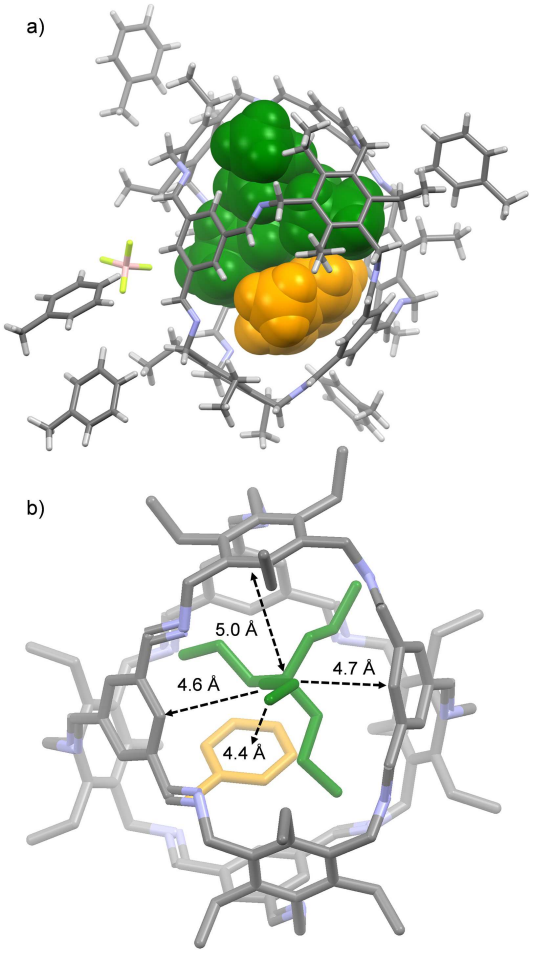


Figure 6. Single-crystal structure analysis of **(NPr_4^+·toluene⊂3-H)BF_4**. a) Stick model of **Et-H** and space filling model of the guest toluene in orange and NPr_4^+ in green. b) Distances of nitrogen to center of the aromatic units.

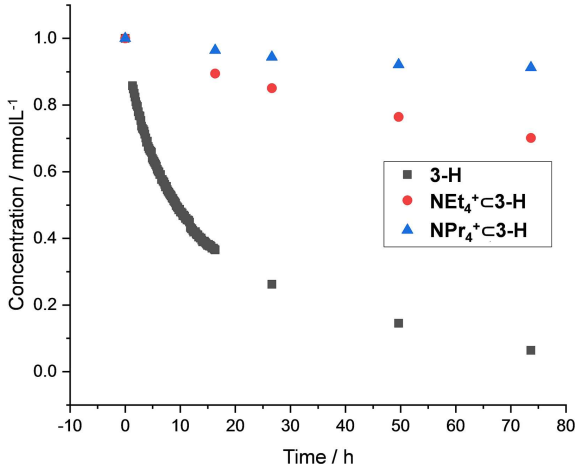


Figure 7. Concentration *vs.* time diagram of the decomposition of **3-H** in $CDCl_3$, followed by ^{1}H NMR spectroscopy (300 MHz).

Table 2. Association constants [M^{-1}] for the guest inclusion depending on the counter ion in DCM-d_2 (298 K).

Host	NMe_4^+ BF_4^-	I^-	NEt_4^+ BF_4^-	I^-	NPr_4^+ BF_4^-	I^-
3-H	$1.9\cdot10^1$	$0.7\cdot10^1$	$2.4\cdot10^3$	$2.2\cdot10^1$	$1.9\cdot10^3$	$9.2\cdot10^2$
3-Me	$4.7\cdot10^1$	$1.5\cdot10^1$	$>1\cdot10^5$	$2.4\cdot10^3$	n. b. [a]	n. b. [a]
3-Et	n. b. [a]	n. b. [a]	$>1\cdot10^5$	$3.5\cdot10^1$	n. b. [a]	n. b. [a]

[a] *n.b.* = no binding was detected.

term of interaction. Similar observations were made before for other host systems.[20g] The smallest change has been observed for NPr_4^+. Here, the association constant with **3-H** slightly decreases from $K_a = 1.9\cdot10^3$ M^{-1} to $K_a = 9.2\cdot10^2$ M^{-1}.

The kinetics of the uptake of tetra-*n*-alkyl-ammonium cations in CD_2Cl_2 was followed by ^1H NMR spectroscopy at 303 K for a solution 0.33 mM of cage **3-H** and 2.2-3.3 mM of ammonium salt (see Supporting Information). The encapsulation of the smallest guest NMe_4^+ in **3-H** reaches equilibrium after only 25 minutes ($k = 6.4\cdot10^{-2}$ $M^{-1}s^{-1}$; Figure 8). Whereas the reaction rates of the larger guests decrease about one order of magnitude with increasing size from $2.5\cdot10^{-3}$ $M^{-1}s^{-1}$ (NEt_4^+) to $1.9\cdot10^{-3}$ $M^{-1}s^{-1}$ (NPr_4^+). The complexation by **3-Me** and **3-Et** was very slow at 303 K, therefore, the kinetics were measured at 314 K. For the cage **3-Me** the rate for the guest uptake dropped by two orders of magnitude for NMe_4^+ ($k = 1.8\cdot10^{-4}$ $M^{-1}s^{-1}$, 314 K) and to $k = 8.4\cdot10^{-4}$ for $M^{-1}s^{-1}$ (NEt_4^+, 314 K). For the **3-Et** with even more narrower window sizes the kinetics for complexation of NEt_4^+ revealed an encapsulation rate of $k = 6.1\cdot10^{-5}$ $M^{-1}s^{-1}$ (314 K).

In principle two different mechanisms for the uptake of the ammonium salts are possible.[35] One possibility is a gate-opening mechanism where a reversible bond cleavage of one or multiple imine bonds occur to 'open the lid' of the cage to enable an encapsulation without or with low barrier of the guest ion, followed by reformation of the imine bonds to close the cage. Indeed, this mechanism has been proposed for an imine based hemicarcerand.[36] The second possibility is a squeezing mechanism.[37] Here, the cage stays intact and the guest is squeezed through the window into the cavity. In an extended study based on experimental observations and theoretical calculations, Raymond *et al.* concluded, that this mechanism is most likely the one tetrahedral metalcatecholate cages take up charged guests. Remarkably, even guests that are intuitively much too big, having to surpass a barrier of 251 kJ/mol, such as $CoCp^*_2{}^+$, seem to enter the cage without any ligand disassociation by this squeezing mechanism.

Considering the large differences in the kinetic uptake of ammonium ions of the same size by more than two orders of magnitude depending on the aperture of the cage windows in combination with similar it is assumed that a squeezing mechanism is more likely than a gate-opening. Therefore, we performed force-field based molecular dynamics simulations (MD) to study the mechanism of complexation behavior by a squeezing mechanism (for details, see Supporting Information). For each cage (**3-H**, **3-Me** and **3-Et**) the dissociation of the two smaller ammonium ions NMe_4^+ and NEt_4^+ from the inner cavity through the windows without bond-breaking were computed. For **NMe_4^+⊂3-H** the barrier was with $\Delta G^{\ddagger} = 61$ kJ/mol approximately half that of **NEt_4^+⊂3-H** ($\Delta G^{\ddagger} = 141$ kJ/mol). As soon as the window apertures get smaller, the calculated barriers increase significantly. For complex **NMe_4^+⊂3-Me** and **NEt_4^+⊂3-Me** the barriers are with $\Delta G^{\ddagger} = 123$ kJ/mol and $\Delta G^{\ddagger} = 241$ kJ/mol nearly double as for the complexes with cage **3-H**. Most interestingly, for the cage with the smallest windows (**3-Et**) the calculated energies drop in comparison to the one with the medium sized windows (**3-Me**) for the uptake of the smallest NMe_4^+ from $\Delta G^{\ddagger} = 123$ kJ/mol to $\Delta G^{\ddagger} = 91$ kJ/mol, whereas for the larger NEt_4^+ the barrier is with $\Delta G^{\ddagger} = 359$ kJ/mol very high and accompanied by a strong deformation of several bonds (Figure 9).

Since the energy is in the regime of covalent C–C bonds at least for the latter **NEt_4^+⊂3-Et** a squeezing mechanism needs to be questioned. Further experiments and calculations need to be done. It is worth mentioning that various amounts of solvent molecules are found in the cavities as co-guests within the thermodynamically most stable host-guest complexes (see Supporting Information). The dynamics of the ammonium complexation for certain is influenced by the dynamics of these co-complexed solvent molecules. Furthermore, not only the thermodynamics and the kinetics of the cation uptake by the cages play a role, but also the solvation of the ammonium salt

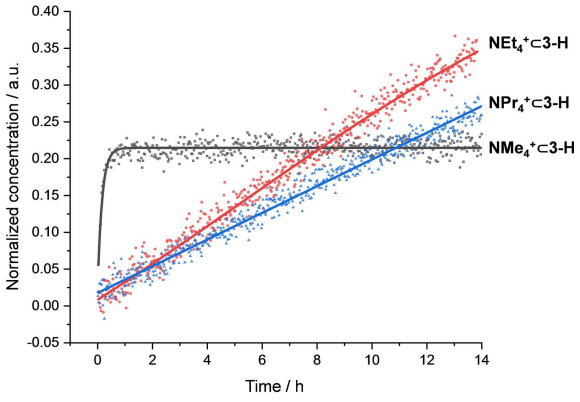


Figure 8. Concentration *vs.* time diagram of the encapsulation of NMe_4^+, NEt_4^+, NPr_4^+ in **3-H** in CD_2Cl_2, followed by ^1H NMR spectroscopy (300 MHz, 303 K).

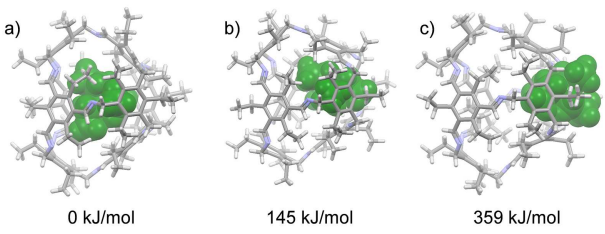


Figure 9. Computed conformations during the squeezing of NEt_4^+ through the window of **3-Et** in CD_2Cl_2. a) NEt_4^+ near the center of **3-Et**. b) NEt_4^+ approaching the window. c) strong deformation of **3-Et** at the transition state.

Table 3. Association constants for the pharmaceutically active ammonia salts in DCM-d_2/acetonitrile-d_3 (ratio 9:1 (v/v)) at 298 K.

Host	association constants K [M^{-1}] acetylcholine$^+$	(±)-muscarine$^+$	denatonium$^+$
3-H	$8.3 \cdot 10^1$	$3.7 \cdot 10^2$	*n. b.* [a]
3-Me	$1.2 \cdot 10^2$	$7.7 \cdot 10^3$	*n. b.* [a]
3-Et	*n. b.* [a]	*n. b.* [a]	*n. b.* [a]

[a] *n.b.* = no binding was detected.

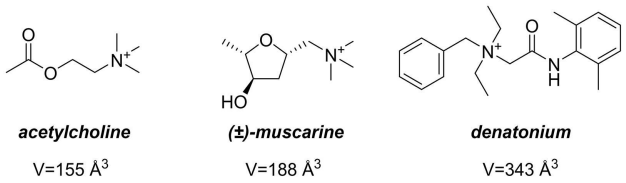


Figure 10. For host-guest chemistry investigated pharmaceutically active ammonium cations with volumes.

in the solvent as well as the strip of the solvation sphere of the ammonium ions to enter the cage needs to be taken into account. The sum of all these energy contributions may explains, why the complexation of the smaller NMe_4^+ within cage **3-Et** is not observed, but the larger NEt_4^+ forms **NEt_4^+⊂3-Et**. It is assumed that the lack of **NMe_4^+⊂3-Et** is of thermodynamic reasons. However, this needs to be proved by further investigations.

Finally, the pharmaceutically active ammonium salts acetylcholine chloride, its agonist (±)-muscarine chloride and denatonium benzoate (Figure 10) were studied as potential guests. The sizes of acetylcholine (155 Å^3) and (±)-muscarine (188 Å^3) differ only slightly and have approximately the size of NEt_4^+ (163 Å^3). The denatonium cation is with 343 Å^3 slightly larger than NBu_4^+ (304 Å^3). The complexation studies were performed in a mixture of DCM-d_2 and acetonitrile-d_3 in a ratio of 9:1 (v/v).

Acetylcholine as well as (±)-muscarine are bound by **3-H** and **3-Me**. For acetylcholine⊂**3-H** an association constant of $K_a = 8.3 \cdot 10^1\ M^{-1}$ was obtained, for (±)-muscarine⊂**3-H** a stronger binding was found ($K_a = 3.7 \cdot 10^2\ M^{-1}$). As expected from the previous experiments **3-Me** binds the two guest's acetylcholine and (±)-muscarine stronger than **3-H** with $K_a = 1.2 \cdot 10^2\ M^{-1}$ for acetylcholine⊂**3-Me** and $K_a = 7.7 \cdot 10^3\ M^{-1}$ for (±)-muscarine⊂**3-Me**, respectively. Simultaneously the selectivity $S = K_a$(muscarine)/ K_a(acetylcholine) changes significant by altering the window size. **3-Me** binds (±)-muscarine with $S = 64$ more selectively than **3-H** with $S = 4.5$. Denatonium as the biggest guest was not bound by any cage as well as **3-Et** also did not bound acetylcholine or (±)-muscarine even after one week at 298 K (Table 3).

Conclusions

To summarize, the complexation of various tetralkylammonium salt ions of different sizes within structurally related [4+4]-cages have been studied. The cages mainly differ in the size of the window apertures. By extended NMR studies, thermodynamic and kinetic data have been generated suggesting that the uptake of ammonium ions is most likely be favored by a squeezing mechanism rather than by a gate-opening mechanism. This is also in line with the previous observation that the [4+4] cages are not thermodynamically but rather kinetically controlled products.[24] Guest uptake mechanisms play a pivotal role for the usage of shape-persistent organic cages as confined molecular reaction vessels and therefore more studies will be pursued to finally pin down the mechanism and use the [4+4] cages as vessels, e.g. for catalytic reactions with cationic transition states.[5c,21j,s,38]

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Keywords: imines · shape-persistent organic cages · host–guest chemistry · molecular mechanics

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