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Specific binding of primary ammoniums in aqueous media by homooxacalixarenes incorporated into micelles

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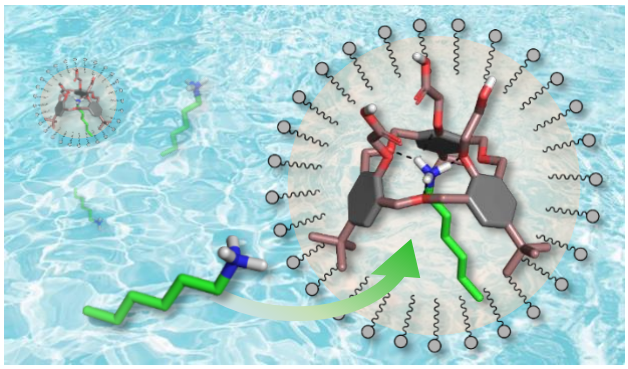
Table of Contents Graphic



Abstract

The development of artificial receptors for efficient recognition of analytes in water is a challenging task. The homooxacalix[3]arene-based receptor **1**, which is selective towards primary ammoniums in organic solvents, was transferred into water following two different strategies: direct solubilization and micellar incorporation. Extensive ^{1}H NMR studies show that recognition of ammoniums is only observed in the case of micellar incorporation, highlighting the beneficial effect of the microenvironment of the micellar core. The selectivity of the system for primary ammoniums over secondary and tertiary ones is also maintained. The hydrophobic effect plays an important role on the recognition properties, which are counter-ion dependent due to the energy penalty for the dissociation of certain ammonium salts in the apolar micellar core. This study shows that the straightforward self-assembly process used for the encapsulation of artificial receptor in micelles is an efficient strategy to develop water soluble nanosized supramolecular recognition systems.

Keywords: Ammonium ions – Micelles – Supramolecular chemistry – Host-guest systems – Molecular recognition in water.

Introduction

The development of strategies for the recognition of primary ammonium cations in water is a topical field of research, with potential applications in biological and environmental monitoring. Primary ammonium groups are indeed present in many natural molecules of biological interests, such as amino acids, peptides, and neurotransmitters.[1] They are also widely used in the industry and are reported, in certain cases, to have a negative impact on our health and the environment.[2–4] A strategy widely exploited for the design of ammonium ion receptors[5] consists in using cavity-based systems such as calixarenes,[6,7] resorcinarenes,[8] hemicryptophanes,[9] or pillararenes.[10,11] These cavitands provide an electron π-rich pocket that can stabilize the ammonium through cation–π and CH-π interactions, while ensuring a size and shape cavity-based selectivity.[12–17] C_{6v} or C_{3v} symmetrical cavitands are of particular interest for primary ammonium groups as, similarly to 18-crown-6 ether derivatives, they can offer a highly complementary H-bonding acceptor site. Examples of C_{3v} symmetrical calix[6]arenes displaying a good selectivity for primary ammonium ions are known,[18,19] however, due to the flattened cone conformation of the receptor framework, these systems are limited to the binding of small or linear guests. Hexahomotrioxacalix[3]arenes present a more open cavity and we have recently shown that receptor **2** (Figure 1) exhibits high affinity and selectivity for primary ammoniums compared to secondary, tertiary and quaternary ones, even in a competitive protic environment (log K_a > 4 for various RNH_3^+ in $CDCl_3/CD_3OD$ 4:1).[20] The elucidated binding mode sees the ammonium ion deeply inserted into the polyaromatic cavity and the NH_3^+ establishing three H-bonds with the ethereal macrocycle.

While selective recognition and efficient binding have been achieved in polar organic solvents, a major challenge is the development of systems that work in an aqueous medium.[21] Water molecules can actively interfere in the recognition processes as they are excellent H-bond donors/acceptors and can efficiently solvate the different binding partners. The high dielectric constant of water is also problematic in the case of coulombic interactions.[22] To the advantage of water, the hydrophobic effect can be beneficial for molecular recognition processes involving less polar guests.[23] A key issue is of course the solubility of the receptor as most receptors developed to date are poorly soluble, if not totally insoluble in water.

Different strategies can be envisaged to transfer molecular receptors into water. The most commonly encountered is the grafting of hydrophilic moieties onto the receptor, as shown in recent reviews devoted to water-soluble macrocyclic synthetic receptors.[24–26] This however requires additional synthetic steps and careful design, as the hydrophilic groups must not interfere with the binding event. A few examples of macrocyclic receptors have been reported to complex ammoniums in water, but none of these show any selectivity towards primary ammonium ions. Calix[5]arenes with sulfonate groups on the large rim have been reported to bind the protonated side chain of the amino acid lysine, but with an affinity two orders of magnitude lower than for quaternary ammoniums.[27–30] Cucurbiturils have been shown to bind various guests among which primary ammoniums.[31–34] Examples of pillararenes (bearing phosphate groups)[35] and cyclophanes (with carboxylates)[36,37] that complex various amino acids, either at the level of their ammonium head groups or of their charged side-chain, have also been reported.

Another strategy, extensively used for the development of chemosensors for analytes in water, consists in the grafting of the receptor onto surfaces or nanoparticles.[38,39] Here too synthetic modifications must be made to ensure efficient grafting onto the chosen surface. Systems which exhibit selectivity for primary ammoniums in water have however, to the best of our knowledge, never been reported. Sulfonatocalix[4]arene modified AuNPs have been reported to aggregate in the presence of lysine, arginine and histidine, via a "sandwich type recognition" with the sulfonate groups interacting with the cationic side chain and ammonium head group of the amino acids.[39] A similar sandwich type recognition and colorimetric detection of diammoniumbenzene isomers by sulfonatocalix[6]arene-modified AuNPs has also been reported.[40] To be noted, the grafting of calix[4]arenes on AuNPs has been reported for the recognition of quaternary ammoniums in organic solvents.[41]

A third strategy, and the one that is investigated in this paper, is the incorporation of the receptors into micelles. It is a straightforward approach, easy to set up experimentally and does not require any synthetic modification of the apolar receptor. Several examples of receptors incorporated in micelles have been reported in the literature, including macrocyclic systems such as calix[6]arenes and resorcinarenes. These systems have shown the ability to bind neutral[42] but also cationic[43–48] and anionic molecules[49]. In certain cases, their binding properties have been shown to be enhanced by the micellar environment in comparison to the water-soluble equivalent. To the best of our knowledge, the only example of recognition of primary ammoniums in micellar systems is the work of Rebek et al.[50] They observed that the conformational reorganization of a water-soluble resorcinarene cavitand when incorporated

into dodecylphosphocholine (DPC) micelles, significantly increased its binding capacity towards different neutral or charged guests, including primary ammoniums.

The binding properties of homooxacalix[3]arenes towards primary ammoniums have never been studied in an aqueous environment. The only report on oxacalix[3]arenes solubilized in water concerns systems functionalized on the small rim with hydrophilic moieties used to transfer fullerenes into water for the photocleavage of DNA.[51,52] Regarding the grafting on surfaces, only two examples of the immobilization of homotrioxacalix[3]arenes have been reported. In both cases, the receptors were adsorbed on gold electrodes through ionic interactions and the receptors were used for the complexation of fullerenes.[53,54]

In this paper, we report the results of our investigation, using ^{1}H NMR, of the binding properties of homooxacalix[3]arene **1** in water. It possesses deprotonable carboxyl groups and we investigate, after studying its properties in organic solvents, two strategies to study it in water: its direct solubilization in water and micellar incorporation.

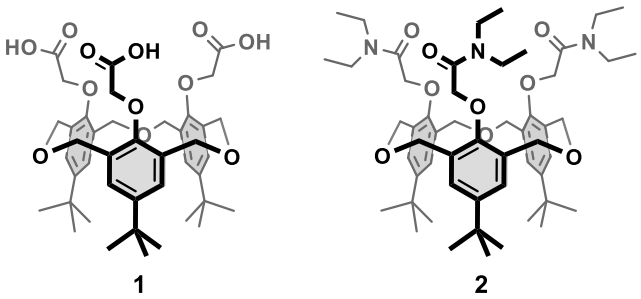


Figure 1. Structure of hexahomotrioxacalix[3]arene-based receptors **1** and **2**.

Results and discussion

Binding properties of 1 towards ammonium ions in organic solvents. The binding properties of homooxacalix[3]arene **1** were studied in organic solvents by ^{1}H NMR. A preliminary study was conducted with hexylammonium picrate ($HexNH_3^+Pic^-$) as the complexation of this guest has already been the object of intense studies with receptor **2**.[20] Receptor **1** (1 mM) was dissolved in a mixture of $CDCl_3/CD_3OD$ (4:1) and aliquots of $HexNH_3^+Pic^-$ were added. New signals for the host-guest complex **1**⊃$HexNH_3^+$ were detected, concomitantly to those of the free receptor and free guest, signature of a slow host-guest exchange process on the NMR chemical shift timescale. The complex was obtained quantitatively after the addition of 1.2 equiv. of the ammonium salt as signals of the free host were no longer visible (Figure 2a vs. 2b), confirming a high binding affinity (log K_a = 4.7 ± 0.2). The ^{1}H signals of the included guest were assigned by recording a COSY NMR spectrum.[55] The intra-cavity complexation of the

ammonium cation is clearly evidenced by the presence of signals in the high field region of the ^{1}H NMR spectrum (< 1 ppm). The downfield shift of Ar*H* and *t*Bu protons of the receptor and the increase in the difference of the chemical shifts of the axial and equatorial ArC*H* protons (from 0.43 ppm to 0.98 ppm) are indicative of a conformational change of the receptor upon complexation when a more open cone conformation is adopted with the oxygen atoms of the bridges directed toward the inside of the cavity (see the structure displayed in Figure 2b). The largest complexation induced shifts (CISs) are observed for the protons in the α-position (-2.72 ppm) and β-position (-1.93 ppm) of the ammonium head, indicating that they are located deep inside the polyaromatic cavity. All these results are comparable to what has been observed with homooxacalix[3]arene **2**.

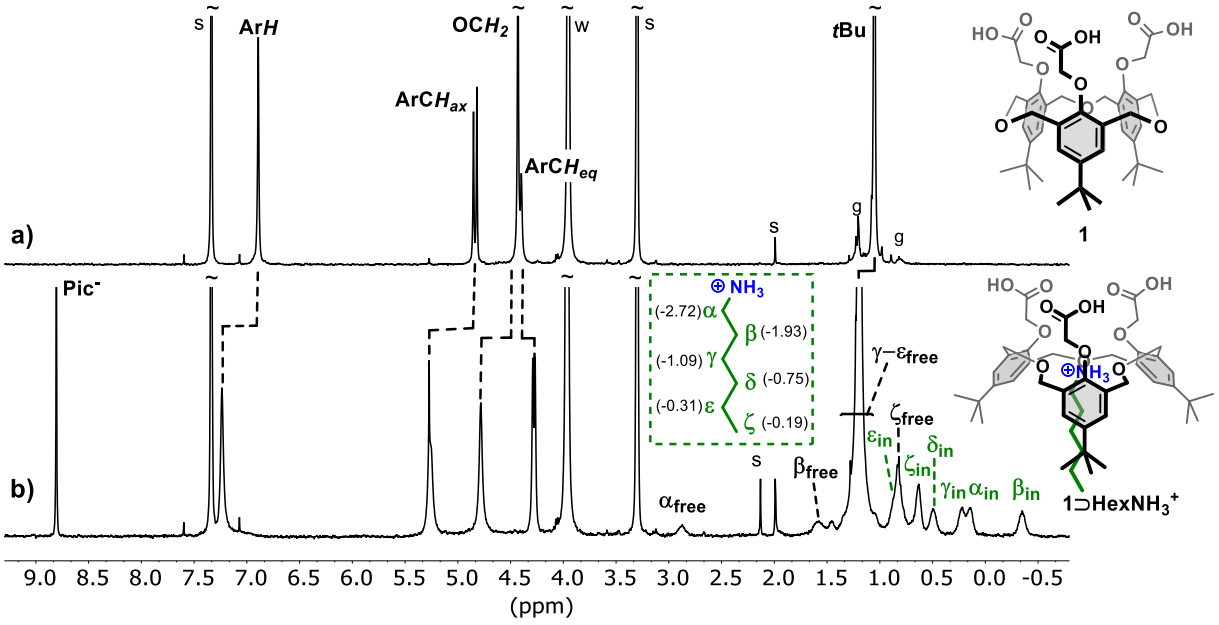


Figure 2. ^{1}H NMR spectra (298 K, 400 MHz) in a 4:1 $CDCl_3/CD_3OD$ solution of a) **1** (1 mM); b) **1** + 1.2 equiv. of $HexNH_3^+Pic^-$. W: water, s: residual solvents, g: grease. Inset: CISs values.

The recognition mode was further investigated by molecular mechanics conformational analysis. The energy minimized structure of the complex **1**⊃$HexNH_3^+$ (Figure 3) is consistent with the information gained by NMR. The host displays an open cone conformation and the ethereal oxygen atoms are pointing towards the center of the cavity. The ammonium head is positioned at the center of the ethereal macrocycle with the nitrogen atom located close to the plane defined by the three oxygen atoms (O1, O2 and O3) of the macrocycle (distance from the centroid defined by the oxygen atoms: 0.213 Å). The complex exhibits a three-point H-bonding complementarity between the NH_3^+ head of the guest and the ethereal oxygens (N-O distances

< 2.99 Å, 136.1°< N-H-O angles < 164.1°).The cation is further stabilized by a fourth H-bond with one of the phenoxy oxygens (O4), ion−dipole interactions with the phenoxy oxygen atoms (N−OAr distances < 2.87 Å), cation−π interaction (N−Ar distances < 3.93 Å) and CH−π interactions with the aromatic walls of the cavity.

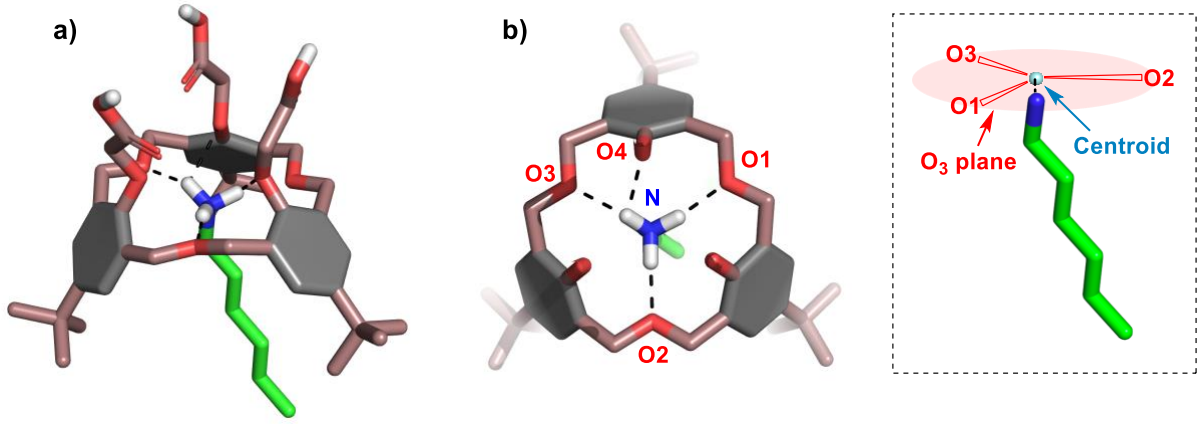


Figure 3. Energy minimized structure of the complex **1**⊃$HexNH_3^+$: a) Side view; b) Truncated top view highlighting the recognition mode. H-bonds are indicated by dashed lines. Inset: schematic representation of the three ethereal oxygen atoms centroid displayed as a blue sphere. Selected distances (Å): 0.213 (centroid-N), 2.87 (O4-N), 2.99 (O3-N), 2.86 (O2-N), 2.91 (O1-N). Selected angles (°): 117.7 (N-H-O4), 136.1 (N-H-O3), 164,1 (N-H-O2), and 159.6 (N-H-O1). Except for the polar H, all the hydrogen atoms of **1** and $HexNH_3^+$ are omitted for clarity.

The binding of several other primary, secondary and tertiary ammonium cations was investigated by ^{1}H NMR in $CDCl_3/CD_3OD$ 4:1 (Figure 4). The bulky *t*-butylammonium,1-adamantylammonium, anilinium and benzylammonium ions were recognized in a similar manner to $HexNH_3^+$, as confirmed by the significant CISs of their α and β protons (Figure 4).[55] The binding constants for these primary ammonium ions, determined by signal integration, are also very similar (see Figure 4) despite the differences in their shape. This can be explained by the high flexibility of the homooxacalix[3]arene host that can adapt the opening of its cavity to the shape of the guest. The nature of the counter-anion (picrate or chloride) has little influence on the binding properties (see $HexNH_3^+Pic^-$ vs. $HexNH_3^+Cl$, Figure 4), in accordance with the high stabilization of anions in protic environments. It is noteworthy to mention that quasi-identical binding constants were reported for host **2** towards these three ammonium ions (log K_a = 4.7 ± 0.2 for $HexNH_3^+Pic^-$, 4.3 ± 0.2 for $HexNH_3^+Cl^-$, 4.2 ± 0.1 for *t*-$BuNH_3^+$ Pic^-), confirming that the substituents on the small rim are not involved in the recognition of the ammonium ion.

No complexation of small secondary or tertiary ammonium ions was detected even in the presence of a large excess of these cations (i.e. 21 equiv. for $Et_2NH_2^+Pic^-$ and 20 equiv. for $Me_3NH^+Pic^-$).[55] As mentioned in the introduction, such a remarkable specificity for primary ammonium ions was also observed in the case of **2** and was ascribed to the fact that only primary ammonium ions can be inserted deeply enough into the cavity to establish H-bonding interactions with the ethereal macrocycle.

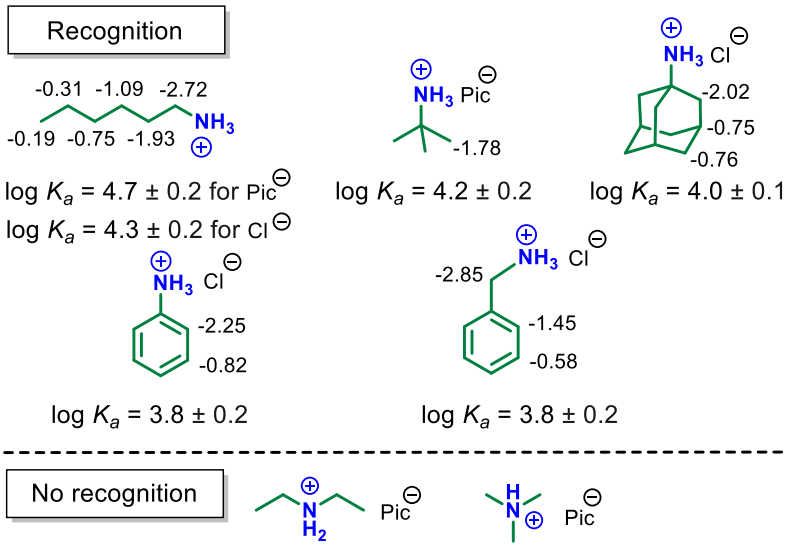


Figure 4. Association constants for host **1** with various ammonium ions in $CDCl_3/CD_3OD$ 4:1. The values displayed on the structures correspond to the CISs determined by ^1H NMR.

The impact of the protonation state of host **1** on its binding properties was further investigated in $CDCl_3/CD_3OD$ 1:2 at 263K.[56] The deprotonation of **1** was monitored by following the shift of the C*H*$_2$COO protons signals upon the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU): from 4.56 ppm to 4.25 ppm after the addition of ~ 3 equiv. of DBU. Very similar affinity constants of *ca.* log K_a = 3.4 ± 0.1 and log K_a = 3.1 ± 0.1 were determined for *t*-$BuNH_3^+Pic^-$ in the absence and in the presence of ca. ~3 equiv. of DBU, respectively.[55] The slightly lower affinity observed when the receptor is deprotonated could be due to the competing exo-coordination of the cationic guest at the level of the negatively charged carboxylates. Again, this result shows that the recognition properties of the receptor are poorly dependent on the nature of the functional groups on the small rim.

Binding properties of 1 toward ammonium ions in pure water. Having demonstrated that host **1** displays similar binding properties as **2** in organic solvents, we moved to its study in water. Receptor **1** was successfully solubilized in D_2O following the addition of diethylamine

whose corresponding ammonium form is not complexed by the receptor (Figure 5a). The receptor needs to be fully deprotonated to be solubilized as 3 equiv. of Et_2NH were necessary to achieve complete dissolution.[55] Upon the addition of $HexNH_3^+Cl^-$, no recognition was evidenced. When $HexNH_2$ was used as base to solubilize the receptor, the complexation of $HexNH_3^+$ was also not evidenced.[55] The strong hydrophilicity of the ammonium head clearly precludes the binding of the guest in pure water.

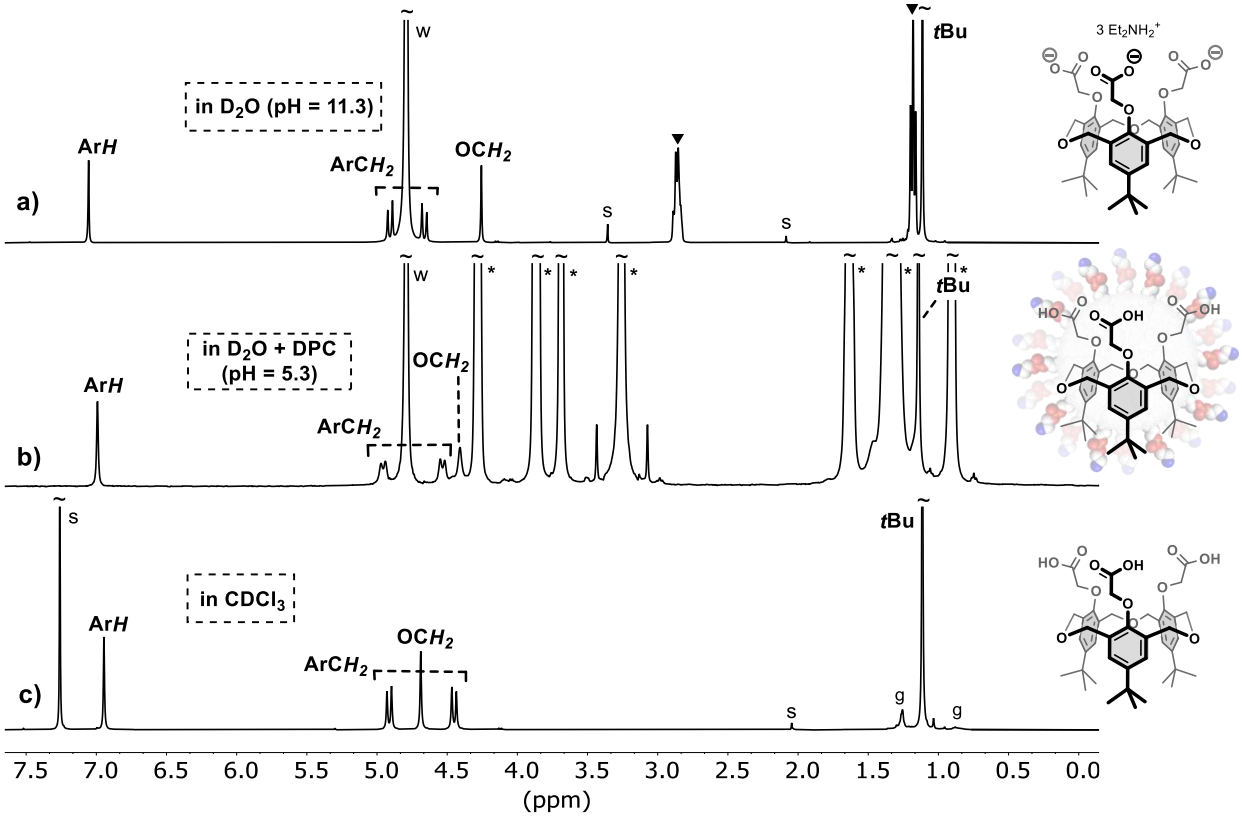


Figure 5. ^1H NMR (298 K, 400 MHz) of a) **1** (~1.5 mM) + Et_2NH (~7 equiv.) in D_2O at pH = 11.3; b) **1** (~0.3 mM) in DPC (20 mM in D_2O at pH = 5.3); c) **1** in $CDCl_3$. w: water, s: residual solvents, g: grease, *: DPC signals, ▼: $Et_2NH/Et_2NH_2^+$ signals.

Binding properties of 1 toward ammonium ions in micellar systems. The second strategy investigated to transfer receptor **1** into water was its incorporation into surfactant micelles. **1** was successfully incorporated into dodecylphosphocholine (DPC) micelles by simple mixing in D_2O at rt. The physicochemical properties of DPC micelles, which have already been used to transfer receptors into an aqueous environment,[42,44,49,50] are well documented in the literature.[57–60] Receptor signals were clearly visible in the ^1H NMR spectra of the micellar solutions, even at pH where the receptor is not soluble in water (i.e. pH < 6), corroborating its successful incorporation.[55] Comparison of the ^1H NMR spectra of **1** in DPC micelles (Figure

5b), in D_2O (Figure 5a) and in $CDCl_3$ (Figure 5c) shows that the receptor adopts a similar C_{3V} symmetrical cone conformation in all three environments (one singlet for Ar*H*, 1 singlet for C*H*$_2$COO, 2 doublets for ArC*H*$_2$ and 1 singlet for *t*Bu protons). Inclusion in DPC micelles was further confirmed by DOSY experiments.[55] The diffusion coefficient of receptor **1** in the DPC solutions, at pH 2.53 and at pH 8.55, ($D_{\mathbf{1}} \approx 0.88 \pm 0.1 \times 10^{-10}$ m^2/s at both pH) was observed to be significantly smaller than the one determined for **1** solubilized in D_2O at pH 12.7 ($D_{\mathbf{1}} \approx 2.9 \times 10^{-10}$ m^2/s). To be noted, the diffusion coefficient of the surfactant ($D_{DPC} \approx 1.08 \pm 0.02 \times 10^{-10}$ m^2/s) is slightly larger than the one of the incorporated receptor as it corresponds to the weighted average of the diffusion coefficients of the aggregated surfactant and of its free monomeric form (CMC of 1.5 mM; $D_{DPC\_monomer} = 4.3 \pm 0.1 \times 10^{-10}$ m^2/s). The chemical shift of the C*H*$_2$COO protons of incorporated **1** changed when increasing the pH (from 4.53 ppm at pH = 2.5 to 4.26 ppm at pH = 7.6), in agreement with the deprotonation of the receptor.[55] PRE experiments,[42,61] using potassium hexacyanochromate as paramagnetic agent, showed that receptor **1** is deeply incorporated into the DPC micellar core both at pH = 8.5 and pH = 2.9.[55]
The binding of $HexNH_3^+$ by **1** incorporated in DPC micelles was investigated by ^{1}H NMR (Figure 6). A receptor concentration of *ca.* 0.35 mM was used, which corresponds to one receptor per micelle.[62] To our delight, when the receptor was deprotonated (pH 7.5), the addition of $HexNH_3^+Cl^-$ led to the formation of complex **1**⊃$HexNH_3^+$ (Figures 6b).[55] As in organic solvents, guest exchange was slow on the chemical shift timescale. The amplitude of the downfield shift of the Ar*H* protons and the CISs measured were similar to those observed in $CDCl_3/CD_3OD$ 4:1, implying the same recognition mode. Surprisingly, when the solution was acidified to a pH at which the receptor is fully protonated (pH = 2.5) no inclusion was evidenced, even in the presence of a large excess of $HexNH_3^+Cl^-$ (12 equiv.).[55] DOSY experiments confirmed however that $HexNH_3^+Cl^-$ partitions into the micelles both at low and high pH.[55] The complexation of $HexNH_3^+$ was however observed, both at pH 7.5 and pH 2.5, when $HexNH_3^+Pic^-$ was added instead of the corresponding chloride salt.[55] The subsequent addition of picrate anion (in the form of $Me_3NH^+Pic^-$) to the solution containing the 12 equiv. of $HexNH_3^+Cl^-$ interestingly also gave rise to the formation of **1**⊃$HexNH_3^+$. Such a counter-ion effect was not observed in organic solvents (i.e. $CDCl_3/CD_3OD$ 4:1). These results can be rationalized based on the nature of the medium (Figure 7):

- in the protic organic environment, anions are stabilized by H-bonds and ion pairs are well separated. The nature of the counter-anion has thus almost no impact on the binding of ammonium ions (Figure 7a);

- in the apolar core of micelles, at pH = 2.5, the binding of the ammonium ion is effective only when a low coordinating anion such as Pic^- is present in the medium (Figure 7b).[63] The energy penalty for the dissociation of the ammonium chloride salt in the apolar micellar environment is indeed too high to enable the intracavity complexation of the cation (Figure 7c);[64]
- in micelles, at pH = 7.5, the receptor is fully deprotonated and can thus play the role of counter-anion and the counter-anion of the ammonium salt has little influence on the host-guest process (Figure 7d).

To our knowledge, such considerations on the impact of a counter-ion on the recognition properties of a synthetic receptor incorporated in micelles have never been reported in the literature.

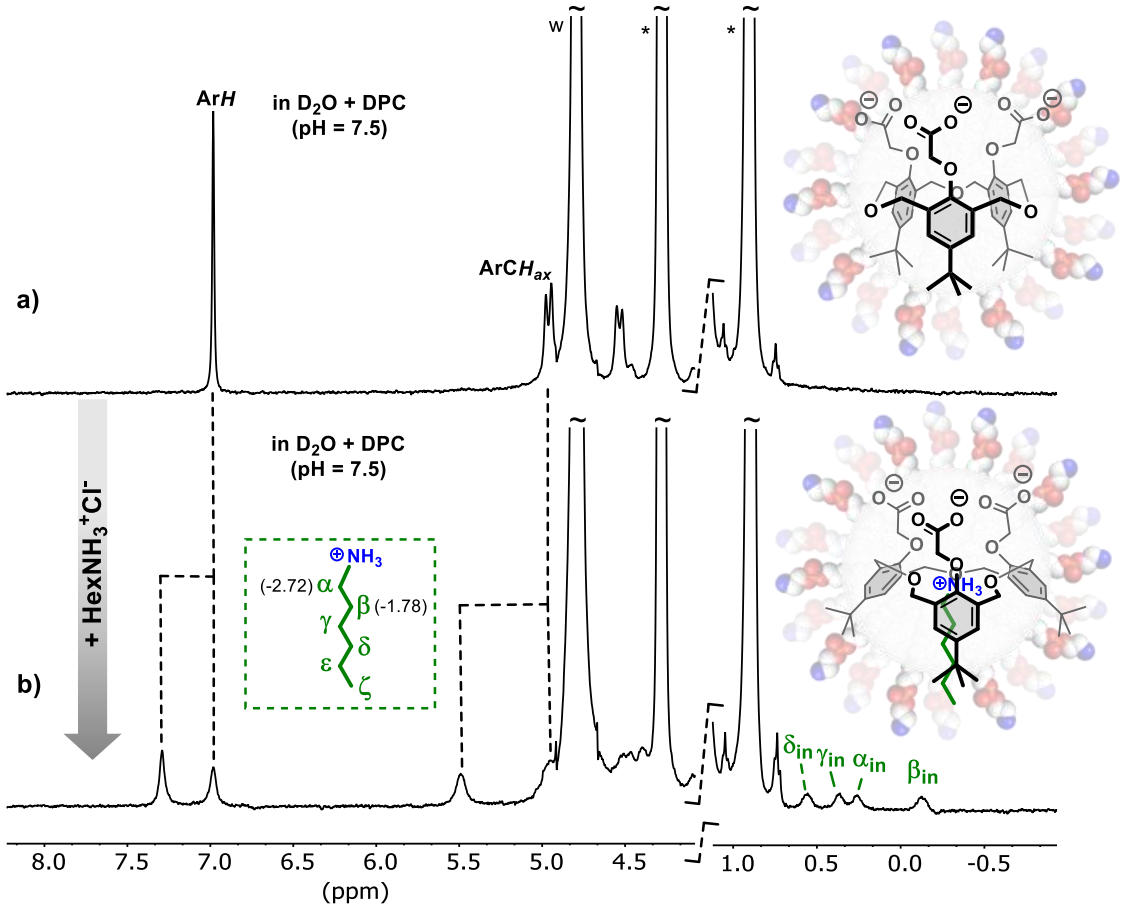


Figure 6. ^{1}H NMR spectra (298 K, 400 MHz) of a) **1** (0.35 mM) in DPC (20 mM in D_2O at pH = 7.5); b) after addition of *ca.* 6 equiv. of $HexNH_3^+Cl^-$. *: surfactant signals, w: water signal.

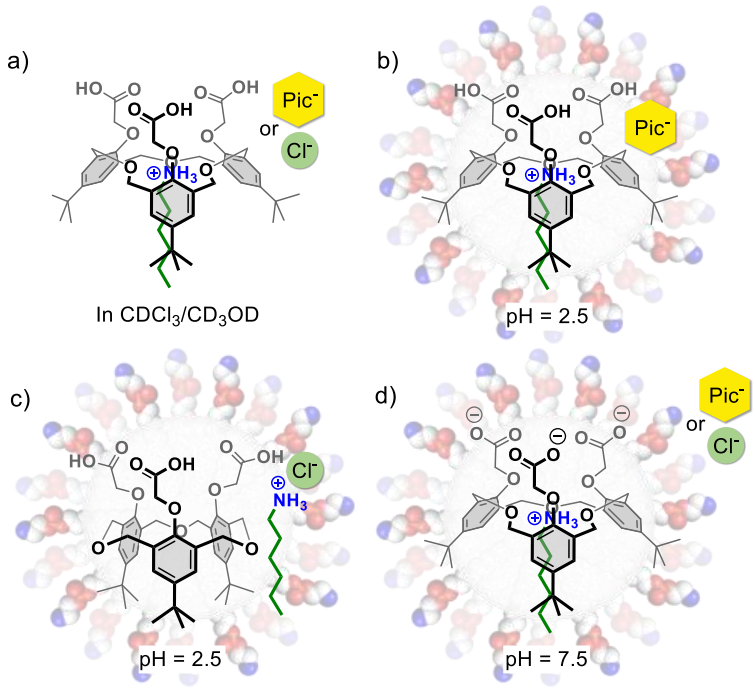


Figure 7. Rationalization of the host guest properties of **1** toward $HexNH_3^+$ in different environments depending on the counter anion (Pic^- or Cl^-).

In a second set of experiments, the binding of different primary ammonium ions by deprotonated **1** (0.35 mM) incorporated in DPC micelles (DPC 20 mM; 10 mM HEPES, pH *ca.* 7.8) was monitored by ^1H NMR (Table 1). In all cases, formation of the inclusion complexes was evidenced, as in $CDCl_3/CD_3OD$ 4:1.[55] The inclusion complexes were observed more readily with the more apolar ammoniums (cLogP > 2; $AdamNH_3^+Cl^-$, $HexNH_3^+Cl^-$ and $DodNH_3^+$). For the more polar guests (cLogP < 1; $PrNH_3^+Cl^-$, *t*-$BuNH_3^+Cl^-$ and *n*-$BuNH_3^+Cl^-$), a significantly larger number of equivalents of the ammonium salt had to be added to observe the signature of the inclusion complex. Signal integration in the presence of ~ 6 equiv. of $AdamNH_3^+Cl^-$ suggested a log K_a of 2.6 ± 0.1 M^{-1} but this value dropped to 2.3 ± 0.1 M^{-1} in the presence of ~30 equiv. of guest (Table 1).[55] This decrease in the measured affinity constant at higher concentrations is due to the saturation of the micelles, which was confirmed with DOSY experiments performed with DPC solutions containing different $AdamNH_3^+Cl^-$ concentrations.[55] To obtain the correct affinity constants it would consequently be necessary to work at very low guest concentrations, conditions which did not allow us to obtain quantitative results (poor S/N ratio). The proportion of **1** with a guest in its cavity, at a given guest concentration, is also given in table 1 and analysis of this data clearly highlights the beneficial influence of the hydrophobic effect: a smaller proportion is observed with the more hydrophilic guests ($PrNH_3^+$, *t*-$BuNH_3^+$ and *n*-$BuNH_3^+$). As mentioned in the introduction, the only example

of a primary ammonium complexed by a host in micellar environment was reported by Rebek et al. who determined a K_a = 11 M^{-1} for the complexation of adamantan-1-methanaminium by a resorcinarene in DPC micelles, a value much lower than the ones obtained in our system for similar primary ammoniums.

Table 1. Measured association constants (K_{mes}) for host **1** with ammonium chloride salts measured by ^{1}H NMR spectroscopy in 20 mM DPC, 10 mM HEPES (pH ca. 7.8) in D_2O at 298 K; [**1**] = 0.35 mM.[65]

Guest (Cl^- salt)	*Equiv.*	*Log K_{mes}*	*$[1 \supset G]/[1_{tot}]$*	*cLogP*
$PrNH_3^+$	38	1.3 ± 0.1	22	0.43
$t\text{-}BuNH_3^+$	35	1.3 ± 0.1	19	0.61
$n\text{-}BuNH_3^+$	27	1.7 ± 0.1	31	0.96
$HexNH_3^+$	6	2.6 ± 0.1	49	2.02
$AdamNH_3^+$	6	2.6 ± 0.1	51	2.03
	30	2.3 ± 0.1	62	
$DodNH_3^+$	1.4	3.4 ± 0.1	42	5.19
$H_3N^+(CH_2)_8NH_3^+$	25	<1	15	1.23
$H_3N^+(CH_2)_{12}NH_3^+$	1.5	3.5 ± 0.1	50	3.35

The complexation of apolar bisammonium ions $H_3N^+(CH_2)_nNH_3^+$ (with n = 8 and 12) was also assessed. A weak binding of the shorter more hydrophilic guest was observed (small inclusion signals detected in the presence of 25 equivalents), while the binding of $H_3N^+(CH_2)_{12}NH_3^+$ was favorable, with a log K_{mes} of 3.5 ± 0.1 (for a smaller number of equivalents), a value similar to the one obtained with the more hydrophobic $DodNH_3^+$. This good affinity may be due to the favorable interaction between the second ammonium group and the phosphate of the DPC headgroup.[66] In the case of the shorter diammonium ion, the second ammonium group would be in the hydrophobic core of the micelle, which is not favorable. The complexation of other primary ammoniums was also evaluated: aniline hydrochloride, benzylammonium chloride, dopamine hydrochloride, histamine monohydrochloride and protonated spermine. Even with large excesses of these guests (respectively 75, 42, 30, 55 and 20 equiv.) no recognition was evidenced at pH = 7.6 – 8.1.[67] The hydrophilicity of these guests means that they are not incorporated into the micelles, as confirmed by DOSY experiments for benzylammonium chloride.[55] The selectivity of **1** in DPC micelles for primary ammonium ions was confirmed as no recognition was observed with the relatively apolar $Me_2NH_2^+$,Et_3NH^+ and Me_4N^+ ammoniums (respectively after the addition of 450, 180 and 180 equiv.).[55]

Host **1** was also successfully incorporated in micelles of other surfactants (i.e. TPGS-750-M, Triton-X-100, SB3-14, SDS and CTABr).[55] No recognition of $HexNH_3^+Pic^-$ was observed in SB3-14 and SDS micelles at pH = 4.8.[55] With SDS micelles, this can be explained by the non-specific interactions between the sulfate head of the surfactant and the ammonium ion. With TPGS-750-M or Triton-X-100 recognition was clearly observed at pH = 5.3-5.4 (respectively with $HexNH_3^+Pic^-$ and $AdamNH_3^+Cl^-$) but the broadness and isochrony of the ^1H signals complicated the studies with these systems. Nevertheless, affinity seem similar to those observed in DPC systems.[55] Recognition of $AdamNH_3^+Cl^-$ was qualitatively evidenced in CTABr at pH = 7.0, but the affinity is low as only 40% of the host was complexed in the presence of a large excess of the ammonium ion (175 equiv.).[55] This low affinity can be rationalized by the electrostatic repulsion between the positively charged head groups of the guest and of the surfactant.[68]

Conclusion

In summary, the binding properties of homooxacalix[3]arene receptor **1** were studied in organic solvents and in water via ^1H NMR spectroscopy. The receptor is, similarly to what we recently observed with a parent receptor,[20] selective for primary ammoniums in organic solvents with. affinity constants up to log K_a = 4.7 ± 0.2 for $HexNH_3^+$ in $CDCl_3/CD_3OD$ 4:1. An *in-silico* study showed that the primary ammonium is deeply inserted into the polyaromatic cavity with the NH_3^+ group almost in the plane defined by the 18-crown-3 moiety. The protonation state of the carboxyl groups on the small rim of **1** does not show a significant impact upon the binding properties in organic solvents, indicating that the substituents on the small rim are not involved in the recognition process.

Receptor **1** was transferred into water following two strategies: 1) direct solubilization through deprotonation of the carboxyl groups and 2) incorporation into micelles. The first strategy shows its limits as no recognition could be evidenced, while in micellar systems, of different surfactants, recognition was observed. Extensive studies were undertaken with the receptor incorporated in DPC micelles. DOSY and PRE experiments confirmed that **1** is located deep in the micellar core, even at pH where it is soluble in water. 1D ^1H NMR studies confirmed that the encapsulated receptor is able to host selectively primary ammoniums, such as $HexNH_3^+$ or $AdamNH_3^+$, which is remarkable considering that primary ammoniums are more hydrophilic than secondary or tertiary ones. The hydrophobic effect does however play an important role in the recognition abilities as the affinity increased by two orders of magnitude from $PrNH_3^+$ to $DodNH_3^+$.

Ammonium recognition by receptor **1** was seen to be counter-ion dependent. With the low coordinating picrate counter anion, recognition was observed whatever the protonation state of the receptor, while with the chloride salt complexation was only observed when **1** is deprotonated, due to the energy penalty for the dissociation of ammonium and chloride in the apolar micellar core. To the best of our knowledge, this behavior has never been described in micellar system.

In conclusion, this study shows that the straightforward encapsulation of artificial organo-soluble receptors in micelles is an efficient strategy to develop water soluble nanosized supramolecular recognition systems. Future work will be directed toward the incorporation of fluorescent receptors in micelles to sense biologically relevant molecules in water.

Experimental Section

General Information. ^{1}H NMR spectra were recorded either on a Jeol 600 MHz spectrometer or on a Varian 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. The NMR spectra were recorded at 298 K unless otherwise stated. Chemical shifts are expressed in ppm. Compound **1** was synthesized as previously described.[69,70]

Procedure to determine affinity constants in organic solvents. Receptor **1** was dissolved in the selected mixture of organic solvents at the desired concentration (typically 1 mM). Stock solutions of the guests were prepared and contained receptor **1,** so as to avoid host dilution during the titrations. Aliquots of these stock solutions were added stepwise to the 600 µL host solution in the NMR tube. Slow host-guest exchange on the NMR chemical shift timescale was evidenced each time and the association constants were determined via integration of the signals of interest.[55]

Micellar preparation. Micellar solutions were prepared by weighting the surfactant and adding D_2O. For receptor incorporation, the solid receptor was weighted (typically 1-2 mg) and the necessary amount of micellar solution was added to reach the desired concentration. The solution was placed under magnetic stirring for at least 6 hours and until a clear solution was observed. The exact concentration of the receptor in the different micellar solutions was determined using an external reference (^{1}H,^{1}H,^{11}H,^{11}H-Perfluoro-3,6,9-trioxaundecane-1,11-diol) of known concentration. When buffered conditions were required, HEPES was added with surfactants and D_2O was added further with further addition of NaOD to reach the desired pH.

Procedure to determine affinity constants in micellar systems. Concentrated solutions of the different guests (in a range of 10-100 mM, depending on the affinity) were prepared with

micellar solutions containing receptor **1** to avoid any dilution effect during the titration. Aliquots of these solution were added stepwise to the 600 µl solution in the NMR. Slow host-guest exchange on the NMR chemical shift timescale was evidenced each time and the association constants were determined via integration of the signals of interest.[55]

DOSY experiments were performed at 298K on the Jeol 600 MHz spectrometer using the BPPDSTE (bipolar pulse pair double stimulated sequence). The diffusion delay was set to 100 ms and the gradient time length to 5 ms. The acquisition time and relaxation delay were chosen to ensure complete relaxation.

The magnitude of the gradient pulses was optimized for each sample so that the final gradient intensity gave rise to a signal which is between 10-20 % of initial signal (gradients ranging between 30 mT/s and 600 mT/s). At least 15 gradient values were used for each experiment.

PRE measurements. For the PRE experiments, aliquots of 5 µl of 5 and 10 mM solutions of $K_3[Cr(CN)_6]$ in D_2O were added to 600 µl of the micellar solution under study. T_1 measurements were undertaken using the classical inversion recovery (180 – τ – 90 – acquisition) sequence, with 20 points and the delay varying between 0 and 6 s. For each signal monitored, the increase in the longitudinal relaxation rate induced by the paramagnetic species (the difference between the longitudinal relaxation rate measured in the presence and in the absence of the paramagnetic species) was plotted as a function of the concentration of paramagnetic species. A linear regression was undertaken with the experimental data points, to obtain the relaxivity parameter (slope of the line).

Molecular Modeling. Monte Carlo multiple minimum (MCMM)[71] conformational searches (100 steps per torsion angle, maximum 1000 steps in total) were performed in Schrödinger Release 2019-1, using the OPLS3e force field[72] with $CHCl_3$ or H_2O as selected solvent in Maestro MacroModel (version 11.9.011).

Supporting information

Additional experimental details and NMR spectra (^1H, COSY, DOSY), graphs and tables obtained from PRE and DOSY experiments are available in the supporting information.

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References

(1) Finlay, J. A.; Callow, M. E. The Toxicity of Alkyl Amines: The Effects of pH. *Biofouling* **1997**, *11* (1), 19–30.

(2) Bühlmann, P.; Pretsch, E.; Bakker, E. Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 2. Ionophores for Potentiometric and Optical Sensors. *Chem. Rev.* **1998**, *98* (4), 1593–1688.

(3) De Leener, G.; Evoung-Evoung, F.; Lascaux, A.; Mertens, J.; Porras-Gutierrez, A. G.; Le Poul, N.; Lagrost, C.; Over, D.; Leroux, Y. R.; Reniers, F.; Hapiot, P.; Le Mest, Y.; Jabin, I.; Reinaud, O. Immobilization of Monolayers Incorporating Cu Funnel Complexes onto Gold Electrodes. Application to the Selective Electrochemical Recognition of Primary Alkylamines in Water. *J. Am. Chem. Soc.* **2016**, *138* (39), 12841–12853.

(4) Inthasot, A.; Le Poul, N.; Luhmer, M.; Colasson, B.; Jabin, I.; Reinaud, O. Selective EPR Detection of Primary Amines in Water with a Calix[6]Azacryptand-Based Copper(II) Funnel Complex. *Inorg. Chem.* **2018**, *57* (7), 3646–3655.

(5) Späth, A.; König, B. Molecular Recognition of Organic Ammonium Ions in Solution Using Synthetic Receptors. *Beilstein J. Org. Chem.* **2010**, *6*, N°32.

(6) Salorinne, K.; Nissinen, M. Calixcrowns: Synthesis and Properties. *J Incl Phenom Macrocycl Chem* **2008**, *61* (1–2), 11–27.

(7) Katsu, T.; Ido, K. Ethylammonium-Selective Membrane Electrode Using *p-tert*-Butylcalix[6]Arene Derivatives. *Anal. Sci.* **2002**, *18* (4), 473–476.

(8) Biros, S. M.; Rebek, J. Jr. Structure and Binding Properties of Water-Soluble Cavitands and Capsules. *Chem. Soc. Rev.* **2007**, *36* (1), 93–104.

(9) Zhang, D.; Martinez, A.; Dutasta, J.-P. Emergence of Hemicryptophanes: From Synthesis to Applications for Recognition, Molecular Machines, and Supramolecular Catalysis. *Chem. Rev.* **2017**, *117* (6), 4900–4942.

(10) Xue, M.; Yang, Y.; Chi, X.; Zhang, Z.; Huang, F. Pillararenes, A New Class of Macrocycles for Supramolecular Chemistry. *Acc. Chem. Res.* **2012**, *45* (8), 1294–1308.

(11) Ogoshi, T.; Nishida, Y.; Yamagishi, T.; Nakamoto, Y. High Yield Synthesis of Polyrotaxane Constructed from Pillar[5]arene and Viologen Polymer and Stabilization of Its Radical Cation. *Macromolecules* **2010**, *43* (17), 7068–7072.

(12) He, Q.; Vargas-Zúñiga, G. I.; Kim, S. H.; Kim, S. K.; Sessler, J. L. Macrocycles as Ion Pair Receptors. *Chem. Rev.* **2019**, *119* (17), 9753–9835.

(13) Gaeta, C.; Troisi, F.; Neri, P. *Endo*-Cavity Complexation and Through-the-Annulus Threading of Large Calixarenes Induced by Very Loose Alkylammonium Ion Pairs. *Org. Lett.* **2010**, *12* (9), 2092–2095.

(14) Arduini, A.; Bussolati, R.; Credi, A.; Secchi, A.; Silvi, S.; Semeraro, M.; Venturi, M. Toward Directionally Controlled Molecular Motions and Kinetic Intra- and Intermolecular Self-Sorting: Threading Processes of Nonsymmetric Wheel and Axle Components. *J. Am. Chem. Soc.* **2013**, *135* (26), 9924–9930.

(15) Brancatelli, G.; Gattuso, G.; Geremia, S.; Manganaro, N.; Notti, A.; Pappalardo, S.; Parisi, M. F.; Pisagatti, I. α,ω-Alkanediyldiammonium Dications Sealed within Calix[5]arene Capsules with a Hydrophobic Bayonet-Mount Fastening. *CrystEngComm* **2015**, *17* (41), 7915–7921.

(16) Brunetti, E.; Picron, J.-F.; Flidrova, K.; Bruylants, G.; Bartik, K.; Jabin, I. Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity. *J. Org. Chem.* **2014**, *79* (13), 6179–6188.

(17) Nyssen, N.; Ajami, D.; Ardelean, A.; Desroches, F.; Li, J.; Luhmer, M.; Reinaud, O.; Jabin, I. Closing a Calix[6]arene-Based Funnel Zn^{2+} Complex at Its Large Rim

Entrance: Consequences on Metal Ion Affinity and Host–Guest Properties. *J. Org. Chem.* **2021**, *86* (17), 12075–12083.
(18) Darbost, U.; Giorgi, M.; Reinaud, O.; Jabin, I. A Novel C_{3v}-Symmetrical Calix[6](aza)cryptand with a Remarkably High and Selective Affinity for Small Ammoniums. *J. Org. Chem.* **2004**, *69* (15), 4879–4884.
(19) Darbost, U.; Rager, M.-N.; Petit, S.; Jabin, I.; Reinaud, O. Polarizing a Hydrophobic Cavity for the Efficient Binding of Organic Guests: The Case of Calix[6]tren, a Highly Efficient and Versatile Receptor for Neutral or Cationic Species. *J. Am. Chem. Soc.* **2005**, *127* (23), 8517–8525.
(20) Lambert, S.; Bartik, K.; Jabin, I. Specific Binding of Primary Ammonium Ions and Lysine-Containing Peptides in Protic Solvents by Hexahomotrioxacalix[3]arenes. *J. Org. Chem.* **2020**, *85* (15), 10062–10071.
(21) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. Supramolecular Chemistry in Water. *Angew. Chem. Int. Ed.* **2007**, *46* (14), 2366–2393.
(22) Cremer, P. S.; Flood, A. H.; Gibb, B. C.; Mobley, D. L. Collaborative Routes to Clarifying the Murky Waters of Aqueous Supramolecular Chemistry. *Nature Chem* **2018**, *10* (1), 8–16.
(23) Biedermann, F.; Nau, W. M.; Schneider, H.-J. The Hydrophobic Effect Revisited-Studies with Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force. *Angew. Chem. Int. Ed.* **2014**, *53* (42), 11158–11171.
(24) Escobar, L.; Ballester, P. Molecular Recognition in Water Using Macrocyclic Synthetic Receptors. *Chem. Rev.* **2021**, *121* (4), 2445–2514.
(25) Leoni, L.; Dalla Cort, A.; Biedermann, F.; Kubik, S. Ion Receptors. In *Supramolecular Chemistry in Water*; Kubik, S., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2019; pp 193–247.
(26) Liu, Z.; Dai, X.; Sun, Y.; Liu, Y. Organic Supramolecular Aggregates Based on Water-soluble Cyclodextrins and Calixarenes. *Aggregate* **2020**, *1* (1), 31–44.
(27) Arena, G.; Casnati, A.; Contino, A.; Lombardo, G. G.; Sciotto, D.; Ungaro, R. Water-Soluble Calixarene Hosts that Specifically Recognize the Trimethylammonium Group or the Benzene Ring of Aromatic Ammonium Cations: A Combined 1H NMR, Calorimetric, and Molecular Mechanics Investigation. *Chem. Eur. J.* **1999**, *5* (2), 738–744.
(28) Arena, G.; Gentile, S.; Gulino, F. G.; Sciotto, D.; Sgarlata, C. Water-Soluble Pentasulfonatocalix[5]arene: Selective Recognition of Ditopic Trimethylammonium Cations by a Triple Non-Covalent Interaction. *Tetrahedron Letters* **2004**, *45* (38), 7091–7094.
(29) Douteau-Guével, N.; Perret, F.; Coleman, A. W.; Morel, J.-P.; Morel-Desrosiers, N. Binding of Dipeptides and Tripeptides Containing Lysine or Arginine by *p*-Sulfonatocalixarenes in Water: NMR and Microcalorimetric Studies *J. Chem. Soc., Perkin Trans. 2* **2002**, No. 3, 524–532.
(30) Bonaccorso, C.; Gentile, S.; Gulino, F. G.; Sciotto, D. Molecular Recognition of Alkylammonium, N-Methylpyridinium Cations and Native L-α-Amino Acids by Water Soluble Penta- and Tetra- Sulfonatocalixarenes. *Lett. Org. Chem.* **2009**, *6*, 598–603.
(31) Kim, Y.; Kim, H.; Ko, Y. H.; Selvapalam, N.; Rekharsky, M. V.; Inoue, Y.; Kim, K. Complexation of Aliphatic Ammonium Ions with a Water-Soluble Cucurbit[6]u ril Derivative in Pure Water: Isothermal Calorimetric, NMR, and X-Ray Crystallographic Study. *Chem. Eur. J.* **2009**, *15* (25), 6143–6151.
(32) Lee, J. W.; Lee, H. H. L.; Ko, Y. H.; Kim, K.; Kim, H. I. Deciphering the Specific High-Affinity Binding of Cucurbit[7]uril to Amino Acids in Water. *J. Phys. Chem. B* **2015**, *119* (13), 4628–4636.

(33) Liu, S.; Ruspic, C.; Mukhopadhyay, P.; Chakrabarti, S.; Zavalij, P. Y.; Isaacs, L. The Cucurbit[*n*]uril Family: Prime Components for Self-Sorting Systems. *J. Am. Chem. Soc.* **2005**, *127* (45), 15959–15967.
(34) Smith, L. C.; Leach, D. G.; Blaylock, B. E.; Ali, O. A.; Urbach, A. R. Sequence-Specific, Nanomolar Peptide Binding via Cucurbit[8]uril-Induced Folding and Inclusion of Neighboring Side Chains. *J. Am. Chem. Soc.* **2015**, *137* (10), 3663–3669.
(35) Grawe, T.; Schrader, T.; Finocchiaro, P.; Failla, S.; Consiglio, G. A New Receptor Molecule for Lysine and Histidine in Water - Strong Binding of Basic Amino Acids by a Macrocyclic Host. In *Proceedings of The 4th International Electronic Conference on Synthetic Organic Chemistry*; 2000; p 1919.
(36) Beaver, J. E.; Peacor, B. C.; Bain, J. V.; James, L. I.; Waters, M. L. Contributions of Pocket Depth and Electrostatic Interactions to Affinity and Selectivity of Receptors for Methylated Lysine in Water. *Org. Biomol. Chem.* **2015**, *13* (11), 3220–3226.
(37) Ingerman, L. A.; Cuellar, M. E.; Waters, M. L. A Small Molecule Receptor That Selectively Recognizes Trimethyl Lysine in a Histonepeptide with Native Protein-like Affinity. *Chem. Commun.* **2010**, *46* (11), 1839–1841.
(38) Sharma, H.; Kaur, N.; Singh, A.; Kuwar, A.; Singh, N. Optical Chemosensors for Water Sample Analysis. *J. Mater. Chem. C* **2016**, *4* (23), 5154–5194.
(39) Patel, G.; Menon, S. Recognition of Lysine, Arginine and Histidine by Novel *p*-Sulfonatocalix[4]arene Thiol Functionalized Gold Nanoparticles in Aqueous Solution. *Chem. Commun.* **2009**, No. 24, 3563.
(40) Han, C.; Zeng, L.; Li, H.; Xie, G. Colorimetric Detection of Pollutant Aromatic Amines Isomers with *p*-Sulfonatocalix[6]arene-Modified Gold Nanoparticles. *Sens. Actuators B: Chem.* **2009**, *137* (2), 704–709.
(41) Arduini, A.; Demuru, D.; Pochini, A.; Secchi, A. Recognition of Quaternary Ammonium Cations by Calix[4]Arene Derivatives Supported on Gold Nanoparticles. *Chem. Commun.* **2005**, No. 5, 645.
(42) Brunetti, E.; Inthasot, A.; Keymeulen, F.; Reinaud, O.; Jabin, I.; Bartik, K. Primary Amine Recognition in Water by a Calix[6]aza-Cryptand Incorporated in Dodecylphosphocholine Micelles. *Org. Biomol. Chem.* **2015**, *13* (10), 2931–2938.
(43) Basilio, N.; Martín-Pastor, M.; García-Río, L. Insights into the Structure of the Supramolecular Amphiphile Formed by a Sulfonated Calix[6]arene and Alkyltrimethylammonium Surfactants. *Langmuir* **2012**, *28* (16), 6561–6568.
(44) Trembleau, L.; Rebek, J. Jr. Interactions between a Surfactant and Cavitand in Water Blur Distinctions between Host and Guest. *Chem. Commun.* **2004**, No. 1, 58.
(45) Mustafina, A. R.; Elistratova, Yu. G.; Syakaev, V. V.; Amirov, R. R.; Konovalova, A. I. Receptor Properties of Calix[4]resorcinarenes toward Tetramethylammonium and Choline Cations in Micellar Solutions of Sodium Dodecyl Sulfate. *Russ Chem Bull* **2006**, *55* (8), 1419–1424.
(46) Elistratova, Yu. G.; Mustafina, A. R.; Amirov, R. R.; Nugaeva, Z. T.; Burilov, A. R.; Konovalov, A. I. Influence of the Structure of Nonionic Surfactants and the Length of Alkyl Substituents of Calix[4]resorcinarenes on Their Solubility, Acid–Base, and Complexation Properties. *Colloid J.* **2004**, *66* (3), 285–291.
(47) Mustafina, A. R.; Amirov, R. R.; Elistratova, Y. G.; Skripacheva, V. V.; Nugaeva, Z. T.; Kazakova, E. K. Solubility, Acid–Base and Complexation Properties of Calix[4]resorcinarene in Aqueous Solutions of Nonionic Surfactants. *Colloid J.* **2002**, *64* (6), 6.
(48) Kim, Y. J.; Lek, M. T.; Schramm, M. P. pH Influenced Molecular Switching with Micelle Bound Cavitands. *Chem. Commun.* **2011**, *47* (34), 9636.
(49) Collin, S.; Parrot, A.; Marcelis, L.; Brunetti, E.; Jabin, I.; Bruylants, G.; Bartik, K.; Reinaud, O. Submerging a Biomimetic Metallo-Receptor in Water for Molecular

Recognition: Micellar Incorporation or Water Solubilization? A Case Study. *Chem. Eur. J.* **2018**, *24* (68), 17964–17974.
(50) Javor, S.; Rebek, J. Activation of a Water-Soluble Resorcinarene Cavitand at the Water–Phosphocholine Micelle Interface. *J. Am. Chem. Soc.* **2011**, *133* (43), 17473–17478.
(51) Ikeda, A.; Hatano, T.; Kawaguchi, M.; Shinkai, S.; Suenaga, H. Water-Soluble [60]Fullerene–Cationic Homooxacalix[3]arene Complex Which Is Applicable to the Photocleavage of DNA. *Chem. Commun.* **1999**, No. 15, 1403–1404.
(52) Ikeda, A.; Ejima, A.; Nishiguchi, K.; Kikuchi, J.; Matsumoto, T.; Hatano, T.; Shinkai, S.; Goto, M. DNA-Photocleaving Activities of Water-Soluble Carbohydrate-Containing Nonionic Homooxacalix[3]arene·[60]Fullerene Complex. *Chem. Lett.* **2005**, *34* (3), 308–309.
(53) Hatano, T.; Ikeda, A.; Akiyama, T.; Yamada, S.; Sano, M.; Kanekiyo, Y.; Shinkai, S. Facile Construction of an Ultra-Thin [60]Fullerene Layer from [60]Fullerene–Homooxacalix[3]arene Complexes on a Gold Surface. *J. Chem. Soc., Perkin Trans. 2* **2000**, No. 5, 909–912.
(54) Ikeda, A.; Hatano, T.; Shinkai, S.; Akiyama, T.; Yamada, S. Efficient Photocurrent Generation in Novel Self-Assembled Multilayers Comprised of [60]Fullerene−Cationic Homooxacalix[3]arene Inclusion Complex and Anionic Porphyrin Polymer. *J. Am. Chem. Soc.* **2001**, *123* (20), 4855–4856.
(55) See Supporting Information.
(56) A more polar solvent mixture was chosen in order to observe more easily a possible change in affinity following deprotonation. Experiments were run at 263K to ensure slow host-guest exchange on the NMR shift timescale.
(57) Kallick, D. A.; Tessmer, M. R.; Watts, C. R.; Li, C. Y. The Use of Dodecylphosphocholine Micelles in Solution NMR. *Journal of Magnetic Resonance, Series B* **1995**, *109* (1), 60–65.
(58) Sikorska, E.; Wyrzykowski, D.; Szutkowski, K.; Greber, K.; Lubecka, E. A.; Zhukov, I. Thermodynamics, Size, and Dynamics of Zwitterionic Dodecylphosphocholine and Anionic Sodium Dodecyl Sulfate Mixed Micelles. *J Therm Anal Calorim* **2016**, *123* (1), 511–523.
(59) Lazaridis, T.; Mallik, B.; Chen, Y. Implicit Solvent Simulations of DPC Micelle Formation. *J. Phys. Chem. B* **2005**, *109* (31), 15098–15106.
(60) George, D. K.; Charkhesht, A.; Hull, O. A.; Mishra, A.; Capelluto, D. G. S.; Mitchell-Koch, K. R.; Vinh, N. Q. New Insights into the Dynamics of Zwitterionic Micelles and Their Hydration Waters by Gigahertz-to-Terahertz Dielectric Spectroscopy. *J. Phys. Chem. B* **2016**, *120* (41), 10757–10767.
(61) Keymeulen, F.; De Bernardin, P.; Dalla Cort, A.; Bartik, K. Paramagnetic Relaxation Enhancement Experiments: A Valuable Tool for the Characterization of Micellar Nanodevices. *J. Phys. Chem. B* **2013**, *117* (39), 11654–11659.
(62) Aggregation number of DPC at 20 mM is 58 (see references 59 and 60). Micelle concentration is thus around 0.35 mM. DOSY experiments confirm that all the receptors are incorporated into the micelles and there is consequently on average one receptor per micelle.
(63) Faramarzi, S.; Bonnett, B.; Scaggs, C. A.; Hoffmaster, A.; Grodi, D.; Harvey, E.; Mertz, B. Molecular Dynamics Simulations as a Tool for Accurate Determination of Surfactant Micelle Properties. *Langmuir* **2017**, *33* (38), 9934–9943.
(64) Marcus, Y.; Hefter, G. Ion Pairing. *Chem. Rev.* **2006**, *106* (11), 4585–4621.
(65) It was decided to compare the complexation abilities at different guests addition, as in case of the most hydrophilic guests, addition of tens equivalents of guests is required to

observe the complexation while with more hydrophobic guests, much less addition is required.

(66) Indeed the length of the hydrophobic tail of DPC is a chain of 12 carbon atoms, same number of carbon as the diammonium. Moreover, we can consider the receptor is located at the center of the micelle. It is therefore quite reasonable to assume the part of the diammonium which is not involved in the recognition process is located at the level of the hydrophilic layer of the micelle.

(67) pKa of aniline is 4.6, therefore the pH decreased to 4.2 with the addition of large amount of aniline hydrochloride.

(68) Keymeulen, F.; De Bernardin, P.; Giannicchi, I.; Galantini, L.; Bartik, K.; Dalla Cort, A. Fluoride Binding in Water with the Use of Micellar Nanodevices Based on Salophen Complexes. *Org. Biomol. Chem.* **2015**, *13* (8), 2437–2443.

(69) Yamato, T.; Zhang, F.; Tsuzuki, H.; Miura, Y. Synthesis and Inclusion Properties Of C3-Symmetrically Capped Hexahomotrioxacalix[3]arenes with Ester Groups on the Lower Rim. *Eur. J. Org. Chem.* **2001**, *2001* (6), 1069–1075.

(70) Yamato, T.; Zhang, F. Synthesis, Conformations and Inclusion Properties of Hexahomotrioxacalix[3]arene Triamide Derivatives Having Hydrogen-Bonding Groups. *J. Incl. Phenom. Macrocycl. Chem.* **2001**, *39*, 55–64.

(71) Chang, G.; Guida, W. C.; Still, W. C. An Internal-Coordinate Monte Carlo Method for Searching Conformational Space. *J. Am. Chem. Soc.* **1989**, *111* (12), 4379–4386.

(72) Banks, J. L.; Beard, H. S.; Cao, Y.; Cho, A. E.; Damm, W.; Farid, R.; Felts, A. K.; Halgren, T. A.; Mainz, D. T.; Maple, J. R.; Murphy, R.; Philipp, D. M.; Repasky, M. P.; Zhang, L. Y.; Berne, B. J.; Friesner, R. A.; Gallicchio, E.; Levy, R. M. Integrated Modeling Program, Applied Chemical Theory (IMPACT). *J. Comput. Chem.* **2005**, *26* (16), 1752–1780.