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Binding of Bioactive Ammonium Ions in Water with a Cavity-Based Selectivity: Water Solubilization versus Micellar Incorporation

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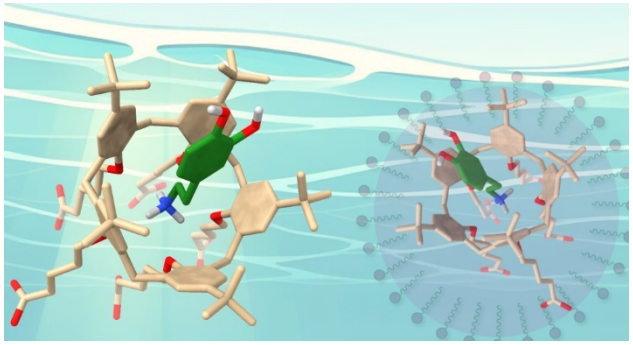


Abstract

Many bioactive molecules contain primary ammonium groups, generating significant interest in developing selective receptors for ammonium ions. A promising strategy involves the use of polyaromatic cavitands to achieve size and shape selectivity through their cavity. However, designing effective receptors for ammonium ions in aqueous media is challenging due to the competitive nature of water. Calix[5]arenes are known to selectively bind primary ammonium ions over secondary, tertiary, and quaternary ammonium ions in organic solvents. Here, we

report on the binding properties of a calix[5]arene, which bears carboxyl groups on its small rim, in organic solvents and aqueous media. This receptor was transferred in water either through deprotonation of its carboxyl groups or by incorporation into dodecylphosphocholine (DPC) micelles. ^{1}H NMR confirmed the *endo*-complexation of various primary ammonium ions not only in organic solvents but also in both aqueous media. Cavity-based selectivity was also observed, validating the cavitand strategy for the selective binding of ammonium ions in water. Unique binding properties, driven by the calix[5]arene's intrinsic recognition ability and the hydrophobic effect, were observed in water. Notably, binding affinities for dopamine and lysine derivatives with log K_a values of > 3.9 were determined. The direct solubilization of the receptor outperformed micellar incorporation due to the hydrophilic nature of the primary ammonium ions, which hinders their uptake into micelles. These results offer promising perspectives for the development of efficient chemosensors for the characterization of bioactive ammonium ions in water.

Keywords: Ammonium ions – Micelles – Supramolecular chemistry – Host-guest systems – Calixarenes – NMR.

Introduction

The development of strategies for the efficient binding of primary ammonium ions in aqueous environments is a topical field of research, with promising applications in biological and environmental monitoring.[1] Amine and ammonium groups are ubiquitous in a wide range of bioactive molecules, including amino acids, peptides, and neurotransmitters. For example, monoamine neurotransmitters such as dopamine and serotonin, along with their structurally related trace amines, play crucial roles in physiological signaling processes within the nervous system.[2] Additionally, amine- and ammonium-containing molecules have broad industrial applications, with some of them (*e.g.*, aromatic amines) being recognized as environmental pollutants or known to be harmful to human health.[3]

An effective approach for designing artificial receptors for ammonium ions involves using macrocyclic compounds with a polyaromatic cavity such as calixarenes,[4–9] resorcinarenes,[10] (hemi)cryptophanes,[11,12] pillararenes[13–15] or prismarenes.[16] Ammonium guests are typically stabilized inside the electron-rich cavity through H-bonds, cation–π interactions and CH–π interactions. The cavity can furthermore provide size and shape selectivity.[17–19] Among these families of polyaromatic cavitands, our groups have specifically studied the binding properties of hexahomotrioxacalix[3]arenes and calix[5]arenes with respect to ammonium ions.

Homooxacalix[3]arenes are bowl-shaped receptors with an 18-crown-3 moiety, enabling the formation of three H-bonds between a primary ammonium group and the ethereal oxygen atoms of the macrocycle.[20] They present an open and flexible cavity capable of accommodating linear but also bulky primary ammonium ions with high affinity, even in a competitive protic environment (log K_a > 4 for various RNH_3^+ in 4:1 $CDCl_3/CD_3OD$).[21] Due to their remarkable complementarity with primary ammonium ions, homooxacalix[3]arenes exhibit specificity for these ions over secondary, tertiary, and quaternary ammonium species. The cavity of calix[5]arenes is less flexible than that of homooxacalix[3]arenes, and calix[5]arenes exhibit selectivity for linear primary ammoniums, with a strong preference for those containing a -$(CH_2)_2NH_3^+$ moiety.[22] They are, however, also to bind linear secondary ammonium cations, albeit with significantly lower binding affinities. A log K_a of > 5 has for example been reported for nBuNH$_3^+$ in $CDCl_3$,[22] while a log K_a <3 has been reported for (nBu)$_2NH_2^+$ in $(CDCl_2)_2$.[23,24] The selective binding of primary ammoniums relies on H-bonds between the ammonium group and the phenolic oxygens of the calixarene, accompanied by cation–π and CH–π interactions. It should be noted that calix[5]arenes have been used as building blocks for various supramolecular assemblies such as heterotopic receptors,[25] capsules,[26] and sensing devices.[27] Very interestingly, the binding of bioactive ammoniums, such as dopamine and lysine derivatives, has been reported with both homooxacalix[3]arene- and calix[5]arene-based receptors in organic solvents.[21,22,28,29]

Exploiting the binding properties of these polyaromatic cavitands in water remains a challenging task. First, the high permittivity of water weakens electrostatic interactions, and in addition, water molecules are excellent H-bond donors and acceptors, efficiently solvating the various binding partners, particularly the primary ammonium ions.[30,31] The hydrophobic effect can however be beneficial for the binding of low-polarity guests.[32,33] Additionally, unlike cucurbiturils[34,35] and cyclodextrins,[36] macrocyclic polyaromatic receptors are not soluble in water. The most classical strategy for transferring these hydrophobic receptors into water consists of the introduction of hydrophilic moieties onto their framework.[37,38] Examples of such water-soluble receptors include pillararenes bearing phosphate groups,[39] cyclophanes[40,41] and prismarenes[42] bearing carboxylate groups, and calix[5]arenes bearing sulfonate groups on the large rim.[43–46] These receptors have shown binding properties with respect to ammonium cations in water, however, they lack selectivity for the primary ammonium group. A notable exception is *p-tert*-butylcalix[5]arene **1** (Figure 1) bearing sulfonate arms on its small rim,[47,48] which has been shown to form complexes with primary ammonium cations inside its cavity in D_2O. It is worth noting that this receptor forms aggregates above a certain concentration (the

"critical micellar concentration", CMC) or in the presence of hydrophobic primary ammoniums included in its polyaromatic cavity. We recently reported on the direct solubilization in water of homooxacalix[3]arene **2** (Figure 1), through deprotonation of its three carboxyl groups.[49] No ammonium ion binding was however evidenced, likely due to the high flexibility of the macrocyclic skeleton, which causes its cavity to "collapse" in water.

An alternative strategy for transferring hydrophobic receptors into water, which we exploited in this work, involves their incorporation into micelles. This approach is simple and straightforward, as it does not require any synthetic modification of the apolar receptor. Studies have suggested that the micellar strategy can even enhance the recognition properties of macrocyclic receptors in water.[50,51] We have used this strategy in several studies, and we recently reported on the selective binding of primary ammoniums mediated by receptor **2** when incorporated in dodecylphosphocholine (DPC) micelles.[49] The highest affinities were observed with the more hydrophobic ammonium ions, highlighting the crucial role of the hydrophobic effect for the partition of the guests inside micelles. We typically observed binding affinities in the range of $1 < \log K_a < 3$. To the best of our knowledge, the only other example of primary ammoniums recognition in micellar systems is the work by Rebek *et al.*, using a resorcinarene, although with lower binding affinity compared to receptor **2**.[50]

In this paper, we report on the new *p-tert*-butyl-calix[5]arene **3** (Figure 1), functionalized with long chain carboxyl arms on its small rim. After investigating and confirming the complexation properties of **3** in organic solvents, we explored the two aforementioned approaches for studying its binding properties in water: (*i*) direct solubilization and (*ii*) incorporation into micelles. The binding properties of **3** toward primary ammonium ions, particularly bioactive ones, were evaluated and compared to those of **1** when directly solubilized in water, and to those of **2** when incorporated into micelles.

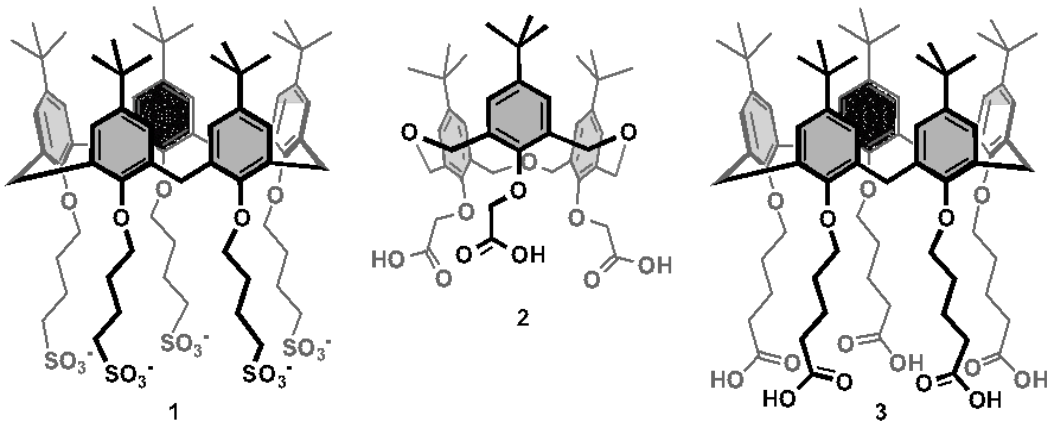
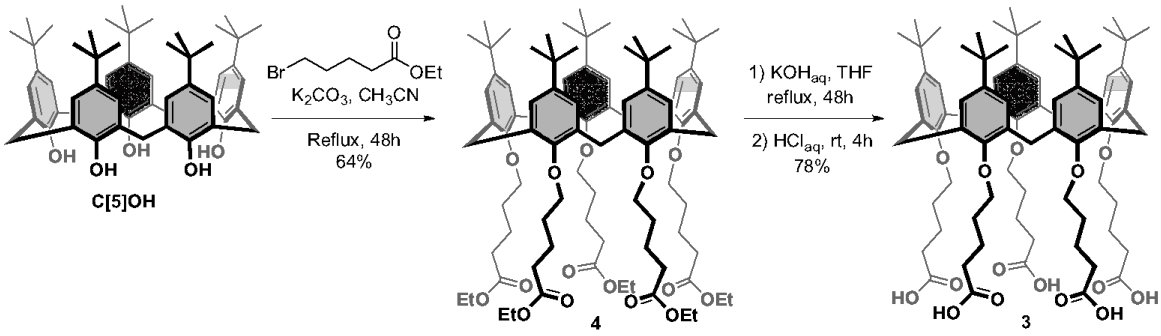


Figure 1. Structures of receptors **1**-**3**.

Results and Discussion

Synthesis. Target receptor **3** was synthesized in two steps from the parent *p-tert*-butylcalix[5]arene **C[5]OH** (Scheme 1). Exhaustive *O*-alkylation of **C[5]OH** with ethyl-5-bromo-valerate in the presence of K_2CO_3 in refluxing CH_3CN afforded pentaester **4** in 64% yield. Subsequent hydrolysis under basic conditions, followed by acidic treatment, afforded receptor **3** in 78% yield. Receptor **3** adopts a major C_{5v} symmetrical cone conformation in $CDCl_3$, as suggested by the presence in its ^{1}H NMR spectrum of a singlet for the Ar*H*, two doublets for the ArCH_2 groups and a singlet for the *t*Bu protons (Figure 2a).



Scheme 1. Synthesis of Receptor **3**.

Binding Properties of host 3 with Respect to Ammonium Ions in Organic Solvents. The binding properties of **3** were first evaluated by ^{1}H NMR in $CDCl_3$, using hexylamine and its corresponding picrate and chloride salts ($HexNH_3^+Pic^-$ and $HexNH_3^+Cl^-$) as model guests. The formation of the host-guest complex **3**⊃$HexNH_3^+$ was observed with both ammonium salts. In the case of free hexylamine, the ammonium ion was generated through protonation of the free amine by the receptor, leading to complex $\mathbf{3}^{-H+}$⊃$HexNH_3^+$ (Figure 2b and Supporting Information). In all cases, host–guest exchange was slow on the NMR chemical shift time scale. The signals characteristic of guest complexation within the polyaromatic cavity of the calix[5]arene were observed in the high-field region of the spectra (between -2 and 1 ppm). These signals were unambiguously assigned through a COSY NMR experiment (see the Supporting Information). The largest complexation induced shifts (CISs) for the alkylammonium protons were observed for the methylene groups at the α- and β-positions relative to the ammonium head (at -4.13 ppm and -3.58 ppm, respectively), consistent with previously reported values,[22,52] suggesting that the guest is located deep inside the polyaromatic cavity. A characteristic downfield shift of the Ar*H* and *t*Bu protons of the receptor was also observed ($\Delta\delta_{ArH}$ = 0.25 ppm and $\Delta\delta_{tBu}$ = 0.14 ppm), which is typical of the cavity opening following *endo*-complexation.

As exchange is slow on the NMR chemical shift time scale, binding affinities were determined by integrating the signals of the free and complexed guest and host (see the Supporting Information).[53] The affinity for the ammonium ion was higher when titrating with the picrate salt (log K_a = 4.4 ± 0.1) than with the chloride salt (log K_a = 3.1 ± 0.1), and also when titrating with the amine (log K_a = 3.8 ± 0.1), where a deprotonated carboxyl group on the small rim plays the role of the counterion. This can be explained by the better dissociation of the picrate ammonium ion pair compared to that of the chloride and carboxylate ammonium pairs in $CDCl_3$.[54]A similar trend has been observed with other conformationally fixed cone calix[5]arenes.[55,56] Titrations were also conducted in 4:1 $CDCl_3/CD_3OD$ to assess the impact of a protic environment on binding affinities. Higher affinities were determined with $HexNH_2$ (log K_a > 5) and $HexNH_3Cl$ (log K_a = 3.7 ± 0.2) in this medium compared to those of $CDCl_3$. This observation can be rationalized by the better solvation of the carboxylate and chloride counteranions in the protic environment, facilitating ion-pair separation. For the weakly coordinating picrate, a lower log K_a (3.9 ± 0.2) was however observed, due to the competitive nature of the medium.

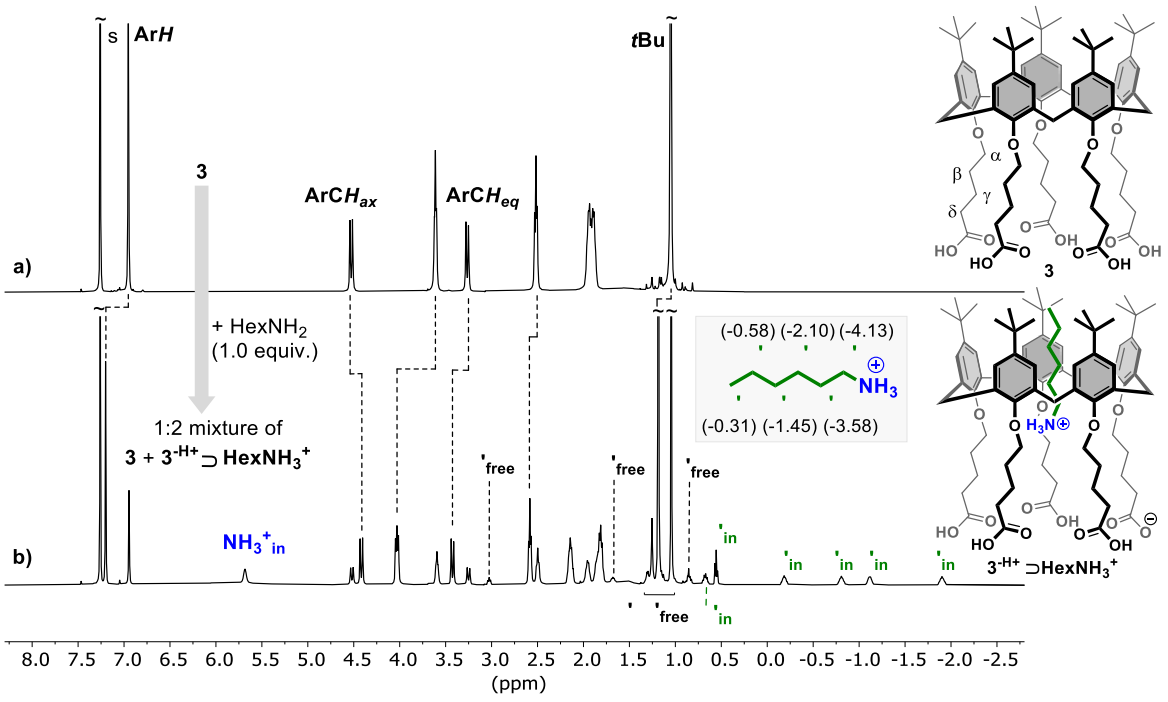


Figure 2. ^{1}H NMR spectra (298 K, 500 MHz) in $CDCl_3$ of a) **3** (1 mM), and b) **3** with 1.0 equiv. of $HexNH_2$. S: residual solvents. The inset shows the CIS values.

The binding properties of host **3** in $CDCl_3$ were then evaluated with a range of primary amines, including biologically relevant ones (Table 1 and Supporting information). Intracavity complexation of the corresponding ammoniums, indicated by characteristic guest signals in the

high field region of the ^{1}H NMR spectra, was observed in nearly all cases. The binding affinities, determined by integrating appropriate ^{1}H signals after the addition of one equiv of amine, followed a clear trend in accordance with the literature. Specifically, conformationally fixed cone calix[5]arenes show a marked selectivity for linear ammonium ions $R(CH_2)_2NH_3^+$, with a preference for shorter ones.[22,57] In our case, the binding affinities decreased in the following order $EtNH_3^+ > PrNH_3^+ \approx nBuNH_3^+ > nHexNH_3^+$. Weaker binding affinities (log $K_a \approx$ 2-3) were observed for phenylalkylammonium ions, likely due to steric hindrance at the level of the *t*Bu groups of the host. Comparable binding affinities for $nPrNH_3^+$ and $nBuNH_3^+$ were confirmed by competition experiments (see the Supporting Information). Weak or no complexation was observed upon addition of $iBuNH_2$ or benzylamine ($BnNH_2$), respectively, as each of these guest lacks the $(CH_2)_2NH_3^+$ motif, which highlights the size and shape selectivity of the calix[5]arene cavity. In contrast, $BnNH_3^+$ and even bulkier primary ammoniums, such as 1-adamantylammonium ion ($AdamNH_3^+$), are well recognized by homooxacalix[3]arenes.[21,49] This can be explained by the ability of homooxacalix[3]arenes to adopt a more open cavity, thereby reducing the number of steric clashes between the included guest and aromatic walls of the cavity. Histamine was also not recognized by host **3**, likely due to the protonation of its imidazole unit by the carboxyl groups of the host and the resulting ion-pairing interactions.[48]

Table 1. Association Constants for Ammonium ions RNH_3^+ Determined by ^{1}H NMR Spectroscopy in $CDCl_3$ or 4:1 $CDCl_3/CD_3OD$ at 298K, following the Addition of 1 equiv of RNH_2 to Host **3** ([**3**] = 1 mM).

	Log K_a	
Guest RNH_3^{+a}	$CDCl_3$	$CDCl_3/CD_3OD$ (4:1)
$EtNH_3^+$	>5	/
$nPrNH_3^+$	4.7±0.2	/
$nBuNH_3^+$	4.8±0.1	/
$nHexNH_3^+$	3.8±0.1	> 5
$nHexNH_3^+Cl^{-b}$	3.1±0.1	3.7 ± 0.2
$nHexNH_3^+Pic^{-c}$	4.4±0.1	3.9 ± 0.2
$iBuNH_3^+$	1.8±0.2	/
$BnNH_3^+$	nd	/
2-PhenylethylNH_3^+	3.1±0.1	/
3-PhenylpropylNH_3^+	2.5±0.2	/
4-PhenylbutylNH_3^+	2.9±0.1	/

3-Methoxytyramine·H^+	3.0±0.1	/
Tryptamine·H^+	2.2±0.2	/
Histamine·nH^{+d}	nd	/

nd: not detected, /: not measured.

[a] The ammonium ion RNH_3^+ is generated through protonation of the corresponding free amine RNH_2 by host **3**.

[b] The chloride salt was added instead of the free amine.

[c] The picrate salt was added instead of the free amine.

[d] A possible protonation of the imidazole unit may explain the absence of *endo*-complexation (see text).

Binding Properties of Host 3 with Respect to Ammonium Ions in Water. Receptor **3** was fully soluble in D_2O at pH above 10. The ^1H NMR spectrum confirmed that it maintains a C_{5v} symmetrical cone conformation, as observed in organic solvents (Figure 3a). When the receptor concentration was increased from 0.2 to 0.8 mM, a broadening of the NMR signals was observed, along with the emergence of a distinct shoulder, indicative of a second signal for the Ar*H* and *t*Bu protons (see the Supporting Information). Similar observations were reported for calix[5]arene **1**, and were attributed to its aggregation in water.[47] DOSY NMR spectra recorded of receptor **3** at concentrations of at 0.2 mM and 0.8 mM confirmed the formation of micellar-type aggregates at higher concentration (see the Supporting Information).

The binding properties of **3** were investigated in D_2O using various linear primary ammonium chloride salts ([**3**] = 0.2 mM, pH = 10.3-10.6). Characteristic signals for intracavity complexation were observed in the high field region of the ^1H NMR spectra (see the Supporting Information). As in the $CDCl_3$ studies, the host-guest exchange process was slow on the NMR chemical shift timescale, enabling the determination of binding affinities (K_a) through signal integration (Table 2). The trend in binding affinities for the linear primary ammoniums in water differed from that observed in $CDCl_3$, with $HexNH_3^+ \approx nDodNH_3^+ \approx H_3N^+(CH_2)_{12}NH_3^+$ (log K_a > 5) > $EtNH_3^+$ > *n*$BuNH_3^+$ > *n*$PrNH_3^+$. The higher affinity observed for *n*$BuNH_3^+$ compared to *n*$PrNH_3^+$ was confirmed by a competition experiment (see the Supporting Information). No evidence of recognition was observed for $BnNH_3^+Cl^-$ and $AdamNH_3^+Cl^-$, due to the cavity-based selectivity of the host. These NMR data clearly underscore the balanced interplay between the hydrophobic effect and the intrinsic binding properties of **3**. Indeed, in organic solvents, the affinity for linear ammonium ions systematically decreases with increasing chain length, from $EtNH_3^+$ to *n*$HexNH_3^+$, while in D_2O the lowest affinities are observed for *n*$PrNH_3^+$ and *n*$BuNH_3^+$ (log K_a = 3.9 and 4.1, respectively). The longer alkylammonium chains benefit from the hydrophobic effect. It is worth noting that binding affinities with log K_a greater than 5 for primary ammonium cations in water is a remarkable feat.

Of true interest is the successful complexation of various bioactive ammonium ions or their derivatives by host **3**. 2-PhenylethylNH$_3^+$, tyramine·H$^+$, dopamine·H$^+$, serotonin·H$^+$, and lysine derivatives were all recognized with high affinities (Table 2), considering the hydrophilic nature of these species. As representative examples, the ^{1}H NMR spectra showing the intracavity binding of *L*-Lysine ethyl ester·2H$^+$ (*L*-Lys-OEt.2H$^+$, log K_a = 3.9) and dopamine·H$^+$ (log K_a = 4.1) are presented in Figures 3b and 3c. Surprisingly, the binding affinities of receptor **3** in D$_2$O were systematically one order of magnitude higher than those of receptor **1** (Table 2). A competition experiment with receptors **1** and **3** with dopamine·H$^+$ as guest confirmed the superior binding abilities of **3** (see the Supporting Information).

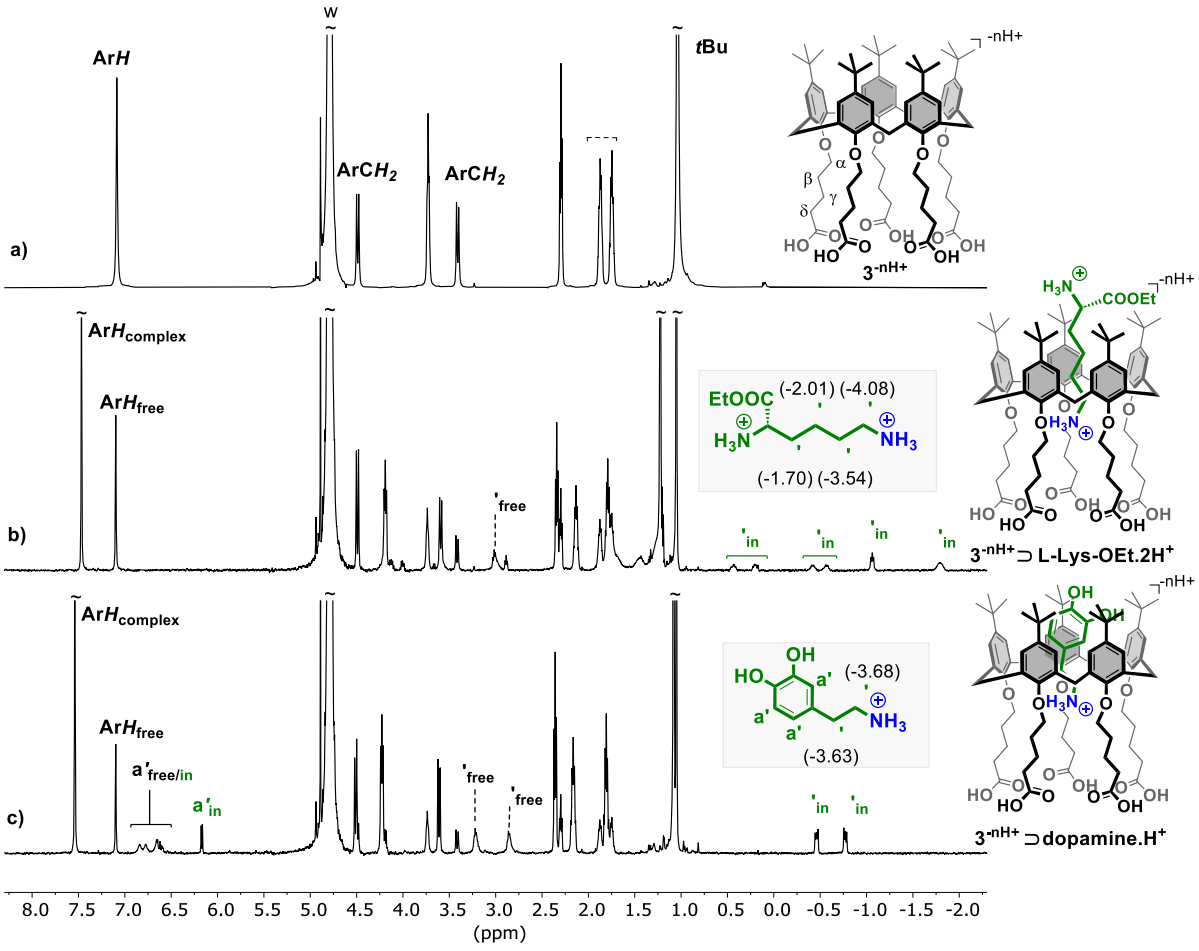


Figure 3. ^{1}H NMR spectra (D$_2$O, 298 K, 600 MHz) of a) **3** (0.2 mM) at pH = 10.4, b) **3** (0.2 mM) at pH = 10.4 with 1.8 equiv. of *L*-Lys-OEt.2HCl, and c) **3** (0.2 mM) at pH = 10.4 with 2.0 equiv. of dopamine·HCl. W: water. The inset shows CIS values (for panel b: obtained by comparison with free *L*-Lys-OEt.2HCl at neutral pH in D$_2$O).

Table 2. Associations Constants (K_a) for Hosts **1** and **3** in D_2O ([**1**] and [**3**] = 0.2 mM, neutral pH for **1** and pH = 10.3-10.6 for **3**) and Measured Association constants (K_m) for host **3** in DPC micelles ([**3**] = 0.35 mM; 20 mM DPC, 10 mM Bis-Tris Propane buffer; pH 9.3-9.5) toward ammonium ions RNH_3^+, determined by ^1H NMR spectroscopy at 298 K.

Guest RNH_3^+ (Cl^- salt)	cLogP[a] RNH_3^+	**1 (in D_2O)** Log K_a	**3 (in D_2O)** Log K_a	**3 (in DPC micelles)** Log K_m	**3 (in DPC micelles)** $[\mathbf{3}\supset RNH_3^+]/[\mathbf{3}_{tot}]$ (%) {Equiv. RNH_3^+}
$EtNH_3^+$	-0.09	/	4.7±0.1	3.1	66 {4.7}
n$PrNH_3^+$	0.43	/	3.9±0.1	2.4	33 {5.2}
n$BuNH_3^+$	0.96	/	4.2±0.1	2.9	51 {4.5}
n$HexNH_3^+$	2.02	/	>5	3.9	48 {0.7}
n$DodNH_3^+$	5.19	/	>5	4.5	68 {0.8}
$H_3N^+(CH_2)_{12}NH_3^+$	3.35	/	>5	3.9	51 {0.8}
$BnNH_3^+$	1.14	/	nd	/	/
$AdamNH_3^+$	2.03	/	nd	nd	nd {70}
2-PhenylethylNH_3^+	1.47	3.3±0.1[b]	4.5±0.1	2.8	55 {5.1}
Tyramine·H^+	0.81	3.7±0.1[b]	4.6±0.2	nd	nd {80}
Dopamine·H^+	0.21	3.3±0.1[b]	4.1±0.1	nd	nd {60}
Serotonin·H^+	0.80	3.1±0.1[b]	4.0±0.2	/	/
O-Methylserotonin·H^+	1.48	/	/	nd	nd {90}
*N*α-acetyl-*L*-Lys-OMe·H^+	-0.74	3.4±0.1	4.2±0.1	nd	nd {23}
L-Lys-OEt·$2H^+$	-0.14	/	3.9±0.1	nd	nd {40}

nd: not detected, /: not measured.

[a] Predicted by ChemDraw Professional 16.0

[b] Data from previous research.[48]

Binding Properties of 3 with Respect to Ammonium ions in micellar systems. The second strategy investigated for transferring receptor **3** into water was its incorporation into dodecylphosphocholine (DPC) micelles. This approach has already been used to transfer numerous receptors into aqueous environments,[50,51,58,59] including homooxacalix[3]arene **2**.[49] The ^1H NMR spectrum of **3** (0.35 mM in buffered D_2O solution, pH = 9.3-9.5) in the presence of DPC (20 mM) is shown in Figure 4a. Full incorporation into the DPC micelles was confirmed by DOSY measurements (see the Supporting InformationOn the basis of the receptor and surfactant concentrations used to acquire the ^1H NMR spectrum, an average of one receptor molecule is expected per micelle (see the Supporting Information). The ^1H spectrum also clearly

shows that **3** adopts a C_{5v} symmetrical cone conformation (one singlet for the Ar*H* and one singlet for the *t*Bu protons) when incorporated into DPC.

The impact of pH on the recognition properties of **3** was studied using $HexNH_3^+Cl^-$ as a model guest (from pH 5.9 to 13.3). As observed in D_2O and in $CDCl_3$, host-guest exchange was slow on the NMR timescale. The characteristic upfield signals for the included guest, along with the downfield shift of the Ar*H* and *t*Bu protons, were observed at the different pHs (see Figure 4b for pH 9.5 in 10 mM Bis-Tris propane buffer). The CISs were almost identical to those recorded in $CDCl_3$, indicating a similar binding mode and deep inclusion of the guest. After the addition of 2.5 equiv. of guest, the aromatic signals were integrated, and maximal inclusion was observed between pH 9.3 and 11.6 (see the Supporting Information). The enhanced performance of **3** under basic conditions can be attributed to its deprotonation, similarly to what was reported for homooxacalix[3]arene **2** in DPC micelles.[49] In the apolar core of micelles, the binding of the ammonium ion is favored when a weakly coordinating anion is present in the medium.[60] As the energy penalty for the dissociation of the ammonium-chloride ion-pair is very high in DPC micelles, complexation is favored when the deprotonated carboxyl arms of **3** play the role of the ammonium counterion. Above pH 11.6, the deprotonation of the ammonium ion (p*Ka* of primary alkylammoniums ≈ 10-11[61]) explains the decrease in the proportion of complexed guest.

The binding of various linear primary alkylammonium ions, $AdamNH_3^+Cl^-$, and bioactive ammonium ions was subsequently studied by ^1H NMR at the optimized pH 9-3-9.5 (10 mM Bis-Tris propane buffer). Recognition was observed with all the linear ammonium ions, including the bis-ammonium $H_3N^+(CH_2)_{12}NH_3^+$, whereas no complexation was detected with the bulky $AdamNH_3^+$ (Table 2). Regarding bioactive guests, no trace of complexation was observed with *L*-Lys-OEt·2HCl, *N*α-acetyl-*L*-Lys-OMe·HCl, dopamine·HCl, tyramine·HCl, *O*-methylserotonin·HCl, even in the presence of a large excess of these guests (> 23 equiv.). It is likely that the micelles do not favor the binding of these hydrophilic guests, as they do not penetrate the hydrophobic micellar core and instead remain in the bulk water. Complexation was only observed with the less hydrophilic 2-phenylethylNH_3^+ (Table 2).

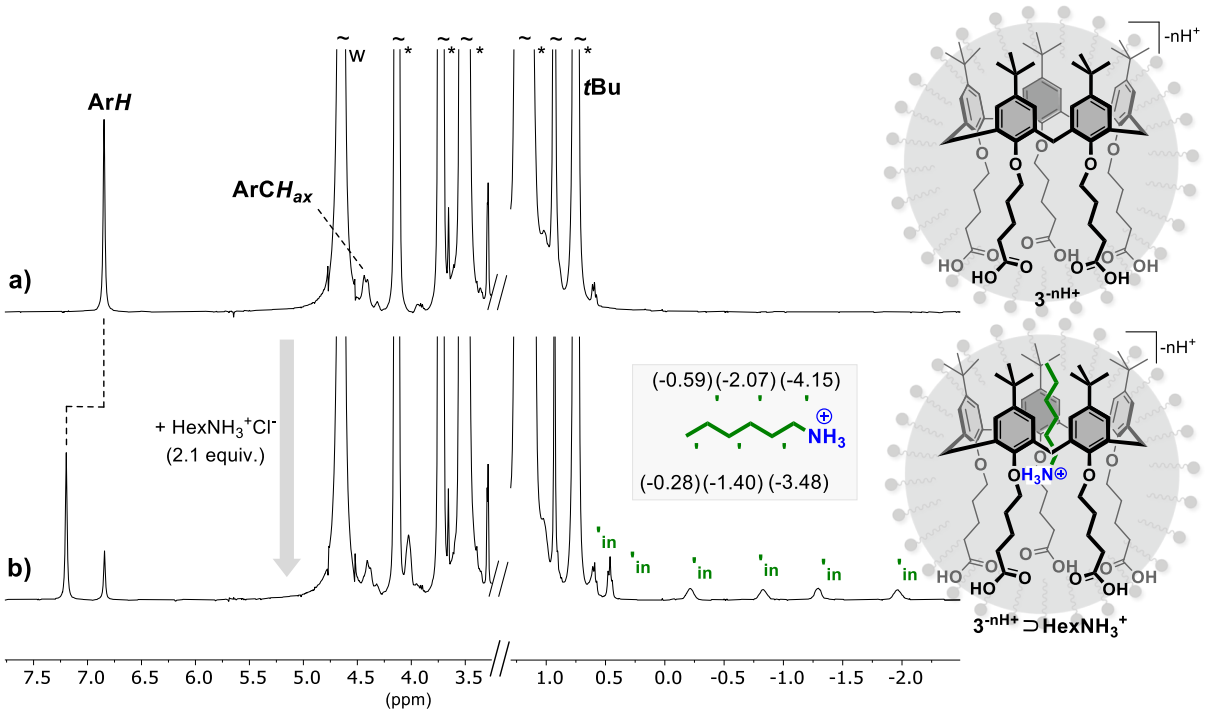


Figure 4. ^{1}H NMR spectra (298 K, 400 MHz) of (a) **3** (0.35 mM) in the presence of DPC (20 mM) and 10 mM Bis-Tris propane (BTP) in D_2O (pH = 9.5); (b) and after addition of *ca.* 2.1 equiv of $HexNH_3^+Cl^-$. *: surfactant signals, w: water signal. The inset shows CIS values (obtained by comparison with free $HexNH_3^+Cl^-$ at neutral pH in D_2O).

As previouly discussed in the study of ammonium complexation by homooxacalix[3]arene **2** incorporated into DPC micelles,[49] we must be careful when evaluating binding affinities due the possible saturation of the micelles with guest molecules. For this reason, the proportions of host-guest complex **3**⊃RNH_3^+, for a given number of equivalents of guest, are listed in Table 2. These values were determined the integration of signals in the NMR spectra. It should be noted that the number of equivalents of guest added was chosen to ensure that a non-negligible fraction of the complex was formed. Inclusion was observed more readily with the more apolar ammonium ions (cLogP > 2), as fewer equivalents of the ammonium ion were needed to detect the signature of the inclusion complex compared to more polar guests (cLogP < 1). As in D_2O, the data clearly highlight the balanced interplay between the hydrophobic effect and the intrinsic binding properties of **3**.

To compare the binding of primary ammonium ions recognized by **3** in micelles with other receptors in micelles or D_2O, a binding constant (K_m) was derived from the proportion of **3**⊃RNH_3^+ and the number of guest equivalents added (Table 2). Upon comparison of these values to those obtained with homooxacalix[3]arene **2** in micelles,[49] it is clear that calix[5]arene **3** exhibits higher affinities. This result is noteworthy because, in organic solvents, receptor **3**

displays binding affinities similar to or even weaker than those of receptor **2**. When the binding abilities of **3** in micelles versus D_2O are compared, it is important to highlight that direct solubilization remains much more attractive in terms of binding affinities and recognition of bioactive ammonium ions. This suggests that receptor **3** could be a valuable candidate for sensing biological species containing primary ammonium groups in aqueous environments, owing to its ability to be grafted onto surfaces or particles through its carboxyl groups.

Conclusion

In this work, we describe the synthesis and recognition properties of a new *p-tert*-butylcalix[5]arene (**3**) functionalized with carboxyl groups on its small rim. Similarly to other calix[5]arenes, this receptor exhibits strong recognition properties with respect to primary ammonium ions in organic solvents, with a preference for short and linear guests due to the shape of its cavity. When solubilized in D_2O above pH 10, **3** exhibits unique binding properties governed by an interplay between the intrinsic recognition properties of calix[5]arenes and the hydrophobic effect. Notably, **3** binds various bioactive ammonium ions such as dopamine, tyramine, and lysine derivatives with affinities ($\log K_a > 3.9$) higher than those of the related receptor (**1)** functionalized with sulfonate arms. A second strategy, consisting in the incorporation of the receptor into DPC micelles, was also investigated to evaluate the binding properties of **3** in aqueous environments. Successful incorporation and recognition of primary ammonium ions are observed, with binding affinities one order of magnitude higher than those observed with homooxacalix[3]arene **2** incorporated into DPC micelles. However, **3** is reluctant with respect to the bioactive guests recognized in water, likely due to their hydrophilicity, which disfavors their incorporation into the micellar core. The transfer of systems previously reported to selectively bind primary ammonium cations in organic solvents to aqueous environments is rare. What is particularly remarkable about the system reported here is that it is not only selective but also exhibits high binding affinities for biologically active ammonium ions in water. We are confident that the excellent results obtained with receptor **3** in D_2O will pave the way for its use in sensing applications in water, notably through it being grafted onto surfaces.

Experimental Section

General Information. ^{1}H NMR spectra were recorded on a Jeol 600 MHz spectrometer, a Varian 400 MHz spectrometer or a Varian UNITY Inova 500 MHz spectrometer. NMR parameters (acquisition time, recycling times and signal accumulation) were chosen so as to ensure that quantitative data could be obtained for signals integration. The NMR spectra were

recorded at 298 K unless otherwise stated. Chemical shifts are expressed in ppm. Structural assignments were made with additional information from gCOSY and gHSQC experiments. High resolution mass spectra were obtained on a quadrupole time of flight (QTOF) spectrometer, used in 4 GHz mode (high-resolution mode). Compound **C[5]OH** was synthesized as previously described.[62] For every titration, slow host-guest exchange on the NMR chemical shift timescale was evidenced and the association constants were determined *via* integration of the appropriate signals. The binding affinities were calculated based on the total amount of guest added, irrespective of its protonation state. pH was measured without correction for the isotopic effect in D_2O.

Procedure to determine affinity constants in organic solvents. Receptor **3** was weighed and a volume of $CDCl_3$ or $CDCl_3/CD_3OD$ 4:1 was added to obtain **3** at the desired concentration (typically 1-2 mM). Concentrated stock solutions of the guests were prepared, and aliquots of these solutions were added stepwise to the 600 µL solution of **3** in the NMR tube.

Procedure to determine affinity constants in D_2O. The desired amount of **2** or **3** was weighed. Then, the volume of D_2O needed to reach a specific concentration (0.1-1 mM) was added. In the case of **3**, aliquots of NaOD were added to allow the solubilization. Concentrated stock solutions of the guests were prepared, and aliquots of these solutions were added stepwise to the 600 µL receptor solution in the NMR tube.

Micellar preparation. Micellar solutions were prepared by weighting the surfactant and adding D_2O to reach 20 mM DPC. 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 overnight and until a clear solution was observed. The exact concentration of the receptor in the different micellar solutions was determined using an external reference (^1H,^1H,^{11}H,^{11}H-Perfluoro-3,6,9-trioxaundecane-1,11-diol) of known concentration or comparing to the peak of DPC surfactant. When buffered conditions were required, Bis-Tris Propane buffer was added with surfactants and D_2O was added with further addition of DCl 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 **3** to avoid any dilution effect of the micelles during the titrations. Aliquots of these solution were added stepwise to the 600 µl solution in the NMR. Slow host-guest exchange on the NMR timescale was evidenced in all cases and the association constants were determined *via* integration of the signals of interest.

DOSY experiments. DOSY spectra were recorded at 298K on the Jeol 600 MHz spectrometer using the BPPDSTE (bipolar pulse pair double stimulated sequence). The diffusion delay was set to 120 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 up to 730 mT/s). At least 15 gradient values were used for each experiment to obtain accurate diffusion coefficients.

ASSOCIATED CONTENT:

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting information Statement

The Supporting Information is available free of charge at https://... Additional experimental details and NMR spectra (synthesis procedures, ^{1}H, ^{13}C, HSQC, COSY, IR, DOSY).

Notes

The authors declare no competing financial interest.

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