Turning on anion and betaine hosting by a small structural change of a biomimetic cavity: a case study

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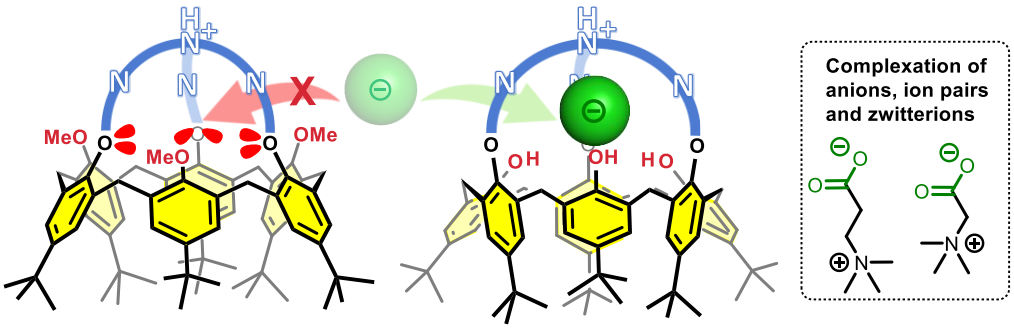


Abstract

This study explores the impact of a small structural modification on a biomimetic receptor. The hosting structure is a calix[6]arene capped by a tetraaza core. Two receptors are compared: one (**1**) presents three anisole units while the other (**2**) has three phenols. The latter was obtained with an excellent yield by selective demethylation thanks to a supramolecular strategy. Complexation studies showed that **[2.H]**$^+$ displays strong affinities for Cl^-, NO_3^-, HSO_4^-, whereas **[1.H]**$^+$ is non-responsive. The anions are embedded at the level of the small rim, hydrogen-bonded to the protonated cap and the phenol groups. Ternary complexes are obtained in the presence of ammoniums. Finally, **[2.H]**$^+$ shows high affinity for small zwitterions presenting a carboxylate and an ammonium groups separated by one or two carbon atom(s), not three, due to multi-point recognition. These results open routes to the design of new receptors for a variety of anionic and zwitterionic guests.

Keywords: Calixarenes – Anions – Supramolecular chemistry – Host-guest systems – Zwitterions.

Introduction

Molecular recognition is at the base of biological catalysis and signal transduction. In both cases, the process is highly sensitive to small structural changes of either the proteic or nucleic target or the ligand itself. These can be observed through structural modification induced by interactions with non-competitive inhibitors or regulators. Mutations can also lead

to activity changes, as well as post-translational modifications (e.g. methylation/demethylation of DNA bases).[1] In enzymes, it can be related to changes of amino acid residues in direct interaction with the substrate, but also can occur at remote distance, leading to a structural change at the active site or entrance channel. One way to study these phenomena and their relative importance from a fundamental point of view, is to design a biomimetic structure large enough to allow small functional and shape modifications and to evaluate their impact on hosting properties. Ultimately, such fundamental studies can open doors to the design of useful probes that are specific and sensitive.[2]

The calix[6]arene macrocycle provides a wonderful platform to design selective biomimetic receptors as well as an excellent tool to evaluate the consequences of small structural changes on hosting properties.[3] Calix[6]tren **1**[4] presents a polarized funnel shape, closed at the small rim by a grid-like nitrogenous core, while its π-basic cavity remains open to the solvent through the large rim (Figure 1). This system revealed itself to be a highly efficient and versatile receptor for neutral or cationic species.[5] Indeed, calixarene **1**, as a neutral but nitrogen-rich host (prone to H-bonding), is efficient for endo-complexation of ammonium guests. Besides, its *per*-protonated derivative, **[1.4H]$^{4+}$**, behaves as a remarkable receptor for small organic molecules presenting a dipolar skeleton, the complexation of which is driven by strong charge-dipole interactions and H-bonds between the polar guest and the tetracationic cap of the calixarene. Finally, coordination of metal ions M^{n+}, such as Zn^{2+} or Cu^{n+} to the tren core leads to selective endo-binding of neutral ligands.[6] This case study demonstrates the efficiency of a polarized receptor, being positively charged at one extremity (poly-aza cap), while presenting a concave π-basic cavity. The resulting combination of charge-dipole, hydrogen bonding, CH-π, and van der Waals interactions is at the base of the stabilization of supramolecular adducts.[5] Surprisingly, complexes **1.M^{n+}** did not display affinity for anions in spite of the strong Lewis acidity of the metal center. This was attributed to a repulsive second

coordination sphere at the small rim, constituted by the oxygen lone pairs directed into the cavity toward the guest coordination site.[7]

The goal of the present study is to evaluate and compare the host-guest properties toward anions of two protonated calix[6]tren receptors, which differ by only a small structural change (Figure 1): while calix[6]tren **1** presents three methoxy groups at the small rim, next to the recognition site, calix[6]tren^{OH} **2** is deprived of these methyl groups, thus presenting three phenolic OH functions, which are prone to H-bonding with included anions.

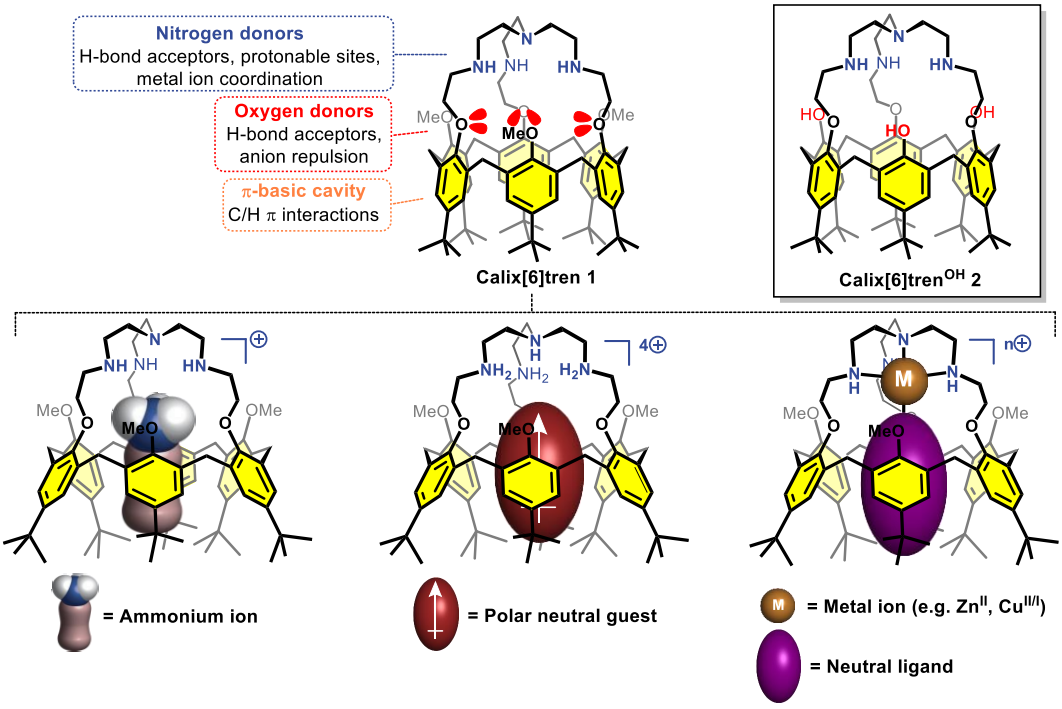


Figure 1. Host-guest properties of calix[6]tren **1**: a highly versatile and efficient receptor.[5] Inset: structure of targeted calix[6]tren^{OH} **2**.

Materials and methods

General Information. All solvents and reagents were obtained commercially. Anhydrous chloroform was purchased from Acros Organics. Other solvents and chemicals were of reagent grade and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded either on a Varian VNMRS 400 or 600, a Bruker Avance 300 or a JEOL JNM-ECZ 400 or 600 MHz

spectrometer equipped with a 5 mm probe. The solvent was used as internal standard for both ^{1}H and ^{13}C chemical shift referencing. $CDCl_3$ was filtered through a short column of basic alumina to remove traces of DCl. Most of the ^{1}H NMR spectra signals were assigned through 2D NMR analyses (COSY, HSQC, HMBC). For the edited-HSQC spectra, the blue signals are negatively phased and the red signals are positively phased. NMR spectra were recorded at 298 K unless otherwise stated. Chemical shifts are quoted on the δ scale and coupling constants (*J*) are expressed in Hertz (Hz). s: singlet, bs: broad singlet, d: doublet, bd: broad doublet, t: triplet, m: massif. MS analyses were performed using an ESI-MS spectrometer (LCQ-Deca, Finnigan, ThermoQuest) equipped with an ion-trap using the following settings: flow rate: 8 $\mu L.min^{-1}$, spray voltage: 5 kV, capillary temperature: 160°C, capillary voltage: –15 V, tube lens offset voltage: –30 V. HRMS analyses were performed using methanol and 0.1% formic acid as solvent on an ESI-MS apparatus (Q-TOF 6520 Agilent Technology) equipped with a TOF detector, or an ESI-MS spectrometer (ThermoScientific Orbitrap Exactive Plus) equipped with an orbitrap detector. IR spectra were recorded on a Perkin Elmer Spectrum One FTIR spectrometer equipped with a MIRacle^{TM} single reflection horizontal ATR unit (germanium crystal) or on a KBr window. Calix[6]tren **1** was prepared as previously reported.[4]

Synthesis of calix[6]tren^{OH} 2. Calix[6]tren **1** (50.3 mg, 0.040 mmol) was dissolved in anhydrous $CHCl_3$ (25 mL). Iodotrimethylsilane (86 µL, 0.605 mmol) was added in one portion and water (4 µL, 0.222 mmol) was added to the reaction mixture. The resulting solution was stirred at 50°C under an argon atmosphere in a sealed reactor for 40 min. The reaction was monitored by ESI-MS. Water (15 mL) was added to the reaction mixture and the organic phase was washed with an aqueous solution of $HClO_4$ (1M, 15 mL) for 30 min. The organic layer was washed with water (2 x 15 mL) and evaporated under reduced pressure to afford a brown solid which was purified by flash column chromatography (CH_2Cl_2 then CH_2Cl_2/CH_3OH 95:5). After evaporation of the solvents, the purified compound was dissolved in CH_2Cl_2 (10 mL) and

vigorously washed with NH_4OH (5%, 10 mL) for 15 min. The organic layer was washed with water (3 x 5 mL) and evaporated to dryness to give calix[6]tren^{OH} **2** as a beige powder (36.8 mg, 76%). Mp 238°C (dec.); IR (ATR): ν 3329, 2956, 1670, 1600, 1481, 1392, 1362, 1293, 1243, 1201, 1120, 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, 298 K): δ (ppm) 1.16 (s, 27H, *t*Bu), 1.19 (s, 27H, *t*Bu), 2.72 (m, 6H, CH_2N_{cap}), 2.94 (m, 6H, OCH_2CH_2), 3.12 (m, 6H, CH_2N_{cap}), 3.45 (d, *J* = 15 Hz, 6H, ArC*H*eq), 3.82 (m, 6H, CH_2O), 4.55 (d, *J* = 15 Hz, 6H, ArC*H*ax), 7.09 (s, 6H, Ar*H*), 7.17 (s, 6H, Ar*H*); ^{13}C NMR ($CDCl_3$, 75 MHz, 298 K): δ (ppm) 30.2, 31.5, 31.6, 34.1, 34.4, 47.6, 48.2, 53.4, 75.4, 77.4, 125.3, 126.3, 128.1, 133.2, 142.6, 147.0, 149.3, 151.3; HRMS calc. for $C_{78}H_{109}N_4O_6$ $[M+H]^+$: 1197.8347, found: 1197.8331.

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

When ^1H NMR spectra revealed two sets of signals for the complex, the guest and for the free receptor in slow exchange on the NMR time scale, association constants (log *K*) were determined *via* integration of the signals of the different species. The association constants were determined as the mean value of the constants calculated based on different spectra and with the integration of different signals. The error was then estimated as the difference between this mean value with the smallest and largest association constants determined.

When ^1H NMR spectra revealed one set of signals for the host-guest complex and for the free receptor in fast exchange on the NMR time scale, the association constants *K* were determined by nonlinear least-square-fitting of 1:1 binding profile to the chemical shift of either the $ArCH_2$ or the ArH protons. The error on the association constant was estimated as the standard

deviation of the association constant values provided by the fitting (10%).

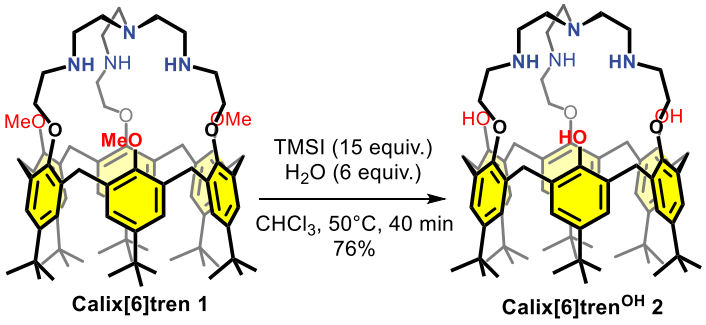
Molecular Modeling. Monte Carlo multiple minimum (MCMM)[8] conformational searches (100 steps per torsion angle, maximum 1000 steps in total) were performed in Schrödinger Release 2018-4, using the OPLS-2005 force field[9] without implicit solvation in Maestro MacroModel (version 11.8.012).

Results and Discussion

Synthesis and characterization of calix[6]tren^{OH} 2.

In order to prepare calix[6]tren^{OH} **2**, a selective *tris*-*O*-demethylation of calixtren **1** was envisioned. In a previously reported study related to the selective demethylation of various 1,3,5-trismethoxy-calix[6]arene derivatives, we showed that the efficiency of the nucleophilic attack of trimethylsilyl iodide (TMSI) was highly dependent on the orientation of the methoxy substituents relative to the macrocyclic cavity.[10] If the OMe groups point toward the inside of the receptor, the reaction is slow and dealkylation takes place at the level of the other phenolic units. If they are projected away from the sterically hindering macrocyclic structure, the demethylation is fast and selective. To force the expulsion of the OMe groups from the cavity, a *supramolecular assistance strategy* was developed. It consists in filling the calix[6]arene-based receptor with a guest that displays a high affinity. With calix[6]tren **1**, we tested the reaction conditions that were found to be optimal for the demethylation of other calix[6]arene systems, i.e. the use of a large excess of TMSI (15 equiv.) in anhydrous chloroform at 50°C. However, these conditions led to the recovery of the starting material with only a few percent of by-products as observed by ESI-MS after 3 hours of reaction (see SI). Actually, the ^{1}H NMR analysis of calix[6]tren **1** in $CDCl_3$ at 298 K indicated an average straight cone conformation ($\Delta\delta_{ArH}$ = 0.10 ppm) with OMe groups partially oriented toward the inside of the cavity (δ_{OMe} = 3.05 ppm), which disfavors the nucleophilic attack of iodide on the methyl groups. Calix[6]tren

1 is known to bind neutral guests, and in particular water, upon protonation of its basic tren cap (*vide supra*).[5] Interestingly, we noticed that the conformation of the calix[6]tren **1** structure changed drastically under acidic and wet conditions (ca. 1 equiv. of PicH and 2.5 equiv. of water). Indeed, a flattened cone conformation ($\Delta\delta_{ArH}$ = 0.56 ppm) with the methoxy groups directed toward the outside of the cavity (δ_{OMe} = ca. 3.6 ppm) was observed most likely due to the binding of a water molecule at the level of the polycationic cap (see SI). The impact of the addition of a few equivalents of water to the reaction mixture was thus evaluated. Besides its role as a guest, it was expected that water could react with TMSI, generating HI and thus the required acidic conditions. To our delight, when 6 equiv. of water were added in the reaction mixture, the reaction of calix[6]tren **1** with TMSI (15 equiv.) led to the fast and selective demethylation of the three anisole units (Scheme 1). Almost no by-products were observed as evidenced through monitoring the reaction by ESI-MS analysis (see SI) and pure calix[6]tren^{OH} **2** was thus isolated in 76% yield after flash chromatography. This result highlights the versatility of the *supramolecular assistance strategy* for the selective dealkylation at the small rim of calix[6]arenes.



Scheme 1. Synthesis of calix[6]tren^{OH} **2**.

Calix[6]tren^{OH} **2** was characterized by NMR spectroscopy in $CDCl_3$ at 298 K in its neutral and monoprotonated states (see SI). All signals were attributed through 2D NMR experiments (COSY, HSQC and HMBC, see SI). The NMR signature of **2** shows a C_{3v}

symmetrical calix[6]arene adopting an average straight cone conformation ($\Delta\delta_{ArH}$ = 0.07 ppm) very similar to calix[6]tren **1** (Figure 2a). The addition of one equiv. of picric acid (PicH) to a solution of **2** generated the *mono*-protonated derivative **[2.H]**$^+$. A larger $\Delta\delta_{ArH}$ shift (0.24 ppm) was then observed, attesting to a more flattened cone conformation, with possible embedment of water molecules, as for the parent compound **[1.H]**$^+$ (Figure 2b).

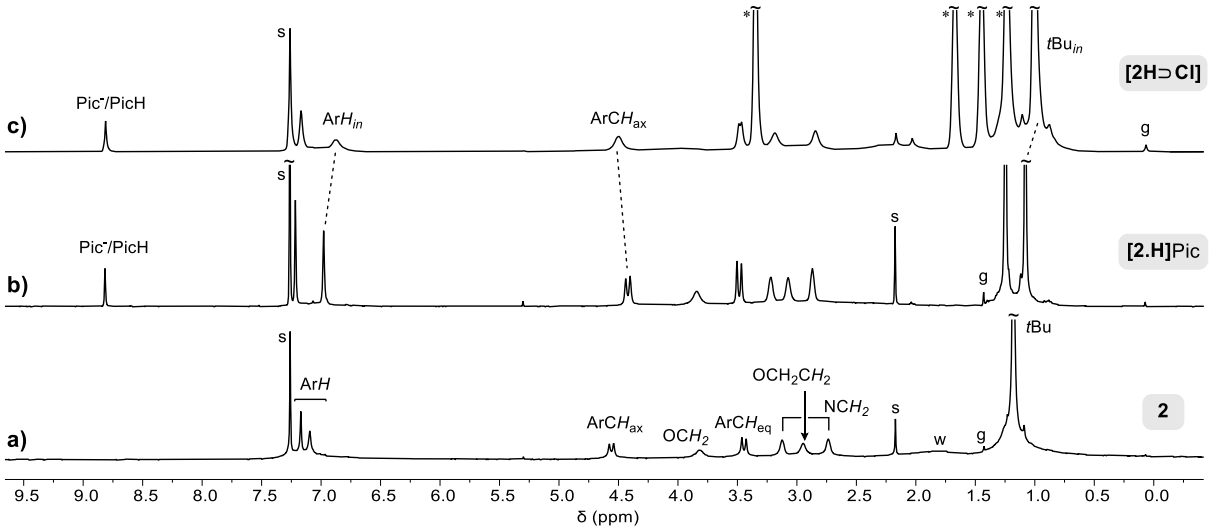
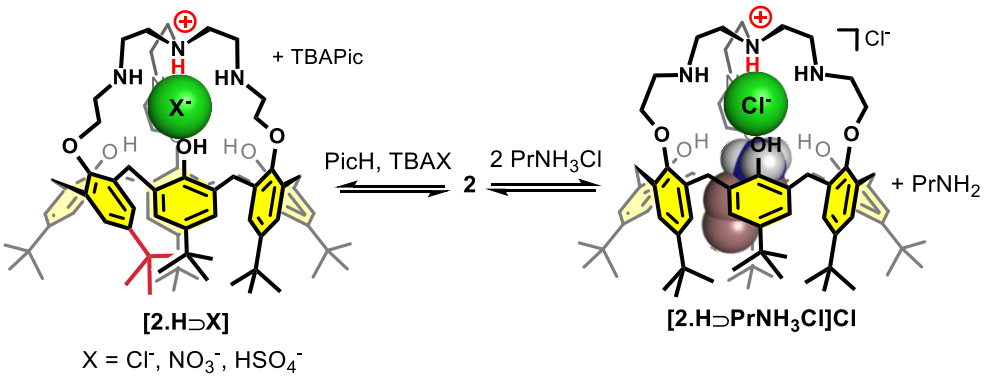


Figure 2. ^{1}H NMR spectra ($CDCl_3$, 400 MHz, 298 K) of a) calix[6]tren^{OH} **2** (3.10^{-3} M); b) **2** + 1.0 equiv. of PicH; c) **2** + 1.0 equiv. of PicH and 4.1 equiv. of TBACl. *: truncated signals belonging to TBA^+. S: solvent, w: water, g: grease.

Host-guest properties of calix[6]tren^{OH} 2 toward anions and ion pairs.

The binding properties of calix[6]tren receptors **1** and **2** towards anions were evaluated in $CDCl_3$ for solubility reasons. Both receptors, in their neutral form, remained unaffected by the addition of a large excess of tetrabutyammonium chloride (TBACl) according to NMR analyses. However, complexation of Cl^- readily occurred with the monoprotonated derivative **[2.H]**$^+$, and not with **[1.H]**$^+$. Indeed, titration of **[2.H]**Pic by TBACl showed a shift of the ^{1}H signals corresponding to the ArH_{in}, ArCH_{ax} and *t*Bu$_{in}$ protons, which is characteristic of a conformational change (Figure 2c and see SI). The conformation of the calixarene core of complex **[2.H⊃Cl]** is more flattened than that of **[2.H]**$^+$ ($\Delta\delta_{ArH}$ = 0.29 vs. 0.24 ppm). Both ArH_{in}

and *t*Bu*in* protons present very broad ^{1}H signals, which is typical of the self-inclusion of one out of the three aromatic units linked to the cap, with a fast exchange process on the NMR shift time scale between the three aromatic units (see structure of **[2.H⊃Cl]** displayed in Scheme 2).[11] Similar shifts of the ^{1}H signals were observed when $TBANO_3$ was used in place of TBACl, showing that the complexation of NO_3^- proceeds through a similar recognition mode. In both cases, almost no change was observed after the addition of more than 1 equiv. of the TBA salt, indicating affinities for these anions that are too high[12] to be determined by NMR. The binding of SO_4^{2-} was also evaluated, but in this case a more complicated binding process was observed (see SI). Indeed, the addition of TBA_2SO_4 to **[2.H]**Pic led to the formation of the neutral complex **[2.H⊃HSO$_4$]** through deprotonation of a fraction of the receptor. An approximately 1:1 mixture of **[2.H⊃HSO$_4$]** and **2** was produced in the presence of *ca.*1 equiv. of TBA_2SO_4. This indicates the much higher stability of the neutral host-guest adduct **[2.H⊃HSO$_4$]** in chloroform (a non-polar solvent) compared to the anionic complex that would result from the embedment of SO_4^{2-}. This first set of experiments shows that the removal of the methyl groups is key for the complexation of anions by protonated calix[6]tren-based receptors. According to the associated NMR changes, it likely occurs within the interior of the cationic tren cap of **[2.H]**$^+$ through ionic interaction associated to multiple H-bonds with the NH^+ and phenol groups present at the small rim (Scheme 2).



Scheme 2. Host-guest properties of calix[6]tren^{OH} **2.H**$^{+}$ toward anions and ion pairs in chloroform.

We next investigated the possibility to host an ammonium ion in the cavity, next to a small anion bound in the protonated tren cap.[13] Hence, a $CDCl_3$ solution of **2** was titrated by $PrNH_3Cl$. The first equivalent of the ammonium salt led to the quantitative protonation of the host and formation of complex **[2.H⊃Cl]** (Figure 3a-b). Above this stoichiometry, a new species, in slow exchange on the NMR shift time scale with **[2.H⊃Cl]**, was detected progressively over the course of the titration (Figure 3c-e). The appearance of high field signals (< 0 ppm) clearly showed the inclusion of the propyl chain of the ammonium ion in the heart of the polyaromatic cavity (see Figure 5e for the complexation induced shift (CIS) values of the guest). This species corresponds to the endo-complex **[2.H⊃PrNH$_3$Cl]Cl** with the anion sandwiched between the NH^+ proton and the ammonium ion accommodated in the cavity (Scheme 2). The complexation of the ion pair $PrNH_3Cl$ was quantitatively obtained upon the addition of *ca.* 4 equiv. of this salt and the 1:1 host-guest stoichiometry was confirmed by the ratio of the integrals of the calixarene and included ammonium signals. The slow *in-out* exchange process of the ammonium ion allowed us to estimate a binding constant of 2.2(±0.5) × 10^3 M^{-1} for the complexation of $PrNH_3^+$ by **[2.H⊃Cl]**.[14] Complex **[2.H⊃PrNH$_3$Cl]Cl** was characterized by 2D NMR experiments (COSY, HSQC, HMBC, see SI). Upon complexation of the ion pair, the calixarene core adopts a flattened cone conformation ($\Delta\delta_{ArH}$ = 0.42 ppm and $\Delta\delta_{tBu}$ = 0.51 ppm). The HMBC spectrum showed that the *t*Bu groups closing the entrance of the cavity belong to the aromatic moieties linked to the cap (see structure displayed in Scheme 2) (see SI). The significant downfield shifts of the OCH_2CH_2 protons (CIS_{OCH2} = 0.78 ppm and $CIS_{OCH2CH2}$ = 0.79 ppm, Figure 3e and Scheme 2) indicate that the ethylene linkers between the tren and calixarene core are projected away from the small rim. This stands in contrast to calixarene **2** and complex **[2.H⊃Cl]**, and is attributable to the presence of the ammonium head

of the guest at the level of the calixarene small rim. Finally, when $PrNH_3Pic$ (up to 6.5 equiv.) was used in place of $PrNH_3Cl$, only protonation of calix[6]tren^{OH} **2** occurred. However, subsequent addition of TBACl (9 equiv.) triggered the inclusion of the ammonium ion into the cavity, leading to formation of the complex **[2.H⊃PrNH$_3$Cl]$^+$** (see SI). This result further highlights that the presence of the anion at the level of the protonated tren cap is crucial for the recognition of the ammonium ion. Interestingly, no endo-complex was observed upon progressive addition of $PrNH_3Cl$ (up to 10 equiv.) to calix[6]tren **1** in $CDCl_3$ even at low T (i.e. 238K) (see SI). Actually, **1** is able to host an ammonium ion only in presence of a low-coordinating anion such as Pic^-. All these results suggest that receptor **2** can recognize $PrNH_3Cl$ as a contact ion pair, thus avoiding the highly energetically unfavorable dissociation of the ion pair.

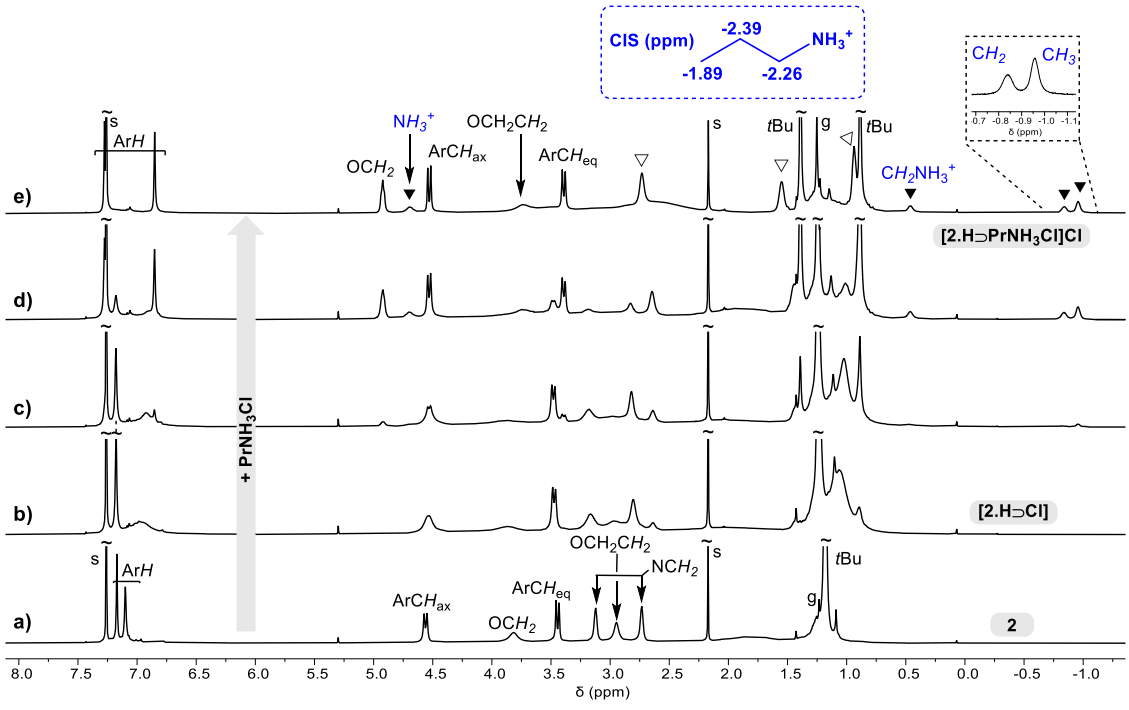


Figure 3. ^{1}H NMR spectra ($CDCl_3$, 600 MHz, 298 K) of a) calix[6]tren^{OH} **2** ($c \approx 3.10^{-3}$ M), and after addition of b) 0.8, c) 1.2, d) 2.2, e) 4.2 equiv. of $PrNH_3Cl$. ▼: $PrNH_3Cl_{in}$, ▽: $PrNH_3Cl_{out}$. s: solvent, g: grease.

To gain a more detailed insight into the recognition modes, structures of the complexes **[2.H⊃Cl]** and **[2.H⊃PrNH$_3$Cl]$^+$** were investigated *in silico* by molecular mechanics conformational analysis (see Experimental section for details). In both cases, the position of the guests and the conformation adopted by host **[2.H]$^+$** are consistent with the NMR data (Figure 4). The calixarene skeleton adopts a flattened cone conformation with the *t*Bu groups of the aromatic units linked to the tren cap in *endo* position relative to the three others, thus defining the cavity entrance. The anion is bound in a highly polar pocket delimited by the protonated tren cap, the three corresponding ethylene linkers and the calixarene small rim. For complex **[2.H⊃Cl]**, the cavity is occupied by one of the *t*Bu groups and the chloride anion is wrapped by an ensemble of well-oriented dipoles provided by N-H, O-H and polarized C-H bonds. In the tren cap, the cationic apical nitrogen and one neutral nitrogen atom orient their N-H bond towards the anion whereas the two other neutral N-H bonds are facing away. Lastly, the flattened conformation adopted by the calixarene projects all three phenol and OCH_2 groups in a direction that allows OH and CH/anion interactions. All in all, we can see 2 NH, 3 OH and 5 CH favorable contacts in addition to the ionic interaction between the cationic N^+ and the anion ($N^+ \ldots Cl^-$ = 3.3 Å). For complex **[2.H⊃PrNH$_3$Cl]$^+$**, the conformation of the calixarene skeleton is slightly different as it is now wrapping the ammonium guest: it is less flat, the aromatic units linked to the tren cap are pushed towards the outside by the alkyl chain of the guest. All four N-H bonds of the cap are directed towards the anion and no appreciable C-H contact can be observed. The oxygen lone pairs at the small rim are now directed inward, towards the ammonium group of the guest except for one phenol O-H that is oriented towards the anion, whereas the two others are H-bonded to the oxygen atom of the neighboring aromatic units. The anion is sandwiched between the two cationic centers with distances of *ca.* 3.2 Å.

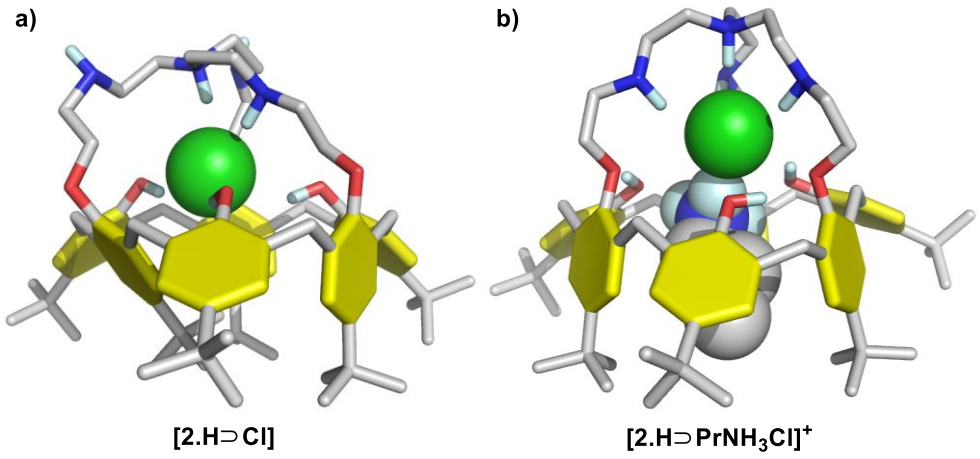


Figure 4. Lowest energy conformers (OPLS-2005) of the complexes a) **[2.H⊃Cl]** and b) **[2.H⊃PrNH$_3$Cl]$^+$**. Stick representation for the host and space filling representation for the guests. Most of the hydrogen atoms are omitted for clarity. Selected contact distances (Å) for **[2.H⊃Cl]**: H_{NH^+}…Cl^-: 2.3, H_{NH}…Cl^- 2.3; H_{NCH2}…Cl^- 3.1, 2.9; H_{OH}…Cl^- 2.4, 2.6, 2.9; H_{OCH2}…Cl^- 2.9, 3.0, 3.0. For **[2.H⊃PrNH$_3$Cl]$^+$** H_{NH^+}…Cl^-: 2.2; H_{NH}…Cl^- 2.2, 2.4, 2.5; H_{OH}…Cl^- 2.6; O_{ArOH}… H_{RNH3+} 1.9, 2.1.

Recognition of zwitterions.

Having shown that **[2.H]$^+$** can bind small anions such as chloride at the level of the protonated tren cap while accommodating an ammonium guest in the cavity, we envisioned that this host could be an excellent candidate for the complexation of ditopic guests as for example small zwitterionic betaine and carnitine derivatives. Indeed, the design of efficient cavity-based hosts for zwitterions remains a challenge and only a few examples have been described over the past two decades.[15] The complexation of three zwitterions differing by the length of the alkyl spacer between the anionic and cationic groups (Figure 5) was thus evaluated by ^{1}H NMR spectroscopy in a $CDCl_3$/CD_3OD 98:2 mixture.

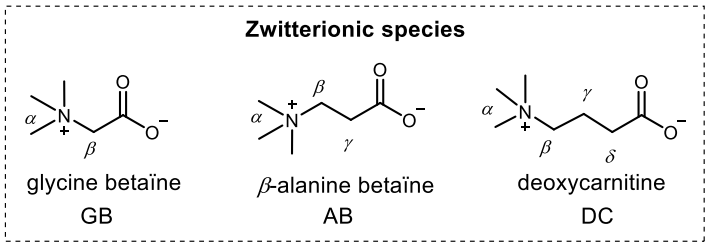


Figure 5. Structure of the zwitterions that were tested.

As expected, the ^{1}H NMR spectrum of the neutral host calix[6]tren^{OH} **2** was not affected by the addition of either GB, AB or DC even after a prolonged time (24 h). However, the progressive addition of picric acid to a solution containing receptor **2** and either GB or AB led, in each case, to the appearance of a new species (in slow exchange on the NMR shift time scale) displaying signals in the high field region of the NMR spectra. The new species were obtained quantitatively after the addition of at least 1 equiv. of the zwitterionic species (Figure 6) and were clearly identified as the 1:1 host guest complexes **[2.H⊃GB]**$^{+}$ and **[2.H⊃AB]**$^{+}$ through 2D NMR experiments (see SI).

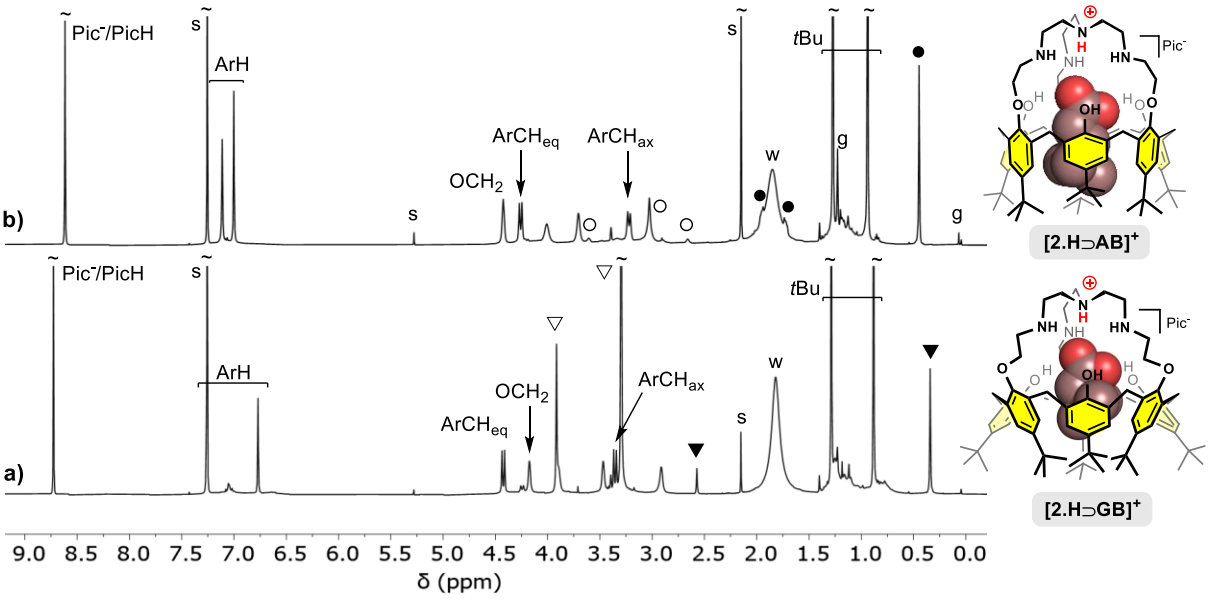


Figure 6. ^{1}H NMR spectra ($CDCl_3/CD_3OD$ 98:2, 600 MHz, 298 K) of calix[6]tren^{OH} **2** (c ≈ 3 mM) in presence of 2.3 equiv. of PicH and a) GB (2 equiv.); b) AB (1 equiv.). ▼: GB_{in}, ▽: GB_{out}, ●: AB_{in}, O: AB_{out}. S: solvent, w: water, g: grease.

In both cases, the high-field singlet (≤ 0.5 ppm) was attributed to the Me_3N^+ group of the guest included in the calix cavity (see Table 1). The higher CIS values for this group, compared to those of the CH_2 or $(CH_2)_2$ spacers of the guests, indicate that the ammonium moiety sits next to the aromatic walls of the calix. A 2D ROESY NMR analysis of complex **[2.H⊃AB]**$^+$ showed correlations between the protons of the ethylene group of bound AB and the $ArCH_{ax}$ and OCH_2 protons of the receptor (see SI). Hence, the recognition process is ditopic, placing the carboxylate group in the protonated tren cap with charge-charge interaction and hydrogen bonding to the aza and phenol functions present at the small rim, while the ammonium group interacts with the cavity through cation-π and CH-π bonds. When compared to **[2.H⊃AB]**$^+$ ($\Delta\delta_{ArH}$ = 0.11 ppm), the larger $\Delta\delta_{ArH}$ (0.49 ppm) and smaller δ_{OCH2} values for **[2.H⊃GB]**$^+$ indicate that the calixarene adopts a much more flattened conformation with a less inflated cap in order to optimize the interactions with the smaller GB guest (see structures in Figure 6). These conformational differences nicely illustrate the induced fit processes operating in the case of such flexible calix[6]arene-based receptors.

Table 1. Chemical shifts and CIS observed by ^{1}H NMR upon complexation of GB and AB by **[2.H]**$^+$.[a]

	[2.H⊃GB]$^+$			**[2.H⊃AB]**$^+$		
	OCH_2	**Me_3N^+ (guest)**	**CH_2 (guest)**	**OCH_2**	**Me_3N^+ (guest)**	**CH_2 (β/γ) (guest)**
δ (ppm)	4.18	0.34	2.57	4.43	0.5	1.94/1.74
CIS (ppm)	–	–2.96	–1.34	–	–2.63	–0.70/–1.85

[a] Signal assignment was performed using 2D NMR spectra (COSY, HSQC, HMBC).

In contrast to GB and AB, no inclusion of DC was observed under the same experimental conditions. The selectivity of receptor **[2.H]**$^+$ in favor of zwitterions having a 1-

or 2-carbon atom spacer highlights the necessary complementarity between the receptor and these guests at both sites of interaction (protonated tren cap and calix cavity). This requires an adequate distance between the carboxylate and ammonium moieties of the guest, as previously observed with other calix[6]arene receptors.[15] From titration experiments, binding constants (to **[2.H]**$^+$) of 12(±4) x 10^3 M^{-1} and 3.0(±0.5) x 10^3 M^{-1} were obtained for AB and GB, respectively. These values are of the same order of magnitude (for GB) or higher (for AB) than that of $PrNH_3^+$ to the chloride complex **[2.H⊃Cl]**$^+$ [2.2(±0.5) × 10^3 M^{-1}]. From competition experiments carried out with GB and AB, a more accurate relative affinity $K_{AB/GB}$ = 5(±0.2) was obtained in the mixture $CDCl_3/CD_3OD$ 98:2, confirming a better fit of the size of AB for ditopic interaction in the calixarene host. Interestingly, the binding of GB and AB was also observed in very competing environment (i.e. $CDCl_3/CD_3OD$ 1:1 mixture) (see SI). In this more polar and protic solvent mixture, the relative affinity $K_{AB/GB}$ reached a value of 12.5(±0.5), as the medium better solvates free GB which is more polar than AB. Finally, no inclusion of ammonium TMA^+ by **[2.H]**$^+$ was detected and no zwitterion complexation was observed with host **[1.H]**$^+$ under similar experimental conditions (see SI).

Comparison between the recognition properties of calix[6]tren 1 and calix[6]tren^{OH} 2.

The above NMR studies show that the cationic tren cap of monoprotonated calix[6]tren^{OH} **[2.H]**$^+$ is able to recognize anions in chloroform. In strong contrast, the related monoprotonated calix[6]tren **[1.H]**$^+$ and the corresponding neutral hosts **1** and **2** are reluctant to bind anions. Protonation of the cap and demethylation of the anisole units are thus keys for the binding of anions. Among the four hosts, only **[2.H]**$^+$ combines these two features. Protonation of the basic tren unit is a prerequisite because it allows the establishment of strong ionic interaction between the cationic cap and the anion. The different behavior between **[1.H]**$^+$ and **[2.H]**$^+$ can be rationalized by conformational considerations at the level of the small rim:

(1) In the case of calixarene **1**, all cationic host-guest complexes that have been characterized so-far adopt the same flatted cone conformation where the anisole units project their methoxy groups away from the cavity, whereas the oxygen atoms connected to the tren cap orient their lone pair toward the center (as schematized in Figure 1), which induces favorable dipole-cation interactions, but unfavorable dipole-anion interactions.

(2) In the case of calixarene **2**, the inclusion of an anion into the protonated tren cap leads to an inversion of the relative position of the aromatic units: those connected to the tren cap project their *t*Bu substituent in *endo*-position, while those of the phenol units are in *exo*-position. For **1**, this conformational change would project the methoxy groups toward the cavity and either a steric clash with the anion or unfavorable dipole-anion interactions with the MeO-lone pairs would result. However, for **2**, it directs the phenol functions next to the binding site. In the case of **[2.H⊃Cl],** all oxygen atoms and their corresponding lone pairs point towards the outside, away from the embedded anion, whereas the three phenol functions are H-bonded to the anion. In the case of **[2.H⊃PrNH$_3$Cl]**$^+$, the *O*-lone pairs are differently oriented, allowing also two favorable OH/Cl$^-$ contacts as well as two H-bonds between phenolic oxygen atoms and the guest ammonium head.

This important difference in behavior illustrates the dramatic impact of a small chemical and conformational change of the recognition site on the binding properties of a molecular receptor.

Conclusion

The goal of this study was to evaluate the impact of a small structural modification of a macrocyclic receptor, next to the main interaction site, on its binding properties. The first challenge was the selective demethylation of three methoxy groups present at the small rim of

the previously described calix[6]arene tren-capped calix[6]tren receptor **1** to yield new receptor **2**. The key was to find conditions to control the conformation of the calix[6]arene structure of **1**, in order to orient properly the methoxy groups for a nucleophilic demethylation by TMSI. Pure compound **2** was isolated with an excellent yield, thus allowing further studies of its host-guest properties. Host-guest studies with anions, ion pairs and zwitterions were undertaken under acidic conditions where the host is mono-protonated. These studies, monitored by ^{1}H NMR, showed that, whereas host **[1.H]**$^{+}$ is totally unreactive toward anions, host **[2.H]**$^{+}$ binds them strongly into the cationic tren-based cap. When an anion such as Cl^{-} is bound to the trenH^{+} cap of host **[2.H]**$^{+}$, the calixarene cavity remains free for accommodating an ammonium ion. Finally, studies with betaines revealed that they are also readily bound by host **[2.H]**$^{+}$. The recognition process is ditopic, placing the carboxylate function in the trenH^{+} cap, at the small rim, and the tertiary ammonium moiety in the heart of the calix[6]arene aromatic core. As a result, the system is highly selective about the spacer length separating the carboxylate and ammonium functions.

Hence, the strong contrast between the hosting properties of **[1.H]**$^{+}$ and **[2.H]**$^{+}$ stems from one single structural modification: OCH_3 vs. OH groups at the small rim, respectively. The host-guest chemistry of **[1.H]**$^{+}$ is under the control of polar interactions with *O*-alkyl groups, as this compound presents inward-oriented lone pairs that are repulsive towards embedded anions. In contrast, the host-guest chemistry of **[2.H]**$^{+}$ is under the control of favorable interactions with the OH phenol groups that orient their dipole outward and provide a H-bonding site for anions.

The high selectivity observed in the case of betaines opens the door to the design of sensitive and selective receptors for a variety of biologically important molecules, as the large rim of receptor **[2.H]**$^{+}$ is functionalizable almost at will.[3]

Disclosure statement

No potential conflict of interest was reported by the author.

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Supporting information

ESI-MS spectra of demethylation reaction of **1**, 1D and 2D NMR spectra of **2** and **[2.H]**$^+$, NMR studies of complexion of anions, ion pairs and zwitterions.

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