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Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity

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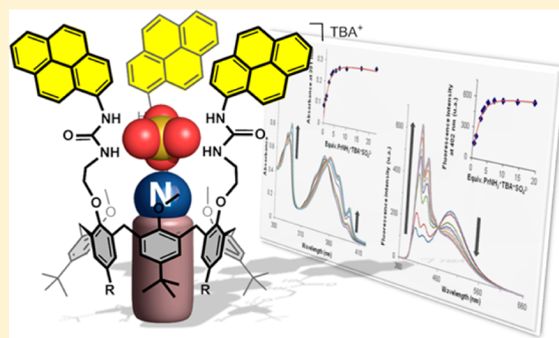
S *Supporting Information*

ABSTRACT: The association of a concave macrocyclic compound to one or multiple fluorophores is an appealing strategy for the design of chemosensors. Indeed, as with biological systems, a cavity-based selectivity can be expected with such fluorescent receptors. Examples of calix[6]arene-based systems using this strategy are rare in the literature, and to our knowledge, no examples of fluorescent receptors that can bind organic contact ion pairs have been reported. This report describes the straightforward synthesis of fluorescent calix[6]arene-based receptors **4a** and **4b** bearing three pyrenyl subunits and the study of their binding properties toward anions and ammonium salts using different spectroscopies. It was found that receptor **4a** exhibits a remarkable selectivity for the sulfate anion in DMSO, enabling its selective sensing by fluorescence spectroscopy. In $CDCl_3$, the receptor is able to bind ammonium ions efficiently only in association with the sulfate anion. Interestingly, this cooperative binding of ammonium sulfate salts was also evidenced in a protic environment. Finally, a cavity-based selectivity in terms of size and shape of the guest was observed with both receptors **4a** and **4b**, opening interesting perspectives on the elaboration of fluorescent cavity-based systems for the selective sensing of biologically relevant ammonium salts such as neurotransmitters.



■ INTRODUCTION

The design of artificial receptors that can selectively bind charged or neutral species with high affinity is a major objective in supramolecular chemistry.[1] Indeed, such receptors could find many applications in various areas and can for example be envisaged for the sensing of chemical species in the fields of biological and environmental analyses.[2] A classical strategy for the design of such chemosensors consists of grafting one or multiple fluorophores onto a molecular receptor that displays a high selectivity for a given guest.[3] Indeed, fluorescence spectroscopy is particularly sensitive and allows the detection of chemical species at nanomolar concentrations.[4] For the elaboration of the molecular receptor, the use of cavity-based macrocyclic compounds[5] is particularly attractive because, in strong relation to natural systems, it can be expected that the cavity will ensure very high selectivity.[6] Calixarenes[7] are well-known concave macrocyclic compounds that have been widely used for the development of fluorescent chemosensors.[8] However, these fluorescent systems have been obtained quasi-exclusively using calix[4]-arenes, whose cavities are too small for the recognition of organic guests, and therefore, they have been mostly used as a platform for the preorganization of a recognition site outside of the cavity.[9] Surprisingly, only a few papers have described the use of fluorescent calix[6]arenes,[10] despite the fact that these larger oligomers can accommodate organic guests in their cavities.[11] Moreover, to our knowledge, there are no examples of fluorescent receptors that can bind organic contact ion pairs,[12] as most of the systems are devoted to the recognition of cations,[13] anions,[14] or neutral guests.[15]

As part of our continuous interest in the synthesis and study of calix[6]arene-based receptors for neutral[16] or charged[17] species, we wanted to exploit the cavity-based selectivity of these receptors for the design of highly selective fluorescent systems. In this regard, we were interested in the elaboration of fluorescent derivatives of calix[6]tris(urea) compounds **1a** and **1b** (Figure 1). Indeed, it was reported that these receptors can efficiently bind either anions or, in a cooperative way, organic contact ion pairs.[18] The three converging urea groups allow strong binding to anions, which in turn can lead to the strong

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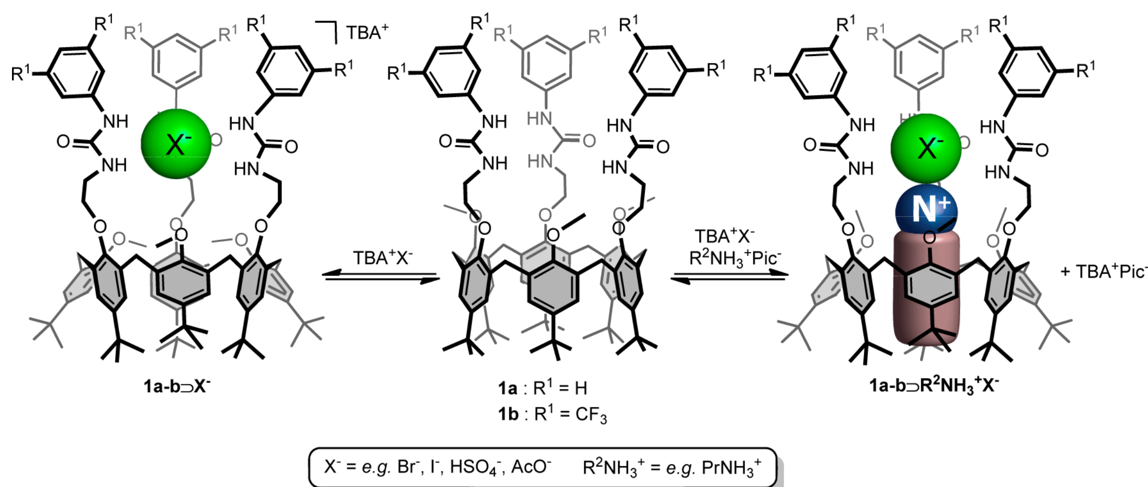
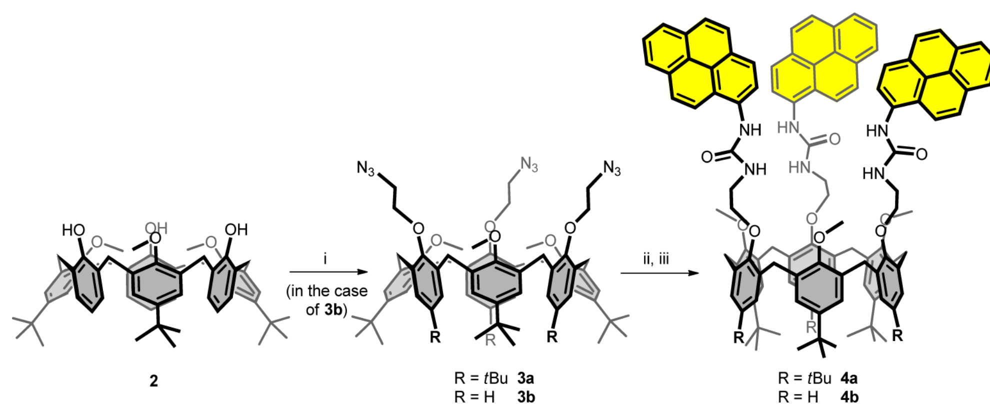


Figure 1. Host−guest properties of calix[6]tris(phenylurea) receptors **1a** and **1b** toward anions and contact ion pairs.

Scheme 1. Synthesis of Calix[6]tris(pyrenylurea) Receptors 4a and 4b[a]



[a]Reagents and conditions: (i) 2-azidoethyl-4-methylbenzenesulfonate, NaH, THF, reflux, 77%; (ii) PPh_3, CO_2, THF, rt; (iii) pyren-1-amine, THF, 50 °C, 70% and 35% overall yields from **3a** and **3b**, respectively.

binding of an ion paired ammonium accommodated in the calixarene cavity. The proximity of the two binding sites is crucial in the recognition process as it circumvents the highly energetically unfavorable separation of the cobound ions.[19] It is noteworthy to mention that calix[6]tris(urea) receptors **1a** and **1b** constitute one of the rare examples of molecular receptors able to bind organic contact ion pairs.[20]

With the aim of studying the potential of transforming system **1a** into a sensor, we have grafted fluorophores in close proximity to the tris(urea) binding site. The pyrenyl group was chosen as the fluorophore[21] as the variation of the excimer to monomer emission intensity ratio can yield information on conformational changes of the receptor upon binding.[22] Besides the synthesis of new fluorescent calix[6]arene-based receptors, the aims of this study were to see (i) whether the binding properties of the receptors were preserved despite the introduction of bulky fluorophores in close proximity to the urea groups, (ii) whether it was possible to give rise to an effective anion or ion-pair sensor in terms of detection by fluorescence spectroscopy, and (iii) whether cavity-based selectivity, notably in terms of size and shape complementarities, could be associated with the sensing process. Herein we describe the synthesis of these new fluorescent calix[6]arene-based receptors and the study of their binding properties toward anions and ammonium salts using NMR, UV−vis absorption, and fluorescence spectroscopy.

■ RESULTS AND DISCUSSION

Synthesis and Characterization of Calix[6]tris(pyrenylurea) Receptors 4a and 4b. The synthesis of two receptors that offer cavities of different size and shape, **4a** and **4b**, was undertaken. Calix[6]tris(azido) **3b** was first obtained in 77% yield through alkylation of the partially de-*tert*-butylated 1,3,5-tris(methoxy)calix[6]arene **2**[23] with an excess of 2-azidoethyl-4-methylbenzenesulfonate.[24] A one-pot, two-step procedure consisting of a domino Staudinger/aza-Wittig reaction (PPh_3/CO_2) and a subsequent addition of pyren-1-amine afforded the desired calix[6]tris(pyrenylurea) compounds **4a** and **4b** in 70% and 35% overall yields from calix[6]tris(azido) precursors **3a**[25] and **3b**, respectively (Scheme 1).

The ^{1}H NMR spectra of calix[6]tris(pyrenylurea) compounds **4a** and **4b** in $CDCl_3$ show broad signals characteristic of C_{3v}-symmetric species (for **4a**, see Figure 2a). When the spectra were recorded in a competing solvent (DMSO-d_6), sharp NMR signals characteristic of a major C_{3v}-symmetric flattened cone conformation were observed for both systems ($\Delta\delta_{ArH}$ = 0.70 and 0.72 ppm for **4a** and **4b**, respectively) with the methoxy groups pointing inside the cavity (δ_{OMe} = 2.12 and 2.60 ppm for **4a** and **4b**, respectively; for **4a**, see Figure 2b). In strong analogy with the parent receptors **1a** and **1b**, this solvent-dependent conformational behavior indicates intramolecular self-association of the urea groups through hydrogen-bonding interactions in apolar

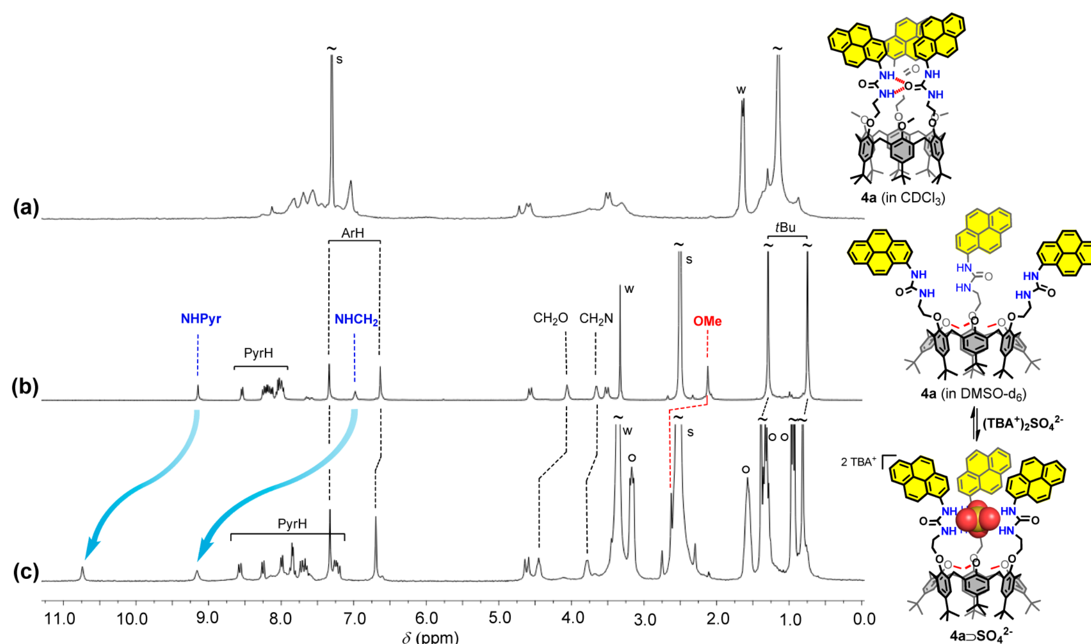


Figure 2. ^{1}H NMR spectra of **4a** at 298 K: (a) in $CDCl_3$ (300 MHz); (b) in DMSO-d_6 (400 MHz); (c) in the presence of 2.5 equiv of $(TBA^+)_2SO_4^{2-}$ in DMSO-d_6 (300 MHz). Labels: ○, TBA$^+$; s, solvent; w, residual water.

Table 1. Affinities of Hosts 4a and 4b toward Different Charged Species at 298 K

complex formed[a]	solvent	log K_{NMR}[b]	log K_{UV-vis}[b]	log K_{fluo}[b]
4a⊃SO$_4^{2-}$	DMSO-d_6	3.4 ± 0.3	3.6 ± 0.4	3.9 ± 0.4
4a⊃SO$_4^{2-}$	$CDCl_3$	>4	5.1 ± 0.5	5.4 ± 0.4
4a⊃PrNH$_3^+$SO$_4^{2-}$	$CDCl_3$	>4	5.3 ± 0.6	5.4 ± 0.1
4a⊃PrNH$_3^+$SO$_4^{2-}$	$CD_3OD/CDCl_3$ (1:11)	3.9 ± 0.1	4.0 ± 0.3	–
4a⊃PyrroNH$_2^+$SO$_4^{2-}$	$CDCl_3$	>4	4.1 ± 0.5	4.5 ± 0.2
4b⊃PrNH$_3^+$SO$_4^{2-}$	$CDCl_3$	>4	5.0 ± 0.2	4.8 ± 0.2

[a]TBA$^+$ was used as the counterion in all cases. [b]K = [complex]/([host][guest]). K_{NMR}, K_{UV-vis}, and K_{fluo} refer to binding constants determined by NMR, UV–vis absorption, and fluorescence spectroscopy, respectively.

solvents such as $CDCl_3$ (see the structure displayed in Figure 2a, the exchange process between the three nonequivalent urea groups being fast on the NMR chemical shift time scale) and, on the contrary, separation of the bulky pyrenylurea groups in competing solvents (see the structure displayed in Figure 2b).[26] It should be noted that in the particular case of **4a** and **4b**, the self-association of the urea arms could also be due in part to π-stacking interactions between the pyrenyl moieties. Calix[6]-tris(pyrenylurea) receptors **4a** and **4b** exhibit UV–vis absorption bands at 280, 346, and 389 nm in chloroform and at 279, 348, and 390 nm (**4a**) or 285, 354, and 390 nm (**4b**) in DMSO.[27]

Anion Complexation Properties of 4a. The ability of calix[6]tris(pyrenylurea) **4a** to bind anions of various geometries (i.e., Cl^-, AcO^-, HSO_4^-, and SO_4^{2-}) was first investigated in DMSO-d_6 by NMR spectroscopy through the progressive addition of the corresponding tetra-n-butylammonium salts ($TBA_n^+X^{n-}$). Except for a weak downfield shift of the urea NH signals, the NMR spectrum of **4a** remained quasi-unchanged upon the addition of a large excess (up to 20 equiv) of either Cl^-, AcO^-, or HSO_4^-. The absence of a conformational reorganization of the calixarene core suggests an extremely weak binding of these anions by only one of the three ureido groups. In strong contrast, upon the progressive addition of $(TBA^+)_2SO_4^{2-}$, downfield shifts of the CH_2O, CH_2N, and OCH_3 signals and upfield shifts of some of the pyrenyl signals were observed (Figure 2c). Significant downfield shifts of the urea NH signals ($\Delta\delta_{NH}$ = 1.60 and 2.18 ppm) were also observed, which clearly indicates strong hydrogen-bonding interactions between the urea groups and the anion. All of these NMR data are compatible with strong binding of the sulfate anion by the convergent NH groups of the pyrenylurea arms, which are thus projected above the small rim upon anion complexation. The resulting host–guest complex **4a⊃SO$_4^{2-}$** displays a major flattened cone conformation ($\Delta\delta_{ArH}$ = 0.63 ppm and $\Delta\delta_{tBu}$ = 0.57 ppm) with the methoxy groups pointing inside the cavity (δ_{OMe} = 2.55 ppm), as illustrated in Figure 2c. It is noteworthy to mention that only one set of signals is apparent over the course of the titration, evidencing fast host–guest exchange on the NMR chemical shift time scale. Job's plot experiments[28] by UV–vis spectroscopy indicate a 1:1 binding stoichiometry.[27] An association constant of log K = 3.4 ± 0.3 (Table 1) was determined for the binding of the sulfate anion by monitoring the complexation-induced shifts (CISs) of appropriate signals of host **4a** (i.e., signals displaying significant shifts upon complexation and no overlap).[27] UV–vis and fluorescence titrations in DMSO afforded K values on the same order of magnitude (log K = 3.6 ± 0.4 and 3.9 ± 0.4, respectively).[27] The fluorescence spectra show that the monomer emission (402 and 420 nm) decreases and slightly shifts to higher wavelengths while the excimer emission (506 nm) increases slightly upon successive addition of $(TBA^+)_2SO_4^{2-}$ (Figure 3 left). These spectral changes are highly compatible with the anion

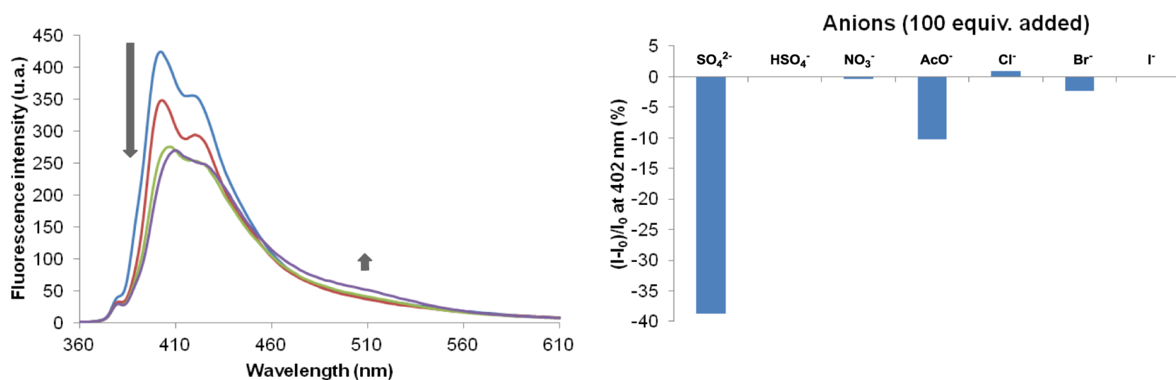


Figure 3. Left: fluorescence spectra of **4a** (∼10 μM) upon the addition of TBA_2SO_4 (0, 20, 100, and 500 equiv) in DMSO (excitation at 348 nm). Right: fluorescence intensity changes [$100\% \times (I - I_0)/I_0$] for **4a** (∼10 μM) in DMSO upon the addition of various anions (100 equiv) (excitation at 348 nm). I_0 is the fluorescence emission intensity at 402 nm for free host **4a**, and I is the fluorescence emission intensity after the addition of the anion.

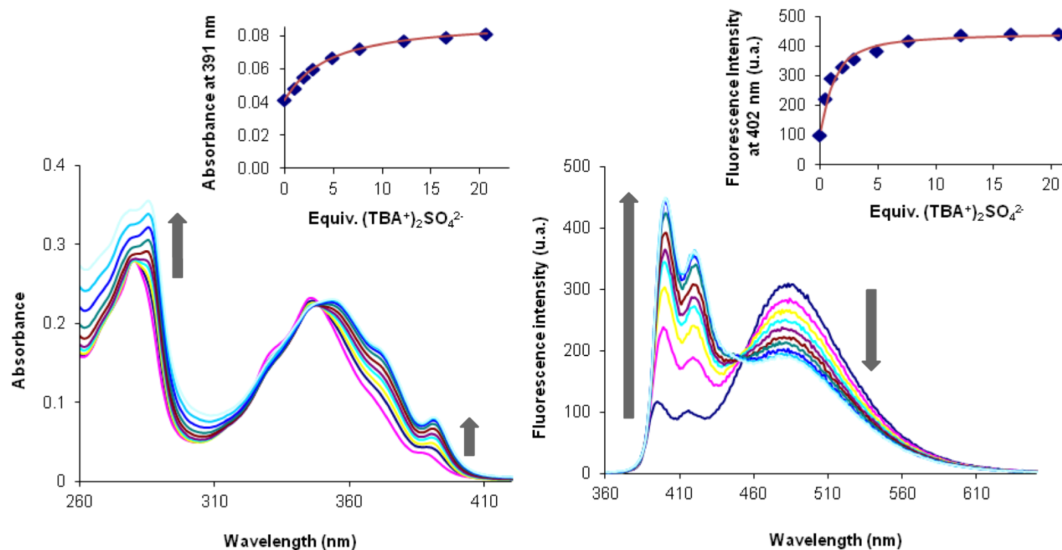


Figure 4. Left: UV−vis spectra of **4a** upon the addition of $(TBA^+)_2SO_4^{2-}$ (0 to 21 equiv) in chloroform. $[\mathbf{4a}]_0 = 4.8 \times 10^{-6}$ M. The inset shows the variation of the absorbance at 391 nm upon the addition of $(TBA^+)_2SO_4^{2-}$. Right: fluorescence spectra of **4a** upon the addition of $(TBA^+)_2SO_4^{2-}$ (0 to 21 equiv) in chloroform. $[\mathbf{4a}]_0 = 4.8 \times 10^{-6}$ M. $\lambda_{ex} = 350$ nm. The inset shows the variation of the fluorescence intensity at 402 nm upon the addition of $(TBA^+)_2SO_4^{2-}$.

coordination at the level of the urea groups resulting in a greater proximity between the pyrene moieties.

To verify that receptor **4a** is indeed a selective sensor for the sulfate anion in DMSO,[29] the binding of common anions of various geometries (i.e., SO_4^{2-}, HSO_4^-, NO_3^-, AcO^-, Cl^-, Br^-, I^-) was investigated by fluorescence spectroscopy. To our delight, only the sulfate anion caused a significant quenching in the emission spectrum of **4a**, confirming the high selectivity for this anion (Figure 3 right).

In a second set of experiments, the binding behavior of **4a** toward anions was evaluated in $CDCl_3$. Again, NMR-monitored titration experiments and Job's plot experiments confirmed the 1:1 complexation of the sulfate anion. The association constant for the host−guest complex **4a⊃SO$_4^{2-}$** (log $K > 4$) was too high to be determined accurately by NMR spectroscopy but could however be determined by absorbance and fluorescence titrations (Figure 4), which afforded log K values of 5.1 ± 0.5 and 5.4 ± 0.4, respectively (Table 1). The fluorescence spectra clearly showed that in this solvent the monomer emission (395 and 415 nm) increases while the excimer emission (484 nm) decreases upon successive additions of $(TBA^+)_2SO_4^{2-}$. These spectral changes are in accordance with a separation of the intramolecularly self-associated urea groups bearing the pyrene moieties upon anion coordination.

In comparison with the results obtained in DMSO, the host−guest properties of **4a** in chloroform are slightly different. The NMR signals of the calixarene, and in particular those of the urea N*H*, are significantly affected upon the successive addition of anions other than sulfate (i.e., Cl^-, Br^-, AcO^-, HSO_4^-, $H_2PO_4^-$), indicating that these anions also bind at the level of the urea groups. However, for these anions the CISs as well as the Job's plot experiments undertaken by NMR spectroscopy were not compatible with a 1:1 binding stoichiometry but more likely corresponded to the formation of mixtures of complexes with different binding ratios.[30] Another surprising difference observed was that the chemical shifts of the protons of the TBA^+ counterion were strongly affected during the titrations with all of the tested anions. In the case of $(TBA^+)_2SO_4^{2-}$, a binding constant with log $K = 2.6 \pm 0.2$ was determined by monitoring the upfield shift of the N^+CH_2 signal of TBA^+ (Table 2).[27] From this titration, it was possible to evaluate the chemical shift for these protons when the TBA^+ cation is complexed, and the obtained value ($\delta_{N^+CH_2} = 1.62 \pm 0.05$ ppm) suggests that this

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Table 2. Affinities of 4a, 4a⊃SO_4^{2-}, and 4a⊃$PrNH_3^+SO_4^{2-}$ toward TBA^+ ($CDCl_3$, 298 K) and Estimated Chemical Shifts of the N^+CH_2 Protons of the Bound TBA^+ Ion

host	log K^a	$\delta_{N^+CH_2}$ (ppm)^b
4a[c]	1.9 ± 0.1	3.11 ± 0.01
4a⊃SO_4^{2-}	2.6 ± 0.2	1.62 ± 0.05
4a⊃$PrNH_3^+SO_4^{2-}$	2.4 ± 0.2	1.74 ± 0.16

[a]K was determined by following the variation of the chemical shift of the N^+CH_2 protons of TBA^+. [b]Estimated by parametric adjustment of the experimental data to the equation $\delta = \delta_{free}y + \delta_{bound}(1 - y)$. [c]The NMR titration was performed with $TBA^+BF_4^-$.

cation is located at the level of the pyrenyl units, where it is stabilized through CH−π interactions. It is noteworthy to mention that a similar interaction of TBA^+ with pyrenyl moieties has been reported previously in the literature.[31] In order to better characterize this interaction, host **4a** was also titrated with TBA^+ associated with a low-coordinating anion (i.e., BF_4^-). As with $(TBA^+)_2SO_4^{2-}$, an upfield shift was observed for the TBA^+ protons, and a binding constant of log K = 1.9 ± 0.1 and a chemical shift of 3.11 ± 0.01 ppm for the N^+CH_2 protons of the bound TBA^+ were determined (Table 2).[27] The value of log K was confirmed through DOSY experiments.[27] The higher binding constant observed for the system in the presence of sulfate can be explained by the favorable electrostatic interaction between the ammonium ion and the cobound sulfate but also by the fact that sulfate complexation at the level of the urea units induces a collapse of the pyrenyl units, which form a "π-electron donor cavity" favorable for cation binding. This hypothesis is confirmed by the fact that the chemical shift for the N^+CH_2 protons of the bound TBA^+ ion is significantly more upfield in the presence of sulfate (1.62 ± 0.05 vs 3.11 ± 0.01 ppm). In other words, the complexation of the anion preorganizes the binding site for the TBA^+ cation, leading to a positive cooperativity. It is important to point out that in the absence of the receptor, no significant chemical shift changes were observed for the TBA^+ signals in $CDCl_3$ as a function of $(TBA^+)_2SO_4^{2-}$ or $TBA^+BF_4^-$ concentration (up to 50 mM), confirming that the observed change in chemical shift is indeed due to the interaction with the receptor.

Altogether, the results obtained with receptor **4a** stand in contrast to those obtained with the parent receptors **1a** and **1b**, which exhibit a much lower selectivity for the sulfate anion. Indeed, **1a** and **1b** strongly recognize a large variety of anions with a 1:1 binding stoichiometry (e.g., log K = 2.2 for Br^- and log K > 3.9 for AcO^-, HSO_4^-, and SO_4^{2-} in the case of **1a** in $CDCl_3$[18]). This difference in the behaviors of the two families of receptors may be rationalized by the presence of steric interactions between the bulky pyrenylurea subunits when the three urea arms have to come in close proximity upon anion complexation. In the case of **4a**, the high selectivity for SO_4^{2-} could be due to the fact that this anion is large and doubly charged and displays good complementarity with the tris(urea) binding site.

Ammonium Salt Complexation. The simultaneous complexation of an ammonium ion in the calixarene cavity and an anion at the level of the tris(urea) binding site was investigated by NMR, UV−vis, and fluorescence spectroscopy in chloroform. In a first set of experiments, $PrNH_3^+$ was chosen as the cationic partner as it is known to display a high affinity for the calix[6]arene cavity.[32] The addition of $PrNH_3^+$ associated with a low-coordinating anion (i.e., picrate, Pic^-) did not have an effect on the NMR spectrum of **4a** in $CDCl_3$, which highlights the poor ability of **4a** to bind ammonium ions independently of a coordinating anion. In contrast, upon the addition of ca. 3 equiv of $PrNH_3^+X^-$ (X^- = Cl^-, AcO^-, HSO_4^-), the intracavity binding of the ammonium ion was evidenced by the presence of high-field signals in the NMR spectra (<0 ppm). However, in addition to the NMR signals for the complex **4a⊃$PrNH_3^+X^-$**, broad signals corresponding to another calixarene species were observed. Even with a larger excess of the ammonium salt (ca. 10 equiv), it was not possible to obtain only the **4a⊃$PrNH_3^+X^-$** complex. The second species could correspond to binding of the ion pair $PrNH_3^+X^-$ with exocomplexation of the ammonium ion (i.e., with the ammonium ion outside of the calixarene cavity at the level of the pyrenyl units). It is noteworthy to mention that the simultaneous addition of ca. 3 equiv of $PrNH_3^+Pic^-$ and $TBA^+HSO_4^-$ did not yield the same NMR spectrum as obtained upon the addition of ca. 3 equiv of $PrNH_3^+HSO_4^-$, as the proportion of complex **4a⊃$PrNH_3^+HSO_4^-$** was even lower in this case. This is probably the result of the concomitant complexation of $TBA^+HSO_4^-$ by the calixarene (vide supra) and highlights the importance of the counterion in the case of receptor **4a**. In other words, all of these data indicate a lack of selectivity for the binding process of ammonium salts $PrNH_3^+X^-$.

In view of the high selectivity of **4a** for the sulfate anion, the ability of the receptor to bind this anion when it is associated with an ammonium ion that can be endocomplexed was evaluated in chloroform. Receptor **4a** was unable to extract $(PrNH_3^+)_2SO_4^{2-}$ in $CDCl_3$, but to our delight, the progressive addition of $PrNH_3^+TBA^+SO_4^{2-}$ led to a new and unique NMR pattern displaying sharp signals characteristic of a C_{3v}-symmetric calixarene species (Figure 5a).

After the addition of 1 equiv of the salt $PrNH_3^+TBA^+SO_4^{2-}$, only the signals of this new species were observed, and the spectrum was not influenced by the further addition of a large excess of the salt (ca. 20 equiv), indicating strong and selective 1:1 binding. All of the signals of this new species were assigned by 2D NMR spectroscopy (i.e., COSY and HMBC),[27] and it was thus possible to make the following observations:

(i) The calixarene core adopts a flattened cone conformation ($\Delta\delta_{ArH}$ = 0.57 ppm and $\Delta\delta_{tBu}$ = 0.58 ppm), and the significant downfield shift of the OMe protons (δ_{OMe} = 4.13 ppm) shows that these groups have been expelled from the cavity.

(ii) The *NH* protons of the ureido groups experience a significant downfield shift (δ_{NHPyr} = 10.48 ppm and δ_{NHCH_2} = 8.88 ppm), highlighting the binding of the sulfate anion through hydrogen-bonding interactions.

(iii) High-field signals belonging to the alkyl chain of $PrNH_3^+$ (i.e., δ = −1.26 and −1.92 ppm) attest to the inclusion of the ammonium ion into the cavity with a 1:1 binding ratio. This host−guest exchange process is slow on the NMR chemical shift time scale. The CIS of the NCH_2 protons of the ammonium ion is quite moderate (i.e., −0.47 ppm) in comparison with those of the rest of the alkyl chain (i.e., −3.03 and −2.84 ppm). This result is highly compatible with the presence of the sulfate anion in close proximity to the ammonium ion and thus with the binding of the two ionic partners as a contact ion pair.

(iv) The HMBC spectrum clearly indicates that the *t*Bu groups of the anisole moieties are directed toward the outside of the cavity (see the structure displayed in Figure 5a).

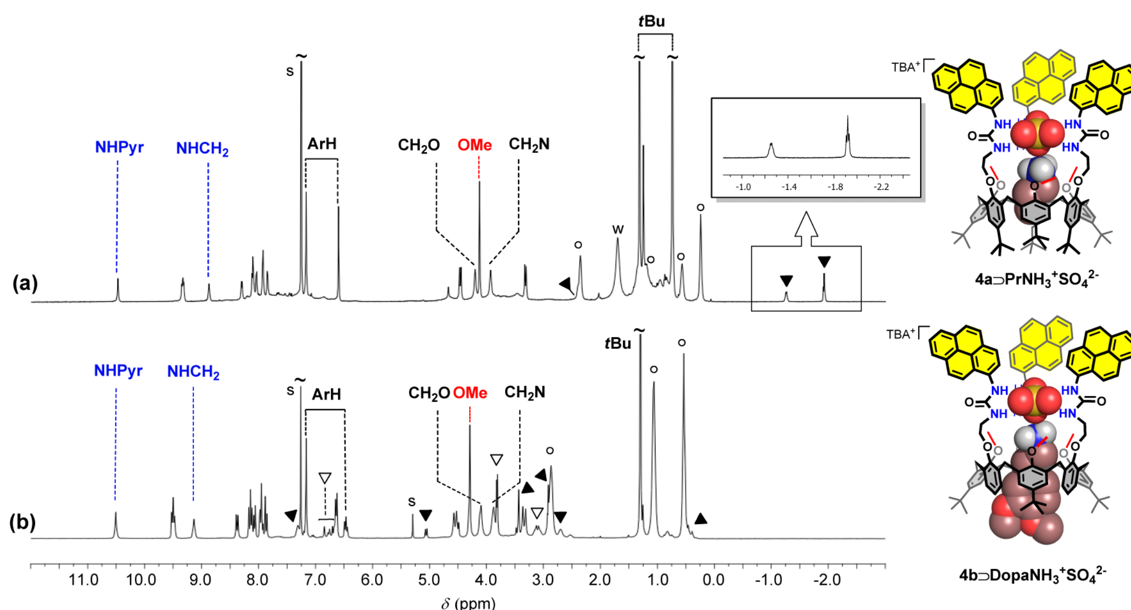

Figure 5. (a) ^{1}H NMR spectrum (600 MHz, 298 K) of **4a** in $CDCl_3$ in the presence of 1 equiv of $PrNH_3^+TBA^+SO_4^{2-}$. (b) ^{1}H NMR spectrum (300 MHz, 298 K) of **4b** in $CDCl_3$ in the presence of ca. 2 equiv of $DopaNH_3^+TBA^+SO_4^{2-}$. Labels: ○, TBA^+;▼, RNH_3^+ in; ▽, RNH_3^+ out; s, residual solvent; w, residual water.

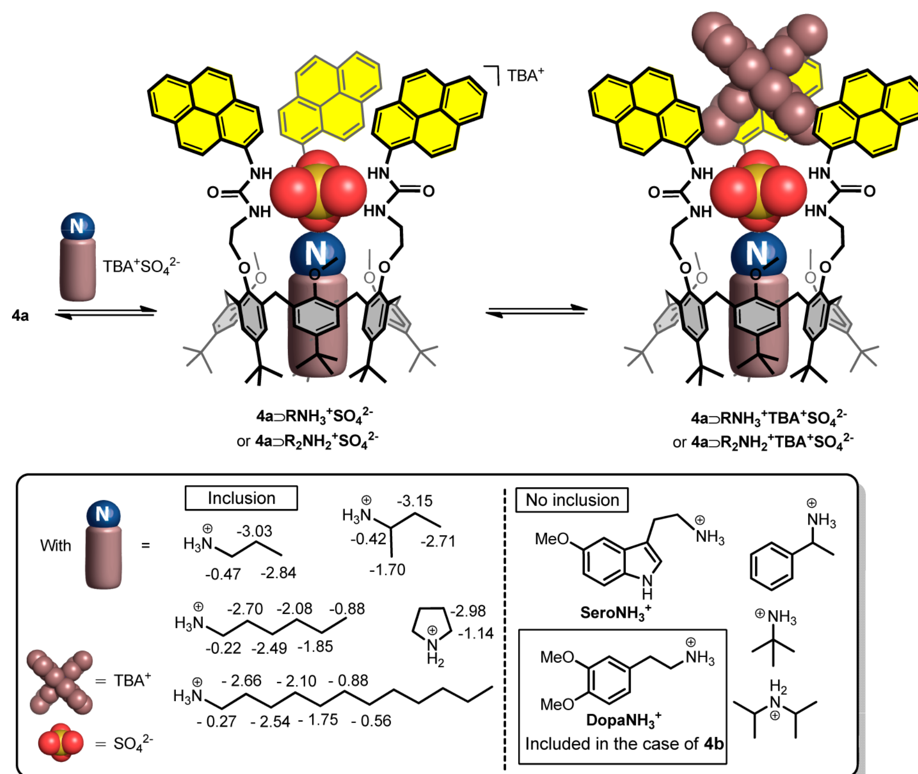

Figure 6. Host−guest properties of calix[6]tris(pyrenylurea) **4a** toward ammonium sulfate salts. Inset: CISs determined by ^{1}H NMR spectroscopy in $CDCl_3$.

(v) The chemical shifts of the TBA^+ protons are strongly affected during the course of the titration as a result of complexation of the TBA^+ cation at the level of the pyrenyl units. The exchange is fast on the NMR chemical shift time scale, and monitoring of the upfield shift of the N^+CH_2 signal yielded a binding constant of log $K = 2.4 \pm 0.2$ and an estimated chemical shift of 1.74 ± 0.16 ppm for the N^+CH_2 protons of the bound TBA^+ ion. These values are comparable to those observed in the presence of the sole sulfate ion (Table 2).

Altogether, these observations show the formation of the ternary host−guest complex **4a⊃PrNH$_3^+$SO$_4^{2-}$** with a weak interaction of the TBA^+ counterion at the level of the pyrenyl units that leads ultimately to the complex **4a⊃PrNH$_3^+$TBA$^+$SO$_4^{2-}$** (Figure 6). In other words, **4a** behaves as a heterotopic receptor

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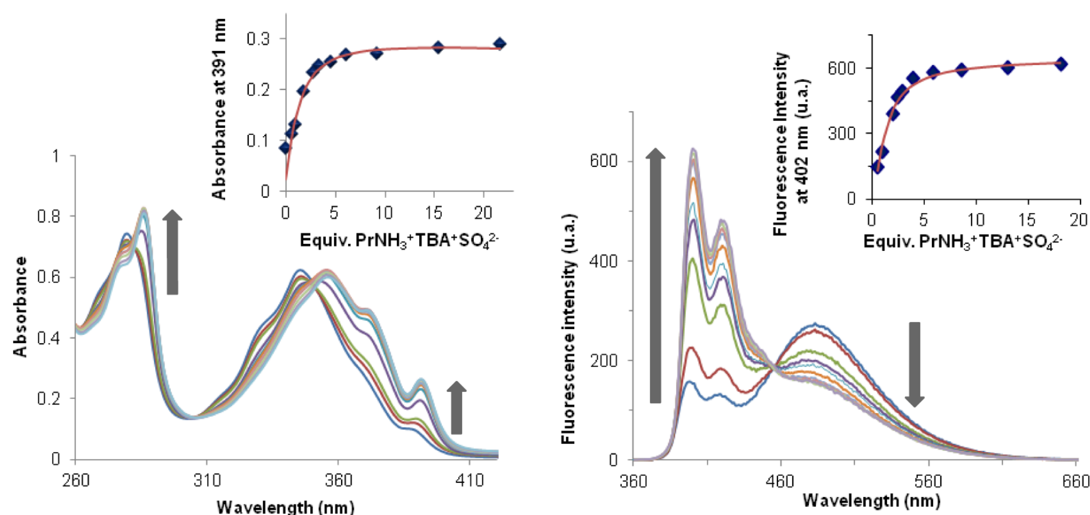

Figure 7. Left: UV−vis spectra of **4a** upon the addition of $PrNH_3^+TBA^+SO_4^{2-}$ (0 to 22 equiv) in chloroform. $[\mathbf{4a}]_0 = 10.0 \times 10^{-6}$ M. The inset shows the variation of the absorbance at 391 nm. Right: fluorescence spectra of **4a** upon the addition of $PrNH_3^+TBA^+SO_4^{2-}$ (0 to 19 equiv) in chloroform. $[\mathbf{4a}]_0 = 4.8 \times 10^{-6}$ M. λ_{ex} = 346 nm. The inset shows the variation of the fluorescence intensity at 402 nm.

for sulfate ammonium salts, with binding of the anion by the urea groups being a prerequisite for the inclusion of the ammonium ion into the calixarene cavity. This positive cooperativity is clearly due to the fact that these two ions can be bound as a contact ion pair, thus avoiding the highly energetically unfavorable dissociation of the ion pair. The association constant for $PrNH_3^+SO_4^{2-}$ was estimated to be log $K > 4$ by NMR spectroscopy (Table 1). Absorbance and fluorescence titrations in chloroform afforded association constants of log K = 5.3 ± 0.6 and 5.4 ± 0.1, respectively (Figure 7). Again, the spectral changes observed in the fluorescence spectra, namely, an increase of the monomer emission and a decrease of the excimer one, are suggestive of the separation of the self-associated urea groups upon anion coordination.

The affinities of receptors **1a** and **4b** for $PrNH_3^+TBA^+SO_4^{2-}$ were also evaluated in $CDCl_3$ in order to make a comparison with host **4a**.[27] Similarly to **4a**, a high affinity constant for the formation of the complexes **1a⊃PrNH$_3^+$SO$_4^{2-}$** and **4b⊃PrNH$_3^+$SO$_4^{2-}$** was determined by ^{1}H NMR spectroscopy (log $K > 4$). In the case of **4b**, absorbance and fluorescence titrations afforded association constants of log K = 5.0 ± 0.2 and 4.8 ± 0.2, respectively, suggesting that **4a** and **4b** display similar recognition properties toward this ammonium salt. Surprisingly, a weak interaction of the TBA^+ counterion at the level of the phenyl units was also observed in the case of **1a** (log K = 2.3 ± 0.2),[27] but a much higher chemical shift for the N^+CH_2 protons of the bound TBA^+ (2.94 ± 0.02 vs 1.74 ± 0.16 for **1a** vs **4a**, respectively) was estimated and no binding of the TBA^+ was observed when this cation was associated with a low-coordinating anion (i.e., BF_4^-). Again, this positive cooperativity shows that the binding of the ion pair $PrNH_3^+SO_4^{2-}$ preorganizes the binding site for the TBA^+ cation by forming an electron-rich tris(phenyl) cavity.

All of these findings prompted us to investigate the ability of the fluorescent receptor **4a** to bind larger ammonium ions associated with $TBA^+SO_4^{2-}$. To this end, additions of hexyl-, dodecyl-, and (±)-*sec*-butylammonium salts as well as the pyrrolidinium salt to $CDCl_3$ solutions of **4a** were performed. In all cases, the formation of the inclusion complex was clearly evidenced by NMR spectroscopy, and the CISs of these ammonium ions were compatible with their inclusion into the calixarene cavity (Figure 6). In particular, all of the methylene protons of the included alkyl chain of $HexNH_3^+$ appeared as well-separated resonances and, in the case of $DodNH_3^+$, the CISs decreased dramatically from the middle of the alkyl chain, indicating that the dodecyl chain protrudes out of the cavity. For the pyrrolidinium cation ($PyrroNH_2^+$), accurate determinations of the association constants by absorbance and fluorescence titrations afforded log K values of 4.1 ± 0.5 and 4.5 ± 0.2, respectively (Table 1). In the case of the $HexNH_3^+$ and $DodNH_3^+$ salts, accurate determinations of the associations constant were not possible because of the competitive formation of **4a⊃SO$_4^{2-}$**.[27] This poor selectivity is likely due to a lower affinity of the calixarene for $HexNH_3^+$ and $DodNH_3^+$ that can be rationalized by the fact that guests with an alkyl chain longer than propyl lead to a steric clash with the introverted *t*Bu groups that close the cavity of the host, forcing the calix[6]arene skeleton to adopt a energetically unfavorable straight conformation.[33] This induced-fit process is clearly evidenced in the NMR spectra of the **4a⊃HexNH$_3^+$SO$_4^{2-}$** and **4a⊃DodNH$_3^+$SO$_4^{2-}$** complexes. Indeed, the calixarene aromatic units bearing the urea groups adopt a conformation more parallel to the C_3 axis with their *t*Bu groups expelled from the cavity by the alkyl chain of the included ammonium ion ($\Delta\delta_{ArH}$ = 0.41 and 0.40 ppm, respectively. Moreover, the inclusion of the bulkier and nonlinear 3,4-*O*-dimethyldopammonium ($DopaNH_3^+$), (±)-α-methylbenzylammonium, and *O*-methylserotonin ($SeroNH_3^+$) ions was not observed, again certainly because of the conformational energy penalty resulting from the steric clash with the *t*Bu groups (Figure 6). Steric hindrance at the level of the ammonium group and its α-position also appeared to be a major selectivity factor because of a steric clash with the small rim of the calixarene. Indeed, the inclusion of *tert*-butylammonium and diisopropylammonium ions was not detected (Figure 6), and the complexation of the (±)-*sec*-butylammonium salt was found to be at least 20 times weaker than that of $PrNH_3^+SO_4^{2-}$. Interestingly, the selective formation of **4a⊃PrNH$_3^+$SO$_4^{2-}$** was obtained in the presence of a large excess of these ammonium sulfate salts.[27] All of these results clearly show that the calixarene cavity controls the recognition of the cationic guest on the basis of its size and geometry. It was thus of particular interest to investigate the binding of the bulky salts by receptor **4b**, which

displays an enlarged and more open cavity (Scheme 1). With this receptor, additions of the $SeroNH_3^+$, (±)-α-methylbenzylammonium, $tBuNH_3^+$, and $(iPr)_2NH_2^+$ ions associated with $TBA^+SO_4^{2-}$ did not yield any inclusion complexes, certainly because of poor shape complementarity between the cationic guest and the cavity. However, to our delight, the addition of $DodNH_3^+TBA^+SO_4^{2-}$ and $DopaNH_3^+TBA^+SO_4^{2-}$ in $CDCl_3$ led to the exclusive formation of the corresponding complexes **4b⊃DodNH$_3^+$SO$_4^{2-}$** and **4b⊃DopaNH$_3^+$SO$_4^{2-}$** (Figure 5b). All in all, these results show that both fluorescent receptors **4a** and **4b** can ensure a cavity-based selectivity at the level of the recognition of the ammonium ion and also that fine-tuning of the recognition process can be obtained through modification of the large-rim substituents.

Studies in a Protic Environment. The next challenge was to evaluate the ability of **4a** to bind ammonium salts in a protic environment. The ^1H NMR spectrum of receptor **4a** in a 1:11 $CD_3OD/CDCl_3$ mixture remained unchanged upon the addition of either $(TBA^+)_2SO_4^{2-}$ or $PrNH_3^+Pic^-$. This highlights the extremely weak anion or ammonium ion recognition properties of **4a** in a protic and thus highly competitive environment. Furthermore, the ^1H NMR spectrum of receptor **4a** in a 1:11 $CD_3OD/CDCl_3$ mixture remained unaffected upon the addition of a large excess of $(PrNH_3^+)_2SO_4^{2-}$ (8 equiv), but the subsequent addition of $(TBA^+)_2SO_4^{2-}$ (ca. 1.5 equiv) led to the quantitative formation of the complex **4a⊃PrNH$_3^+$SO$_4^{2-}$**.[27] It is noteworthy to mention that an identical NMR pattern was obtained upon the addition of $PrNH_3^+TBA^+SO_4^{2-}$ to **4a**. This remarkable result highlights once again the importance of the counterion and shows that the presence of a dissociated cation such as TBA^+ is required for the complexation of ammonium sulfate salts by host **4a**. The association constant for $PrNH_3^+SO_4^{2-}$ ($\log K = 3.9 \pm 0.1$) was determined by integration of the ^1H NMR spectrum recorded after the addition of 1 equiv of $PrNH_3^+TBA^+SO_4^{2-}$ (Table 1). A similar log K value of 4.0 ± 0.3 was obtained by UV−vis titration.[27] It should be noted that in this solvent the binding constant for the complexation of the TBA^+ was too small to be determined accurately. Considering the inertness of the receptor toward $(TBA^+)_2SO_4^{2-}$ and $PrNH_3^+Pic^-$ in a 1:11 $CD_3OD/CDCl_3$ mixture, the strong and simultaneous binding of both partners (i.e., $PrNH_3^+$ and SO_4^{2-}) in this protic environment highlights a remarkable mutual cooperativity. Indeed, the complexation of the sulfate anion can only proceed when an ammonium ion is present in the calixarene cavity, and conversely, without SO_4^{2-} the receptor **4a** is inefficient at binding the ammonium ion. Finally, a certain affinity of receptor **4b** for **DopaNH$_3^+$TBA$^+$SO$_4^{2-}$** in 1:10 $CD_3OD/CDCl_3$ was also observed (log $K \sim 1.5$ obtained by NMR spectroscopy), showing a remarkable behavior of this receptor for the recognition of biological ammonium salts in a protic environment.

■ CONCLUSION

The straightforward syntheses of the first fluorescent calix[6]-tris(urea) hosts **4a** and **4b** was achieved efficiently. In comparison to the parent receptors **1a** and **1b**, the introduction of bulky pyrenyl groups in close proximity to the anion binding site did not inhibit the binding properties of the receptors toward anions and contact ion pairs. In contrast to **1a** and **1b**, it was observed that receptor **4a** exhibits a remarkable selectivity for the sulfate anion in DMSO, enabling its selective sensing by fluorescence spectroscopy. In $CDCl_3$, **4a** was able to bind ammonium ions efficiently only in association with the sulfate anion. To our delight, this cooperative binding of ammonium sulfate salts was also observed in a protic environment. The binding constants determined by UV−vis absorbance, fluorescence, and NMR titrations were found to be in agreement in all cases. Corroborating conformational changes of the receptor upon binding were evidenced by fluorescence and NMR spectroscopies. Interestingly, cavity-based selectivity in terms of the size and shape of the guest was observed with both receptors **4a** and **4b**. While **4a** displays a strong affinity for small or linear ammonium salts, **4b** was found to have the ability to recognize the 3,4-*O*-dimethyldopammonium sulfate salt and not the corresponding serotonin derivative. In other words, this work opens interesting perspectives on the elaboration of unique fluorescent cavity-based systems for the selective sensing of anions or biologically relevant ammonium salts such as neurotransmitters. Current efforts are now being directed toward the design of water-soluble fluorescent calix[6]arene-based receptors.

■ EXPERIMENTAL SECTION

General Experimental Methods. All of the reactions were performed under an inert atmosphere. Anhydrous THF was obtained through distillation over Na/benzophenone. Silica gel (230−400 mesh) was used for flash chromatography purifications. Chloroform (both deuterated and nondeuterated) was filtered prior to use over a short column of basic alumina to remove traces of HCl/DCl. ^1H NMR spectra were recorded on a 600, 400, or 300 MHz spectrometer, and ^{13}C NMR spectra were recorded on the 300 or 400 MHz spectrometer at 75 or 100 MHz, respectively. 2D NMR spectra (COSY, HSQC, HMBC) were recorded to complete signal assignments. DOSY experiments were recorded on the 600 MHz spectrometer. NMR parameters (acquisition time, recycling times, and signal accumulation) were chosen to ensure that quantitative data could be obtained from signal integration in the 1D ^1H spectra. Traces of residual solvent were used as an internal chemical shift reference. Chemical shifts are quoted on the δ scale. The NMR, UV, and fluorescence spectra were recorded at 298 K, unless otherwise stated. De-*tert*-butylated 1,3,5-tris(methoxy)calix[6]arene **2**,[23] calix[6]tris(azido) **3a**,[25] and 2-azidoethyl-4-methylbenzenesulfonate[24] were prepared as previously described.

Calix[6]tris(azido) 3b. De-*tert*-butylated 1,3,5-tris(methoxy)-calix[6]arene **2** (1.81 g, 2.14 mmol) was dissolved in freshly distilled THF (100 mL), and NaH (60 wt % in oil, 0.510 g, 12.82 mmol) was added. The mixture was heated to reflux for 30 min. Then 2-azidoethyl-4-methylbenzenesulfonate (2.060 g, 8.54 mmol) was added, and the heating was maintained for 48 h. After the mixture was cooled to room temperature, methanol (15 mL) was added, and the solution was stirred for 30 min. Then the solvents were evaporated, and the residue was dissolved in a mixture of CH_2Cl_2 (80 mL) and HCl (80 mL, 1 M). The organic layer was separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed twice with brine, filtered through a WA filter, and evaporated under vacuum. The crude product was then purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 100:0 then 95:5) to give calix[6]tris(azido) **3b** (1.730 g, 77% yield). Mp: 125−130 °C (dec.). IR (KBr): ν 2956, 2105, 1455 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 298 K): δ (ppm) 1.28 (s, 27H, *t*Bu), 2.80 (s, 9H, OCH_3), 3.46 (m, 6H, CH_2N_3), 3.75 (m, 6H, OCH_2), 4.02 (s, 12H, $ArCH_2$), 6.33 (s, 9H, ArH), 7.21 (s, 6H, ArH). ^{13}C NMR ($CDCl_3$, 75 MHz, 298 K): δ (ppm) 30.6, 31.5, 34.2, 51.2, 59.8, 70.9, 123.9, 126.8, 128.2, 133.2, 134.5, 146.1, 153.9, 154.2. ESI-HRMS (CH_2Cl_2/CH_3OH): calcd for $C_{63}H_{79}N_{10}O_6$ $[M + NH_4]^+$ 1071.6184, found 1071.6162.

Calix[6]tris(pyrenylurea) 4a. Calix[6]tris(azido) **3a** (0.350 g, 0.29 mmol) was dissolved in anhydrous THF (10 mL), and triphenylphosphine (0.450 g, 1.72 mmol) was added. Then CO_2 was bubbled through the solution for 5 min. The solution was stirred for 19 h at room temperature and kept under a CO_2 atmosphere. The medium was then purged with argon for 5 min, and 1-aminopyrene (0.273 g, 1.26 mmol) was added. The mixture was then stirred under argon for 48 h at 50 °C. The solvent was evaporated under vacuum, and acetonitrile

(1 mL) was added. The suspension was sonicated for 15 min and then centrifuged for 15 min, and the supernatant was removed. This operation was repeated six times. The crude product was then purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 100:0 then 99:1 then 95:5) to afford calix[6]tris(pyrenylurea) **4a** (0.370 g, 70% yield). Mp: 208–212 °C (dec.). IR (KBr): ν 3375, 2962, 1655, 1524, 1482 cm^{-1}. ^1H NMR (DMSO-d_6, 298 K, 400 MHz): δ (ppm) 0.75 (s, 27H, *t*Bu), 1.29 (s, 27H, *t*Bu), 2.12 (s, 9H, OMe), 3.50 (d, 6H, $ArCH_2^{eq}$, ^2J = 15.4 Hz), 3.65 (br s, 6H, CH_2N), 4.05 (br s, 6H, CH_2O), 4.56 (d, 6H, $ArCH_2^{ax}$, ^2J = 15.0 Hz), 6.61 (s, 6H, ArH^{Urea}), 6.96 (s, 3H, N*H*CH_2), 7.31 (s, 6H, ArH^{OMe}), 7.92–8.05 (m, 12H, PyrH), 8.07–8.24 (m, 12H, PyrH), 8.51 (d, 3H, PyrH, ^3J = 8.4 Hz), 9.11 (s, 3H, N*H*Pyr). ^{13}C NMR (DMSO-d_6, 298 K, 100 MHz): δ (ppm) 29.0, 30.7, 31.3, 33.5, 33.9, 59.4, 71.9, 120.2, 121.0, 121.2, 123.0, 124.2, 124.5, 124.7, 125.2, 125.3, 126.2, 126.4, 127.3, 128.1, 130.5, 131.1, 132.5, 133.2, 133.7, 144.8, 145.2, 151.4, 153.8, 155.8. ESI-HRMS (CH_2Cl_2/CH_3OH): calcd for $C_{126}H_{132}N_6O_9Na$ $[M + Na]^+$ 1895.9954, found 1895.9949.

Calix[6]tris(pyrenylurea) 4b. Calixarene **4b** was prepared using the same procedure as for **4a** starting from calix[6]tris(azido) **3b** (0.500 g, 0.47 mmol), which gave calix[6]tris(pyrenylurea) **4b** (0.280 g, 35% yield). Mp: 225–230 °C (dec.). IR (KBr): ν 3319, 2961, 1651, 1557, 1485 cm^{-1}. ^1H NMR (DMSO-d_6, 298 K, 300 MHz): δ (ppm) 1.19 (s, 27H, *t*Bu), 2.60 (s, 9H, OMe), 3.61 (br s, 6H, CH_2N), 3.77–4.26 (m, 18H, $CH_2O/ArCH_2^{eq}/ArCH_2^{ax}$), 6.51 (d, 6H, ArH^{Urea}, ^3J = 7.5 Hz), 6.67 (t, 3H, ArH^{Urea}, ^3J = 8.0 Hz), 6.91 (t, 3H, N*H*CH_2, ^3J = 5.0 Hz), 7.23 (s, 6H, ArH^{OMe}), 7.80–8.06 (m, 12H, PyrH), 8.07–8.28 (m, 12H, PyrH), 8.51 (d, 3H, PyrH, ^3J = 8.4 Hz), 9.09 (s, 3H, N*H*Pyr). ^{13}C NMR (DMSO-d_6, 298 K, 75 MHz): δ (ppm) 30.9, 32.1, 34.7, 60.4, 110.0, 120.9, 121.7, 122.0, 124.2, 125.0, 125.3, 125.4, 125.4, 126.1, 126.2, 127.0, 127.3, 127.6, 128.1, 128.2, 131.4, 131.9, 133.6, 134.6, 135.2, 146.4, 154.7, 155.0, 156.7. ESI-HRMS (CH_2Cl_2/CH_3OH): calcd for $C_{114}H_{109}N_6O_9$ $[M + H]^+$ 1706.8290, found 1706.8256.

^1H NMR Characterization of Various Host–Guest Complexes. **4a⊃SO_4^{2-}**: ^1H NMR (DMSO-d_6, 298 K, 300 MHz): δ (ppm) 0.79 (s, 27H, *t*Bu), 1.36 (s, 27H, *t*Bu), 2.55 (s, 9H, OMe), 3.31–3.41 (m, 6H, $ArCH_2^{eq}$)*, 3.75 (br s, 6H, CH_2N), 4.43 (br s, 6H, CH_2O), 4.59 (d, 6H, $ArCH_2^{ax}$, ^2J = 15.0 Hz), 6.67 (s, 6H, ArH^{Urea}), 7.14–7.26 (m, 3H, PyrH), 7.30 (s, 6H, ArH^{OMe}), 7.51–8.18 (m, 18H, PyrH), 8.23 (d, 3H, PyrH, ^3J = 9.0 Hz), 8.55 (d, 3H, PyrH, ^3J = 9.0 Hz), 9.14 (s, 3H, N*H*CH_2), 10.71 (s, 3H, N*H*Pyr). **4a⊃$PrNH_3^+SO_4^{2-}$**: ^1H NMR ($CDCl_3$, 298 K, 600 MHz: δ (ppm) −1.92 (t, 3H, $CH_3^{PrNH_3^+{}_{in}}$, ^2J = 7.4 Hz), −1.26 (m, 2H, $CH_2^{PrNH_3^+{}_{in}}$), 0.74 (s, 27H, *t*Bu), 1.32 (s, 27H, *t*Bu), 2.40 (s, 2H, $CH_2NH_3^{+PrNH_3^+{}_{in}}$)*, 3.32 (d, 6H, $ArCH_2^{eq}$, ^2J = 14.4 Hz), 3.93 (br s, 6H, CH_2N), 4.13 (s, 9H, OMe), 4.20 (br s, 6H, CH_2O), 4.46 (d, 6H, $ArCH_2^{ax}$, ^2J = 14.4 Hz), 6.60 (s, 6H, ArH^{urea}), 7.17 (s, 6H, ArH^{OMe}), 7.80–8.25 (m, 18H, PyrH), 8.30 (d, 3H, PyrH, ^3J = 9 Hz), 9.30–9.40 (m, 6H, PyrH), 8.88 (s, 3H, N*H*CH_2), 10.48 (s, 3H, N*H*Pyr). **4b⊃$DopaNH_3^+SO_4^{2-}$**: ^1H NMR ($CDCl_3$, 298 K, 300 MHz): δ (ppm) 0.46 (m, 2H, $CH_2NH_3^{+DopaNH_3^+{}_{in}}$)*, 1.30 (s, 27H, *t*Bu), 2.70 (br s, 2H, $CH_2^{DopaNH_3^+{}_{in}}$), 2.91 (s, 3H, $OMe^{DopaNH_3^+{}_{in}}$)*, 3.34 (d, 6H, $ArCH_2^{eq}$, ^2J = 14.4 Hz), 3.43 (s, 3H, $OMe^{DopaNH_3^+{}_{in}}$), 3.87 (m, 1H, $ArH^{DopaNH_3^+{}_{in}}$ and br s, 6H, CH_2N)*, 4.09 (br s, 6H, CH_2O), 4.11 (m, 1H, $ArH^{DopaNH_3^+{}_{in}}$)*, 4.29 (s, 9H, OMe), 4.55 (d, 6H, $ArCH_2^{ax}$, ^2J = 14.4 Hz), 5.06 (d, 1H, $ArH^{DopaNH_3^+{}_{in}}$, ^3J = 8.4 Hz), 6.47 (t, 3H, ArH^{urea}, ^3J = 9.0 Hz), 6.64 (d, 6H, ArH^{urea}, ^3J = 9.0 Hz), 7.16 (s, 6H, ArH^{OMe}), 7.31 (m, 3H, $NH_3^{+DopaNH_3^+{}_{in}}$)*, 7.82–8.20 (m, 18H, PyrH), 8.38 (d, 3H, PyrH, ^3J = 9.0 Hz), 9.13 (t, 3H, N*H*CH_2, ^3J = 5.1 Hz), 9.43–9.55 (m, 6H, PyrH), 10.51 (s, 3H, N*H*Pyr). Asterisks (*) denote signals assigned thanks to the COSY spectrum.

NMR Titration Experiments. All of these experiments were undertaken following a similar protocol. A known volume (~600 μL) of a solution of known concentration of the host (~2 mM) was placed in an NMR tube, and the ^1H NMR spectrum was recorded. Aliquots of a stock solution of the guest (~5 μL, corresponding to 0.25 equiv of host) were successively added, and the ^1H NMR spectrum was recorded after each addition. Aliquots were added until no changes in the host signals were observed.

UV–Vis and Fluorescence Titration Experiments. All of these experiments were undertaken following a similar protocol. A known volume (~2 mL) of a solution of known concentration of the host (~10^{-5} M) was placed in a cell, and the absorbance or emission spectrum was recorded. Aliquots of a stock solution of the guest (~5 μL, corresponding to 0.5 equiv of host) were successively added, and the spectrum was recorded after each addition. Aliquots were added until no changes in the spectrum were observed. The values obtained for the absorbance/emission were corrected for dilution.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H NMR, UV–vis, and fluorescence studies of the complexing properties of **4a** and **4b** in $CHCl_3$ and DMSO; Job's plot analysis of the association between host **4a** and SO_4^{2-}; ^1H NMR study of the complexing properties of **1a**; and 1D and 2D NMR spectra of **3b**, **4a**, and **4b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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