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Colorimetric Recognition of Anions Using Preorganized Tetra-Amidourea Derived Calix[4]arene Sensors

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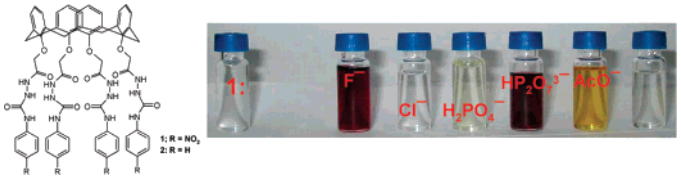

The synthesis and the spectroscopic studies of the amidourea based calix[4]arene sensors **1** and **2** are described. The 4-nitrophenyl based sensor **1** was synthesized in two steps from the corresponding calix[4]arene tetraethyl ester and shown to give rise to color changes in the UV−vis spectra in DMSO upon recognition of pyrophosphate and fluoride. Fitting the changes in the absorption spectra using nonlinear regression analysis indicated strong binding of several anions by **1** such as acetate and hydrogen phosphate in 1:1 (Host:Guest) stoichiometry, and at higher concentration in 1:2 stoichiometry. The preorganized calix-cavity was, however, not found to host chlorine while binding of bromide was determined. At high concentrations of these anions, significant colorimetric changes were also observed that were clearly visible to the naked eye for both pyrophosphate and fluoride. The phenyl analogue **2** was made to enable analysis of the anion recognition using ^{1}H NMR titrations and showed that ions such as phosphate were bound in 1:1 stoichiometry, whereas the "urea" protons were shown to be significantly affected upon coordination to the anion.

Introduction

The design and the use of luminescent and colorimetric molecules for anion recognition and sensing is a fast growing field of research within supramolecular chemistry.[1] There is no doubt that there currently exists a major need for developing both sensitive and selective sensors for anions.[2] Anions play a central role in physiology and, therefore, in healthcare. They are prevalent in both heavy industry and in farming, and as such in the environment. They are often harmful pollutants, for example, phosphates from fertilizers, etc., and many anions are also used as, or are the products of, the degradation (hydrolysis) of chemical warfare agents. Hence, the need for fast, either as "one-off" or for real-time monitoring, and accurate detection of ions such as fluoride (F^-), which is the breakage product of

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sarin gas and organophosphates found in many nerve agents, has become an unfortunate reality.[3] Hence, sensors that selectively detect these anions using manageable techniques, such as colorimetric sensors,[4] which can be incorporated into simple hand-held instruments or even "dip-sticks", giving rise to naked eye "*no-yes*" response, are highly advantageous. Traditionally, anion sensing has been more difficult to achieve in comparison to that of cations.[5,6] This is mostly due to their wide range of geometries, which are often pH sensitive, as in the case of phosphate, which therefore renders them sensitive to spatial arrangements and orientation of binding groups. This has been difficult to achieve except with cumbersome synthetic targets.[7] Consequently, anion sensing has often been achieved using charged receptors,[8] such as metal complexes,[9] which is disadvantageous due to lack of anion selectivity. Targeting anions using charge neutral receptors,[9] which recognize the anions through hydrogen bonding, has recently become both highly successful[10] and possible even in highly competitive media, such as in buffered water.[11] Charge neutral anion receptors,[12] such as amides,[12] thiourea,[13] and amidoureas,[14,15] are particularly attractive for such recognition, as they are good hydrogen bonding donors, possessing directionality, and are synthetically undemanding to incorporate into larger preorganized structures. We have developed many examples of anion sensors possessing such structural motifs.[16] However; these are usually based on simple receptors, where the anion recognition occurs through either a one or two hydrogen bonding donor. In this paper, we demonstrate that incorporation of four such anion recognition moieties in the form of amidoureas into a preorganized calix[4]arene host,[17] in an easy two-step synthesis, gives rise to both selective anion sensing and strong binding, as well as in some cases colorimetric responses, which are visible to the naked eye. These color changes occur from "colorless → deep-red" and are the equivalent of a "*no-yes*" response in the case of both F^- and pyrophosphate, whereas other competitive ions such as acetate (AcO^-), hydrogen phosphate ($H_2PO_4^-$), nitrate (NO_3^-), and other halides (Cl^- and Br^-) give either weak binding or no colorimetric responses. These are the first examples of the use of tetra-amidourea receptors in such a highly ordered structure for the recognition and colorimetric sensing of anions.

Results and Discussion

Synthesis. The synthesis of the 4-nitrophenyl-based sensor **1** and the phenyl analogue **2** is outlined in Scheme 1. The aminolysis of the calix[4]arene tetraethyl ester **3**,[18] using hydrazine hydrate in ethanol at 40 °C, the reaction was left stirring for 12 h, to give the desired product, **4** (Figure S1, Supporting Information), in near-quantitative yield by evaporation of methanol and excess hydrazine and washing with methanol and water. The hydrazide **4** was found to be insoluble in organic solvents of moderate polarity, e.g., CH_2Cl_2, THF, and Et_2O. However, it was found that reaction of a suspension of **4** in chloroform with **6** occurred by simply stirring overnight under an inert atmosphere, giving a pearly suspension that was concentrated under reduced pressure, and to which excess methanol was added. Filtration, followed by methanol and water washes, gave **2** in 60% yield, requiring no further purification.

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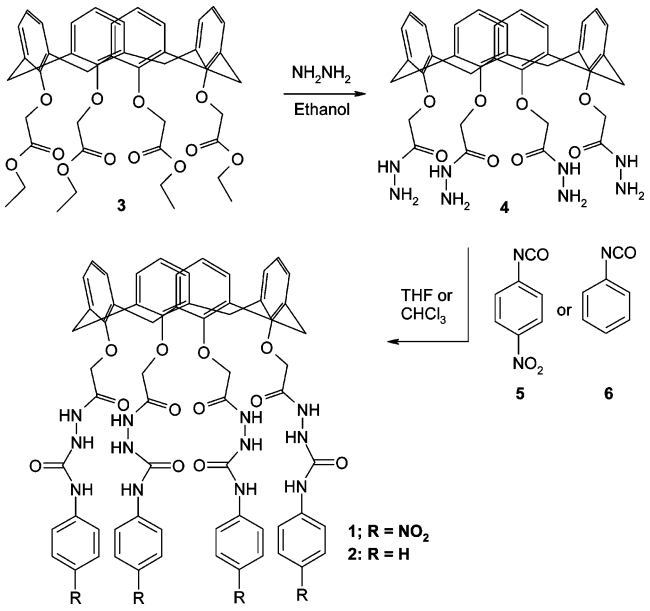
SCHEME 1. Synthesis of Sensors 1 and 2 from the Calix[4]arene Tetraethyl Ester 3



Similarly, using THF, a suspension of **4** and **5** was stirred at reflux overnight, upon which **1** precipitated from solution once the reaction had run to completion. This gave **1** in 72% yield after workup. The use of the amidourea moiety is very attractive for anion recognition as it has been shown to give rise to both stronger binding as well as additional hydrogen bonding donors in comparison to the simple urea structure. These sensors were found to be only soluble in polar solvents, such as DMSO and DMF. Attempts to prepare crystals for X-ray study were on all occasions unsuccessful. Such insolubility may be attributed to strong intramolecular hydrogen bonds for these receptors. However, addition of anions to suspensions of these compounds led on all occasions to their immediate solubilization in a range of medium-to-high polarity organic solvents, for example, CH_2Cl_2, THF, Et_2O, and CH_3CN. We propose that perturbation of the aforementioned hydrogen-bonding motifs by the anions is responsible for this effect.[19] Slow evaporation of the solutions containing these complexed anions led, however, to the formation of oils on all attempts. The ^{1}H NMR (Figure S2 and S3, Supporting Information) of both sensors showed the presence of "simple spectra", indicating the presence of C_4 symmetry, with the three N−H protons appearing at 9.91, 8.83, and 8.09 for **1**, whereas for **2** these resonated at 9.99, 9.51, and 8.44 ppm, respectively.

Anion Binding Studies of 1. The calix[4]arene scaffold offers a rigid and structurally preorganized cavity, which has previously been employed as a structural scaffold for anion recognition and for the formation of anion directed self-assembly formation.[17,19,20] Sensor **1** was developed to give a highly ordered hydrogen donating cavity, which could give rise to colorimetric changes upon anion recognition, as the 4-nitrophenyl moiety is part of the amidourea anion receptor moiety, giving rise to an internal charge transfer (ICT) absorption band in the absorption spectra, which was centered at ca. 335 nm (ϵ = 66 346 $m^{-1}cm^{-1}$) when recorded in DMSO. Upon adding anions such as $H_2PO_4^-$ (Figure 1) or AcO^- to a solution of **1**, a gradual shift to longer wavelength was observed, signifying a perturbation in the ICT character of the sensor, due to the recognition of the anion at the amidourea moiety. This enhances the push−pull character of the ICT state, and consequently a red-shift is observed, upon hydrogen bonding of the anion to the receptor array of the sensor. Fitting these changes using the nonlinear regression analysis program SPECFIT gave good fit, with two binding constant $\log \beta_1 = 5.46$ (± 0.17) and $\log \beta_2 = 4.87$ (± 0.35). The speciation distribution diagram is shown as an insert in Figure 1 and demonstrated that the 1:1 stoichiometry (**H**ost:**G**uest) is the most stable species in solution, and only at higher concentration does the 1:2 (**H:G**) become dominating. In the case of AcO^- (Figure 2), unlike that seen for $H_2PO_4^-$, an isosbestic point was observed at 377 nm, with the formation of a broad shoulder at longer wavelength, lingering into long wavelength. Again, the main interaction here is the hydrogen bonding of the anion to the receptor, which gives rise to enhanced ICT character for the bound receptor. Analysis of these changes using SPECFIT gave two binding constants: $\log \beta_1 = 5.11$ (± 0.04) and $\log \beta_2 = 4.29$ (± 0.12), for the 1:1 and the 1:2 complexes, respectively. No higher order binding was observed, indicating that, most likely, each of the two AcO^- is coordinating to two urea moieties. This analysis also demonstrates strong binding for AcO^- in DMSO to **1**, which is still, however, less than that observed for phosphate, enabling the selective detection of the latter. By simply comparing the changes in the UV−vis spectra with that of $H_2PO_4^-$, it is obvious that the binding mode must be different, and perhaps reflect the tetrahedral nature of the $H_2PO_4^-$ anion, which can be better hosted within the cavity of the calix sensor. Substituents appended to the lower rim have, as in the case of **1**, the option of *endo*- or *exo*- binding, that is, that the binding can take place within the cavity formed, or outside this cavity, following free rotation in solution of the urea moieties. For the *endo*- binding array, the anions would be encapsulated by the cooperation of all receptor moieties. This is both the intuitive and the desirable conformation in light of the inherent rigidity of the calix[4]arenes. This would provide a convergent anion binding array, offering eight "urea" protons for anion binding. However, the *exo*- binding mode may also lead to strong binding of anions, where the calixarene scaffold would merely provide several, non-cooperative binding sites. *Exo*-binding may also provide a contribution from the amido protons, which we expect to be absent in the *endo* binding. Giving the structural nature of the anion, it is possible that $H_2PO_4^-$ binds *via* the *endo* motive, whereas AcO^- would bind possibly by both modes, or more so *via exo*-binding. However, the binding of the second equivalent of $H_2PO_4^-$ could occur *via exo* binding. To analyze these binding possibilities further, we attempted to grow crystals for X-ray crystallographic analysis of the 1:1 and the 1:2 stoichiometries of these ions with **1**. However, on all occasions, we were unable to form crystals of good enough quality for such analysis, and hence, we were unable to proof exclusively the correct binding geometry. Analysis of the species distribution for AcO^- interaction is shown as an insert in Figure 2, and it demonstrates, as in the case of $H_2PO_4^-$, that only at higher concentration of the anion does the 2:1 complex dominate. However, unlike that seen for $H_2PO_4^-$, the tail in the absorption spectra of AcO^- gave rise to light colorimetric response that was, however, visible to the naked eye.

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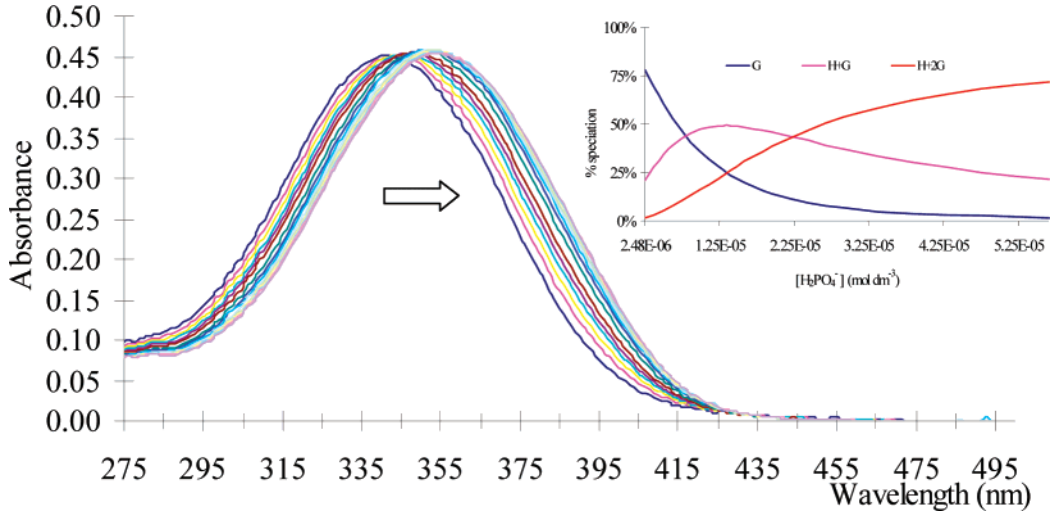

FIGURE 1. Changes in the absorption spectra of **1** (6.7×10^{-6} m) and upon titrating with $H_2PO_4^-$. (Inset) Speciation distribution diagram for the binding of $H_2PO_4^-$ to **1**.

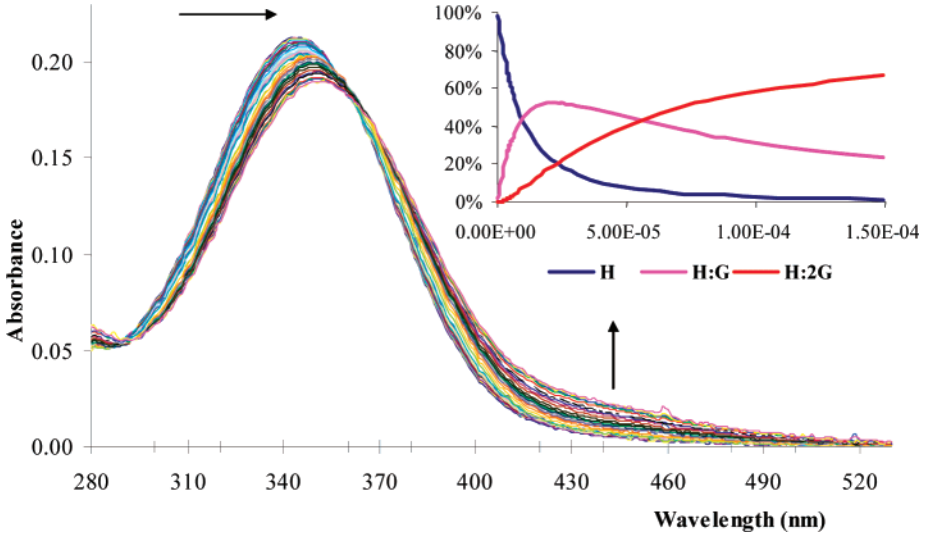

FIGURE 2. Changes in the absorption spectra of **1** (3.2×10^{-6} m) and upon titrating with AcO^-. (Inset) Speciation distribution diagram for the binding of AcO^- to **1**.

The most interesting results were obtained for pyrophosphate and fluoride, respectively. For the former, the absorption spectra was shifted to longer wavelength, with ca. 10% overall enhancement. However, the absorption spectra were also red-shifted, with λ_{max} being shifted from *ca.* 340 nm → 356 nm. Analysis of these changes revealed that pyrophosphate bound to the sensor in only one binding mode through the desired 1:1 binding. Both the changes in the absorption spectra at 345 nm and the corresponding fitting, obtained using SPECFIT and the speciation distribution diagram, are shown in Figure 3. From these changes, the binding constant $\log \beta_1 = 5.34$ (± 0.04) was obtained. This is of similar magnitude to that observed above in Figure 1 for $H_2PO_4^-$. However, no evidence for the 1:2 stoichiometry was observed within the concentration range shown. These changes were also accompanied with the formation of red color change that was visible to the naked eye. However, this color change was less visible than we have observed in related systems based on the use of 1,3-disubstituted calix[4]arene. Nevertheless, upon addition of excess anion, those visible color changes was more apparent, giving rise to red color formation. These results will be discussed below.

In the case of F^-, significant changes were observed in the absorption spectra as well as visible colorimetric changes. Here, the absorption was similar to that observed for AcO^-: the main absorption band was reduced in intensity with concomitant shift to longer wavelength and with the formation of a tail or shoulder, which extended toward the red, Figure 4. Analysis of these changes using SPECFIT gave good fitting, with two binding constants of $\beta = 5.68$ (± 0.20) and $\log \beta = 4.78$ (± 0.39), respectively, by fitting the data to 1:1 and 1:2 **H:G** formation. Although this gives reasonable fit, and the speciation distribution diagram indicates that the 1:2 complex is the dominant species in solution, we do not anticipate this to be the case, and rather that the second equivalent of F^- participates in the formation of biflouride, HF_2^-. Such deprotonation has previously been shown to occur in related systems by ourselves, and by Gale et al.[21] In contrast to these results, the titrations of **1** using either Cl^- or Br^- did not give rise to significant changes in the

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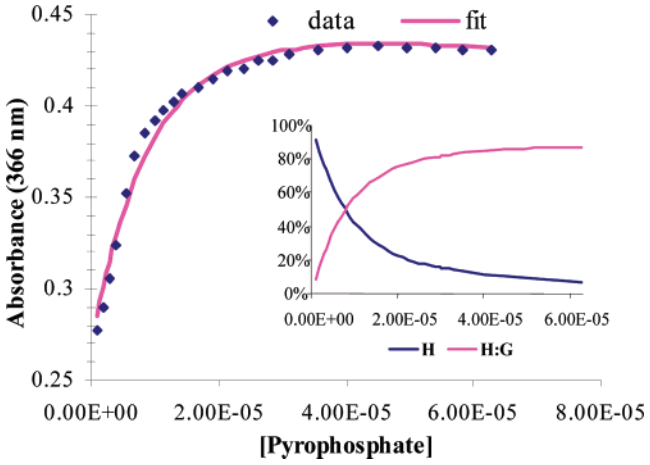

FIGURE 3. Changes in the absorption spectra of **1** (3.2×10^{-6} m) at 345 nm upon titrating with pyrophosphate. (Inset) Speciation distribution diagram for the binding of pyrophosphate to **1,** showing only the formation of the 1:1 complex.

absorption spectra. Initially, we had predicted that **1**, having the four amidourea receptors preorganized in a cyclic manner, would indeed show selectivity toward large spherical anions. However, as said, only minor changes were observed in the absorption spectra, which in the case of Cl^- were too small to allow for accurate fitting. However, initial ^1H NMR studies (in DMSO-d_6) did suggest that Cl^- was hosted within the cavity of the sensor; consequently, the reason for the lack of colorimetric changes could be due to the fact that the anion coordinates through a hydrogen bonding array, most likely, to all four of the amidourea receptors. Unlike that seen above, where the anion recognition is more directional, the effect on the ICT of the receptor is much less. However, in the case of Br^-, small (ca. 8%) but uniform absorption changes were observed, which allowed for evaluation of the binding affinity of **1** toward this anion. Bearing in mind that there is a significant error associated with the measurement of Br^-, a 1:1 binding constant that fitted well the binding isotherms was determined as $\log \beta_1 = 4.15$ (± 0.05). However, no measurable colorimetric change was observed. Similarly, for nitrate, no changes were observed in the absorption spectra that allowed for the detection of this anion by **1**. As was discussed above, **1** gave rise to colorimetric responses with several anions, though these were not striking color changes at lower concentration of the receptors. Upon increasing the concentration of **1**, the aforementioned anion selectivity and significantly more intense color changes were visible, as can be seen in Figure 5. These color changes clearly follow the same trend observed in the anion titrations discussed above, where only F^- and pyrophosphate give rise to large colorimetric response, followed by AcO^-. As the sensor has no visible absorption, its solution is transparent, whereas in the presence of F^- and $H_2PO_4^-$, the red color is clearly visible; consequently, these color changes can be considered as being the equivalent of a naked eye "*no-yes*" response for these anions. We are currently improving the design principle presented herein with the aim of making these striking color changes more visible at lower concentrations. The binding constants discussed above are summarized in Table 1.

The anion recognition event was also evaluated using ^1H NMR as previously discussed for Cl^-. A titration was first undertaken using **1** in DMSO-d_6. However, due to the ICT nature of the receptor, even after the addition of 0.1 equiv of the anion, the characteristic NH resonances appeared to lose resolution. This situation deteriorated through the titration, and therefore no signal could be successfully used to track the changes which were occurring upon binding. Because of this, we synthesized **2**, as detailed above. The titration of **2** using phosphate showed that the high symmetry of the spectra remained during the titration, indicting that on the NMR time scale, the anion was not binding directly to a single receptor and the overall changes were in slow exchange. Moreover, all of the N−H resonances were clearly visible and were significantly shifted upon adding anions. For $H_2PO_4^-$, the ^1H NMR (Figure S4, Supporting Information) gave rise to the most significant changes. For the urea proton adjacent to the phenyl ring, the addition of 0.3 equiv of $H_2PO_4^-$ led to significant broadening, whereas the distal urea protons were shifted *ca.*

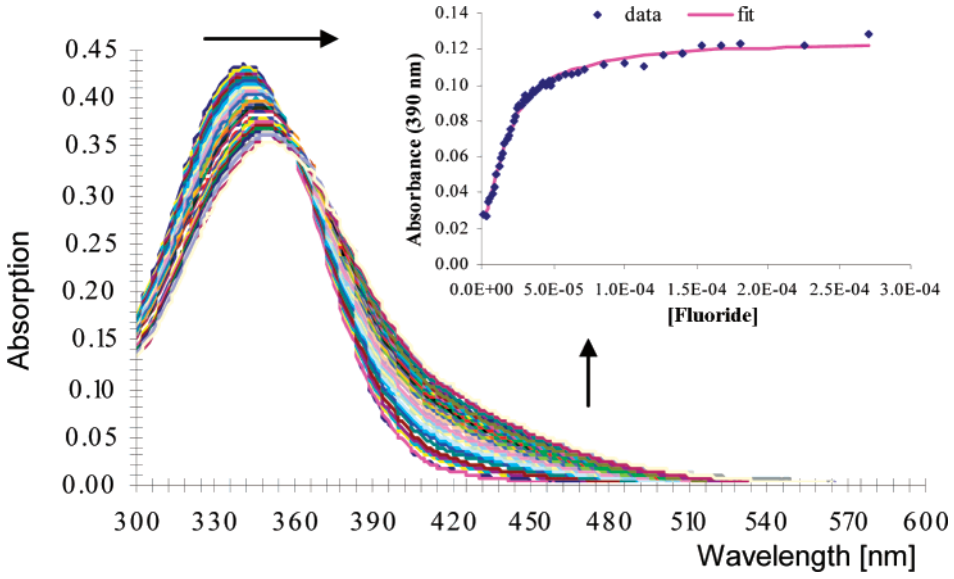

FIGURE 4. Changes in the absorption spectra of **1** (6.6×10^{-6} m) and upon titrating with F^-. (Inset) Fitting of absorption changes at 390 nm for the titration of F^- with **1**.

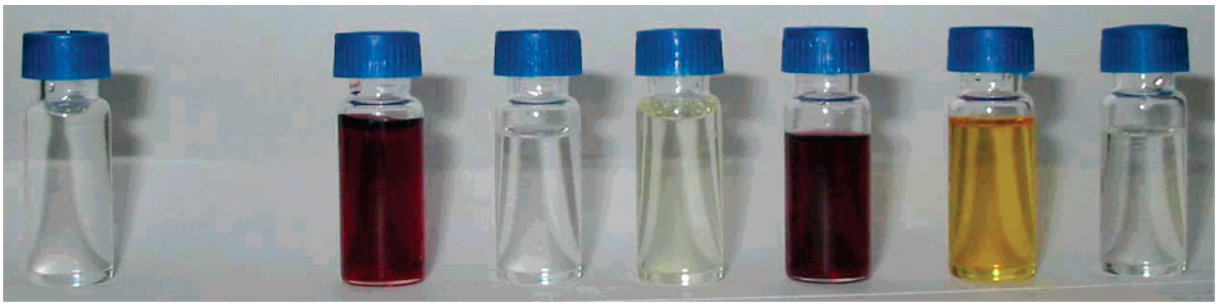

FIGURE 5. Colorimetric effect upon addition of TBA salts of anions (1 mM) to DMSO solutions of **1**; left to right: **1**, F^-, Cl^-, $H_2PO_4^-$, $HP_2O_7^{3-}$, AcO^-, and NO_3^-.

TABLE 1. Binding Constants Obtained from the Changes in the Absorption Spectra of 1 upon Titrations with Several Anions Using the Nonlinear Regression Analysis Program SPECFIT

Ion	log β_1 1:1 stoichiometry	log β_2 2:1 (guest to host) stoichiometry
AcO^-	5.10 ± 0.04	4.29 ± 0.12 (HG_2)
$H_2PO_4^-$	5.45 ± 0.17	4.84 ± 0.35 (HG_2)
Pyrophosphate	5.34 ± 0.04	-
F^-	5.68 ± 0.20	4.79 ± 0.39 (HG_2)
Br^-	4.15 ± 0.05	-

0.8 ppm, for the urea protons after the addition of ca. 1.2 equiv. In comparison, the amido proton, appearing at 9.3 ppm, was only slightly affected and clearly visible even after the addition of 1 equiv. Plotting these changes as a function of added $H_2PO_4^-$ gave a gradual curve that began to plateau after the addition of 1 equiv, Figure 6, for one of these urea protons. These changes demonstrate that the desired 1:1 stoichiometry is the most favorable one under these experimental conditions. The data obtained was also fitted using the computer program WinEQNMR, which calculates equilibrium constants based on NMR shift data.[22] Fitting of the data gave binding constant data of $K = 1 \times 10^4$ ($\pm 4 \times 10^3$). The isotherm and fit, as generated using WinEQNMR, are shown in Figure 6 as an insert and showed a good correlation with the experimental data. Nevertheless, this binding constant was determined with an exceptionally large error that is beyond the 15% error margin of the technique, as estimated by Gale.[23] It is thus difficult to say with accuracy that this is the binding affinity for the anion. However, it is, as expected, significantly smaller than observed in the UV−vis titration of **2**, as it lacks the electron withdrawing nitro group, which makes the urea protons less acidic and, as thus less capable hydrogen bonding donors. These results confirm the changes observed in the absorption titrations above, that the anion is bound within the cavity in a 1:1 stoichiometry. We are currently developing related systems with the aim of achieving better selectivity for the anion sensing as well as more visibly striking colorimetric changes.

Conclusion

We have developed the first examples of colorimetric anion sensors, **1** and **2**, based on the introduction of four amidourea based receptors into the lower rim of a calix[4]arene structure, in high yielding synthesis. This gives rise to the formation of a highly symmetrical and preorganized cavity, as shown by ^1H NMR, for anion recognition through hydrogen bonding. The binding of several anions was investigated in DMSO using UV−vis spectroscopy, and it was established that ions such as $H_2PO_4^-$ and pyrophosphate were recognized by **1** through 1:1 host−guest complex formation (which was verified using ^1H NMR for **2**) with high binding constants. In contrast to these results, anions such as F^- and AcO^- gave rise to strong 1:2 binding, whereas for the former, the anion recognition was most likely due to the formation of HF_2^-, which is formed upon deprotonation of the urea moiety. Sensor **1** was principally developed as a colorimetric naked-eye sensor for anions. This was indeed found to be the case for anions such as F^- and

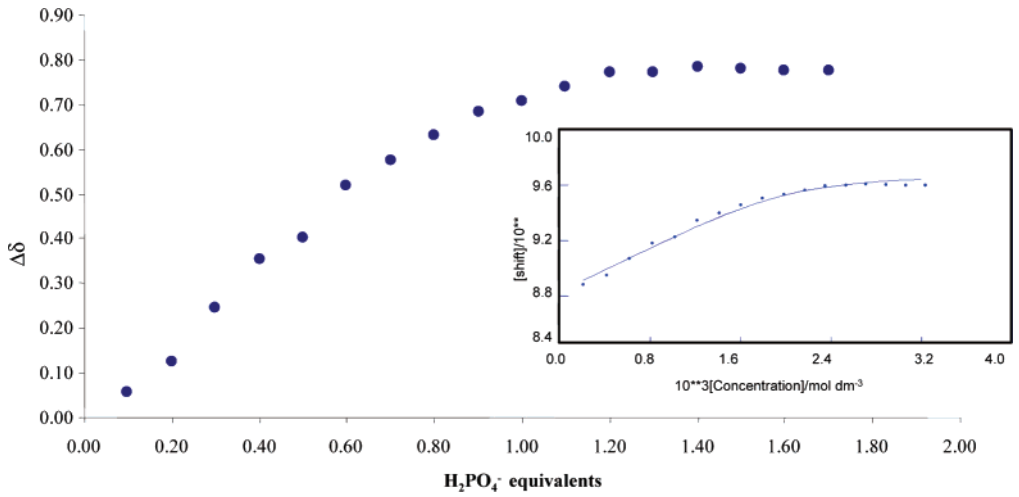

FIGURE 6. Changes in the chemical shifts one of the urea protons of **2** in the ^1H NMR titration of **2** using $H_2PO_4^-$. (Inset) Fitting of the experimental data using WinEQNMR.

pyrophosphate where the color changed from colorless to red. For AcO^-, the changes occurred from colorless to light yellow, enabling the distinction of this anion from the above. These changes were assigned to the disturbance in the ICT nature of the anion receptor, which gives rise to red shifts in the absorption spectra. We are currently further investigating the use of the calix[4]arene structure in the formation of novel anion hosts and with the aim of using anions to direct the formation of larger self-assemblies between such structures.

Experimental

Synthesis: 25,26,27,28-Tetrakis[(hydrazidocarbonylmethyl)-oxy]calix[4]arene. To a solution of calix[4]arene ethyl ester (3) in methanol was added hydrazine hydrate 10 mL (large excess), and the solution was stirred at 40 °C overnight. The solvents and excess hydrazine were removed at reduced pressure, giving the crude product as an off-white solid. The residue was triturated sequentially with water and methanol and the product collected by filtration. The desired product **4** was obtained in 96% yield from **3**. mp 201 °C. Required for $C_{36}H_{40}N_8O_8 \cdot H_2O$: C, 59.17; H, 5.79; N, 15.33; Found: C, 59.60; H, 5.53; N, 14.63; Accurate MS (*m*/*z*): Calculated for $C_{36}H_{40}N_8O_8Na$ (M + Na): 735.2867; found 735.2838; ^{1}H NMR (400 MHz, methanol-d_4), δ_H: 9.50 (s, 4H, NH), 6.85 (d, J = 8 Hz, 8H), 6.65 (t, J = 8 Hz, 4H), 4.47 (d, J = 13 Hz, 4H), 4.42 (s, 4H), 3.24 (d, J = 13 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C): 167.5, 155.1, 134.6, 128.6, 123.4, 73.4, 30.1; IR (ν/cm^{-1}): 3313, 3035, 2920, 1672, 1528, 1459, 1442, 1291, 1248, 1196, 1094, 1006, 982, 730.

General Procedure for the Synthesis of 1 and 2. The hydrazide **4** was suspended in dry THF. Phenyl isocyanate, **6** (4.0 equiv) was added neat, whereas nitrophenyl isocyanate, **5** (4 equiv), was added as a solution in dry THF. The mixture was stirred overnight, giving a thick precipitate. The reaction was quenched by addition of CH_3OH, and the precipitate filtered and washed with CH_3OH and water to yield the desired sensors which were suitable for use without further purification.

25,26,27,28-Tetra[3-(4-nitrophenyl)ureidocarbamoyl]methoxy calix[4]arene (1). Obtained from **4** and **5** in 72% yield. mp: 244 °C. Required for $C_{64}H_{56}N_{16}O_{20} \cdot 2H_2O$: C, 54.70; H, 4.30; N, 15.95. Found: C, 54.66; H, 4.10; N, 15.62; ESMS (*m*/*z*, ES−): 1369.0, expect 1368.4 (M); ^{1}H NMR (400 MHz, DMSO-d_6,δ_H): 9.99 (s, 4H, NH), 9.51 (s, 4H, NH), 8.44 (s, 4H, NH), 7.98 (d, J = 9.36 Hz, 8H,), 7.57 (d, J = 8.76 Hz, 8H), 6.78 (d, J = 7.00 Hz, Ar−H), 6.67 (t, J = 7.00 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ_C): 168.9, 155.5, 154.6, 146.0, 141.0, 134.3, 128.6, 125.4, 124.7, 123.1, 117.7, 72.8, 30.6; IR (ν/cm^{-1}): 3269, 2927, 1709, 1674, 1543, 1500, 1459, 1329, 1228, 1192, 1177, 1111, 1095, 1043, 1007, 852, 840.

25,26,27,28-[Tetra(3-phenylureidocarbamoyl)methoxy] Calix-[4]arene (2). Obtained from **4** and **6** in 60% yield. mp: 232 °C Required for $C_{64}H_{60}N_{12}O_{12-} \cdot CH_3OH$: C, 63.92; H, 5.28; N, 13.76. Found: C, 63.94; H, 5.14; N, 13.73; ^{1}H NMR (400 MHz, DMSO-d_6,δ_H): 9.91 (s, 4H, NH), 8.83 (s, 4H, NH), 8.09 (s, 4H), 7.40 (d, J = 7.04 Hz, 1H), 7.20 (t, J = 7.04 Hz), 6.92 (t, J = 6.52 Hz, 4H), 6.80 (d, J = 7.52 Hz, 8H), 6.66 (d, J = 7.56 Hz, 1H), 4.74 (d, J = 12.48 Hz, 4H, 2H), 4.69 (s, 8H, 2H) 3.29 (d, J= 13.08 Hz, 4H,); ^{13}C NMR (100 MHz, DMSO-d_6, δ_C): 168.8, 155.6, 155.4, 139.3, 128.7, 128.6, 123.1, 122.0, 118.7, 72.9, 30.7; IR (ν/cm^{-1}): 3239, 1660, 1541, 1444, 1204, 749, 692.

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Supporting Information Available: Figures S1−S4. General experimental description. This material is available free of charge via the Internet at http://pubs.acs.org.

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