Second Generation Calixpyrrole Anion Sensors

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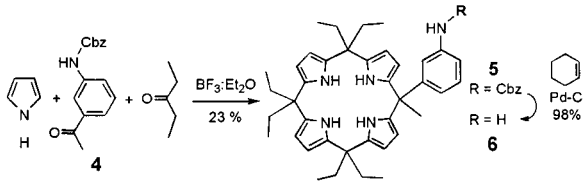
While traditional methods of anion sensing such as ion selective electrodes continue to hold their ground, increasing attention is being devoted to finding alternative ways of effecting anion detection. Here, sensors based on anion-induced changes in fluorescence appear particularly attractive. They offer the potential for high sensitivity at low analyte concentration coupled with obvious ease of use.[1] Unfortunately, few, if any, fluorescent anion sensors exist that display high phosphate/chloride selectivities, emit in the visible region, or function in aqueous media over a wide range of pH. Such attributes, however, would be desirable. They might allow, among other things, the study of metabolic processes in biological milieus without interference from endogenous substrates such as chloride anion or aromatic amino acids.

Recently we described a new class of fluorescent anion sensors[2] that are based on the use of octamethyl calix[4]pyrrole[3] as the anion recognition element.[4] In these first generation sensors, an anthracene derivative was used as the fluorescent signaling device. Unfortunately, drawbacks, including low phosphate:chloride selectivity ratios and less-than-ideal generalized affinities for anions, prompted us to search for improved systems. In this communication, we report the synthesis of three second generation calixpyrrole-based fluorescent anion sensors, compounds **1**−**3**. These systems bind anions with greater affinity than previous systems while displaying a more efficient fluorescent response.

In the design of sensors **1**−**3**, a rigid aromatic spacer was used so as to fix the distance between the quencher (anion) and the signaling moiety. This spacer element contained either a sulfonamide[5] (compound **1** and **2**) or thiourea[6] (sensor **3**) group. These linker moieties were introduced with the expectation that they might provide additional hydrogen bond donor sites that would act in concert with the calixpyrrole NH protons to enhance the overall anion binding affinities.

The choice of fluorescent label was guided by two considerations. First, to target biological analytes, it was appreciated that the sensors would have to function in the presence of water (i.e., either in water itself or in a solvent in which water is miscible). Second, to avoid possible interference from fluorescent impurities, such as aromatic amino acids, it was considered desirable to use labels that would allow for excitation by visible light ($\lambda_{Abs} >$ 300 nm). Given these considerations, we decided to use dansyl, Lissamine-rhodamine B, and fluorescein as fluorescent labels.[7] These labels show appreciable fluorescence intensity in aqueous solutions even at very low concentrations (in this work the concentrations of the sensors were always kept ≤ 5 μM).

Scheme 1



The synthesis of sensors **1**−**3** departs from the general precursor **6**. This key intermediate may be prepared in multigram scale in two steps from Cbz-protected 3-aminoacetophenone **4**, 3-pentanone, and pyrrole in the presence of BF_3:Et_2O (Scheme 1). Deprotection of the initial product **5** then produces **6** in 21% overall yield. Sensors **1**−**3** were then prepared using standard labeling methodologies[8] and were isolated in 92%, 68%, and 93% yields, respectively.

All three sensors **1**−**3** proved soluble in a wide range of organic solvents. Acetonitrile was selected because it is water miscible, meaning it would allow the sensing of anions added in the form of aqueous solutions. In the case of sensors **1** and **2,** acetonitrile containing 0.01% water (which corresponds to a water concentration of ca. 5.6 mM) was used. In the case of sensor **3**, a system designed in such a way that it actually requires the presence of water to hydrolyze its nonfluorescent precursor (i.e., the corresponding lactone), studies were carried out in solutions of acetonitrile containing 4% water by volume ($[H_2O] = 2.2$ M). Under these conditions, sensor **3** was found to operate at pH 6.5−8.5, with neutral pH 7.0 ± 0.1 being used for quantitative studies.

Anions tested as potential substrates for sensors **1**−**3** included fluoride, chloride, dihydrogenphosphate, and hydrogen pyrophosphate. These anions, studied in the form of their tetrabutylammonium (TBA^+) salts, were chosen because of their biological importance (especially Cl^-, $H_2PO_4^-$, and $HP_2O_7^{3-}$).

^{1}H NMR spectroscopic analyses were used to establish 1:1 binding stoichiometries.[9] They were also used to carry out qualitative binding titrations. Here, for instance, concerted downfield shifts were observed for the protons attached to C2 of the aromatic spacer, the pyrrole, and the sulfonamide nitrogen as receptors **1** and **2** were exposed to increasing concentrations of anions. Likewise, the multiplets corresponding to the C4, C5, and C6 protons of the phenyl spacer were seen to be shifted to higher field as the concentration of anions was increased. Taken together, these concerted changes support the contention that all the

(1) (a) *Chemosensors of Ion and Molecular Recognition*; Desverne, J.-P., Czarnik, A. W., Eds.; NATO ASI Series, Ser. C; Kluwer: Dordrecht, The Netherlands, 1997; Vol. 492, (b) de Silva, A. P.; Guanarante, H. Q. N.; Gunnlaugson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (c) Beer, P. D. *Chem. Commun.* **1996**, 689−696, (d) Dickens, R. S.; Gunnlaugson, T.; Parker, D.; Peacock, R. D. *Chem. Commun*. **1998**, 1643−1644. (e) Fabbrizzi, L.; Faravelli, I.; Francese, G.; Licchelli, M.; Perotti, A.; Tagletti, A. *Chem. Commun*. **1998**, 971−972. (f) Black, C. B.; Andrioletti, B.; Try, A. C.; Ruiperez, C.; Sessler, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 10438−10439.

(2) Miyaji, H.; Anzenbacher, P., Jr.; Sessler, J. L.; Bleasdale, E. R.; Gale, P. A. *Chem. Commun.* **1999**, 1723−1724.

(3) Baeyer, A. *Ber. Dtsch. Chem. Ges.* **1886**, *19*, 2184−2185.

(4) Gale, P. A.; Sessler, J. L.; Král, V.; Lynch, V. *J. Am. Chem. Soc.* **1996**, *118*, 5140−5141. Gale, P. A.; Sessler, J. L.; Král, V. *Chem. Commun.* **1998**, 1−8.

(5) Kavallieratos, K.; Bertao, C. M.; Crabtree, R. H. *J. Org. Chem*. **1999**, *64*, 1675−1683.

(6) Sasaki, S.; Mizuno, M.; Naemura, K.; Tobe, Y. *J. Org. Chem.* **2000**, *65*, 275−283. Bühlmann, P.; Nishizawa, S.; Xiao, K. P.; Umezawa, Y. *Tetrahedron* **1997**, *53*, 1647−1654. Scheerder, J.; Engbergsen, J. F. J.; Casnati, A.; Ungaro, R.; Reinhoudt, D. N. *J. Org. Chem.* **1995**, *60*, 6448−6454.

(7) Sensor **1** (fluorescent label: dansyl) has absorption maximum $\lambda = 350$ nm, emission $\lambda = 520$ nm, $\Phi = 0.36$; sensor **2** (fluorescent label: Lissamine) has absorption maximum $\lambda = 550$ nm, emission $\lambda = 575$ nm, $\Phi = 0.18$; sensor **3** (fluorescent label: fluorescein) has absorption maximum $\lambda = 510$ nm, emission $\lambda = 525$ nm, $\Phi = 0.42$. Fluorescence quantum yields (Φ) were measured following the method described by Demas and Crosby (Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991−1024) with quinine sulfate as standard (2 μM solution in 0.05 M sulfuric acid, $\Phi = 0.546$).

(8) Haughland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes, Eugene-Leiden, 1996. Lefevre, C.; Kang, H. C.; Haughland, R. P.; Malekzadeh, N.; Arttamangkul, S.; Haughkand, R. P. *Bioconj. Chem*. **1996**, *7*, 482−489.

(9) Tsukube, H.; Furuta, H.; Odani, A.; Takeda, Y.; Kudo, Y.; Inoue, Y.; Liu, Y.; Sakamoto, H.; Kimura, K. *Determination of Stability Constants*, in *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., Macnicol, D. D., Vogtle, F., Eds.; Elsevier Science Ltd.: New York, 1996; Vol. 8, pp 425−482.

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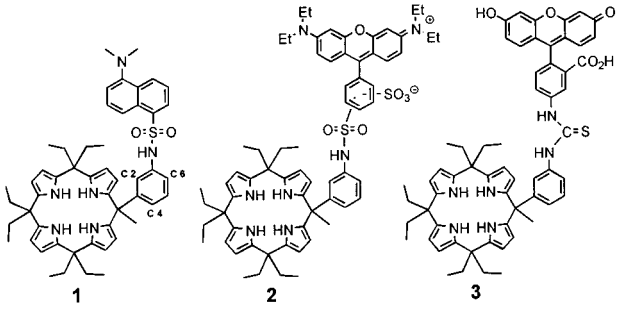

Figure 1. Structures of second generation sensors **1**−**3**. These systems contain a rigid spacer and rely on the use of dansyl (**1**), Lissamine-rhodamine B (**2**), and fluorescein (**3**) moieties as the fluorescent elements, respectively.

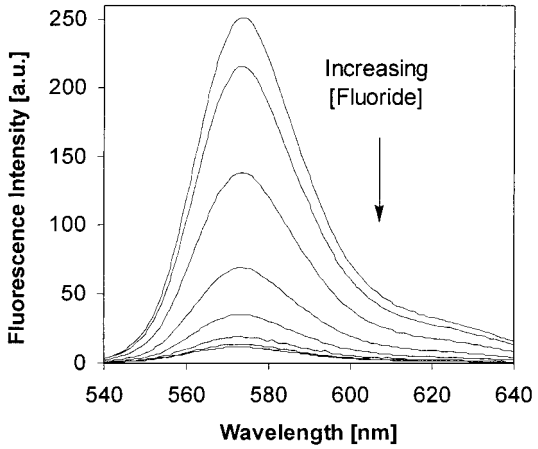

Figure 2. Decrease in fluorescence emission intensity observed when sensor **2** (0.1 μM in acetonitrile containing 0.01% v/v water) is titrated with increasing concentrations of tetrabutylammonium fluoride. From top to lowest trace, [F^-] = 0, 0.30, 0.76, 1.53, 2.30, 3.06, 3.83, 4.60 μM.

Table 1. Affinity Constants[a] for Sensors **1**−**3** and Anionic Substrates As Determined in Acetonitrile (0.01% v/v water) for Sensors **1** and **2** and Acetonitrile−Water (96:4, pH 7.0 ± 0.1) for Sensor **3**

	association constants (mol^{-1}) determined by emission quenching		
	sensor **1** (5×10^{-6} M)	sensor **2** (1×10^{-6} M)	sensor **3** (5×10^{-6} M)
F^-	222 500	>1 000 000	>0 200 000
Cl^-	10 500	18 200	<10 000
$H_2PO_4^-$	168 300	446 000	682 000
$HP_2O_7^{3-}$	131 000	170 000	>2 000 000

[a] For a detailed description of the experimental conditions used for these titration experiments see the Supporting Information.

hydrogen bond donors within the molecule of sensors **1**−**3** act in a cooperative fashion.

More quantitative assessments of the anion affinities came from observing the extent to which the fluorescence intensity of sensors **1**−**3** was quenched in the presence of anions (Figure 2).

From the extent of this quenching, appreciable for all three sensors and all four anions, affinity constants were calculated.[1f] The resulting values are listed in Table 1. While, as expected,[4] fluoride anion gave rise to the largest response, an inspection of Table 1 reveals that sensors **1**−**3** are remarkably selective for phosphate and pyrophosphate anions relative to Cl^-. The high selectivity for phosphate and pyrophosphate, something not seen previously for any calixpyrrole-based system,[2,4] is potentially advantageous in biological sensing applications where a high concentration of Cl^- (but not F^-) pertains. In the case of sensors **1**−**3**, this selectivity can be explained by the presence of multiple

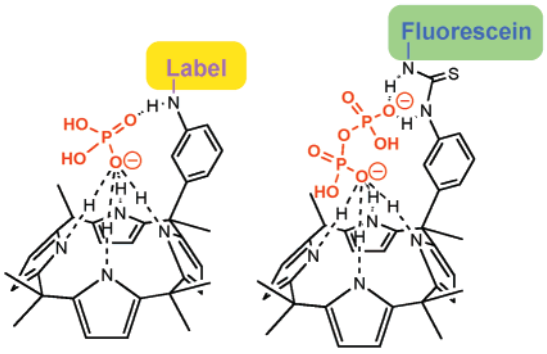

Figure 3. Schematic representation of the multiple hydrogen bonding interactions that are believed to account for the high phosphate and pyrophosphate affinities observed for sensors **1** and **2** and **3**, respectively.

hydrogen bonding interactions involving these two nonspherical anions. Such effects, illustrated in Figure 3, are likely to be particularly pronounced in the case of sensor **3** and pyrophosphate dianion where the coordination of the second anionic center within the pyrophosphate by the thiourea moiety is believed to be responsible for the dramatic increase in affinity.[10]

One of the more interesting findings to emerge from the present study was that for sensors **1**−**2**, the sensing efficiency[11] was actually improved by the presence of water in acetonitrile (up to 0.01% v/v), presumably as the result of improved solvation of the charged/ionic moieties present in the fluorescent labels, a feature that also prevents the formation of sensor aggregates. Consistent with this proposal is the finding that, in the absence of water, the relevant anion binding isotherms display biphasic character. This is rationalized in terms of sensor aggregate dissociation and anion binding occurring at the same time. Addition of water, on the other hand, results in binding isotherms of nearly ideal hyperbolic shape; it also gives rise to higher numeric values for the affinity constants under consideration. The presence of water was also found to be beneficial for sensor **3**. This latter species was found to operate as a viable fluorescent sensor at concentrations of water in acetonitrile of up to 20% (v/v).

In summary, sensors **1**−**3** display the highest anion binding affinities for anions yet recorded for calixpyrrole-type receptors. They are also the first to show high phosphate/chloride selectivity (2 orders of magnitude) and, in the case of sensor **3**, the first such systems to operate successfully in the presence of water at physiological pH.

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Supporting Information Available: Synthetic experimental details for all **1**−**6**, Scott plots for the fluorescence titrations, and representative Job plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Related arguments can be advanced to explain the differences in binding strength between receptors **1**, **2**, and **3**. Sensor **2** contains a sulfonic acid residue that increases the acidity of the sulfonamide NH and, relative to **1**, its presumed efficacy as a hydrogen bond donor. Likewise, the presence of two ancillary hydrogen bond donors in the thiourea moiety is expected to increase the general anion binding efficiency of **3**.

(11) Sensing efficiency as used here is a qualitative term meant to imply improvements in a range of desirable features including linear response of the sensors to the presence of anions and a higher degree of quenching at lower anion concentrations.