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Sensing of Antipyretic Carboxylates by Simple Chromogenic Calix[4]pyrroles

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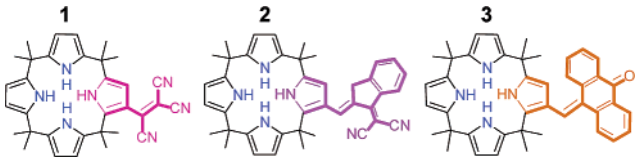
The importance of anions in biological and industrial processes requires the development of inexpensive and reliable anion sensors.[1] In this regard, sensors that utilize a change in optical properties for signaling are being increasingly appreciated.[2]

Octamethylcalix[4]pyrrole (OMCP),[3] an easy-to-make, colorless macrocycle containing four pyrrole NHs as hydrogen bond donors is an ideal candidate for the preparation of optical anion sensors. Indeed, several OMCP derivatives displaying anion-induced changes in either color[4a] or fluorescence[4b] have been synthesized by attaching pre-existing chromophores to the OMCP. Unfortunately, this approach proved too costly, and together with the fact that these materials could not be used in aqueous environments, precluded their application as anion sensors.

We were not ready to give up on OMCP, yet, and designed chromogenic anion sensors utilizing a combination of the OMCP pyrrole with nonchromophoric dye precursors to form the reporter chromophore. These sensors can be synthesized in a few steps and detect anions administered in the form of aqueous solutions, as well as in the presence of other ionic species/electrolytes.

The obvious advantages of this approach are the ease and high yield of the OMCP synthesis together with its electron-rich pyrrole moieties, a variety of available dye precursors, and time-proven reliable transformations. Additionally, this approach is generally applicable to other receptors comprising aromatic moieties.[5] Also, an effective change in color upon anion binding is expected because the anion binding to the pyrrole moiety of the dye is expected to induce a large change of electronic density in the chromophore as a result of partial negative charge ($\delta-$) transfer.[3a] Three examples of such materials are sensors **1**−**3**.



Sensor **1** was prepared by an electrophilic aromatic substitution reaction of OMCP with tetracyanoethylene. Sensors **2** and **3** were obtained by condensation of formyl-OMCP[6] with 1-indanylidene-malononitrile and anthrone, respectively.

The anion sensing ability of sensors **1**, **2**, and **3** was studied on a qualitative level by visual examination of the anion-induced color changes in the solution of sensors **1**−**3** (50 μM in DMSO/0.5% water) before and after the addition of an anion. Sensors **1**−**3** showed dramatic color changes in the presence of fluoride, acetate, pyrophosphate, and also phosphate, suggesting strong binding (Figure 1). Conversely, the addition of chloride, bromide, iodide, or nitrate resulted in no change in color. To demonstrate the relevance of sensors **1**−**3** to health care applications,[1] we performed the sensing experiments at a high electrolyte concentration and in blood plasma. Furthermore, studies with carboxylates of medical

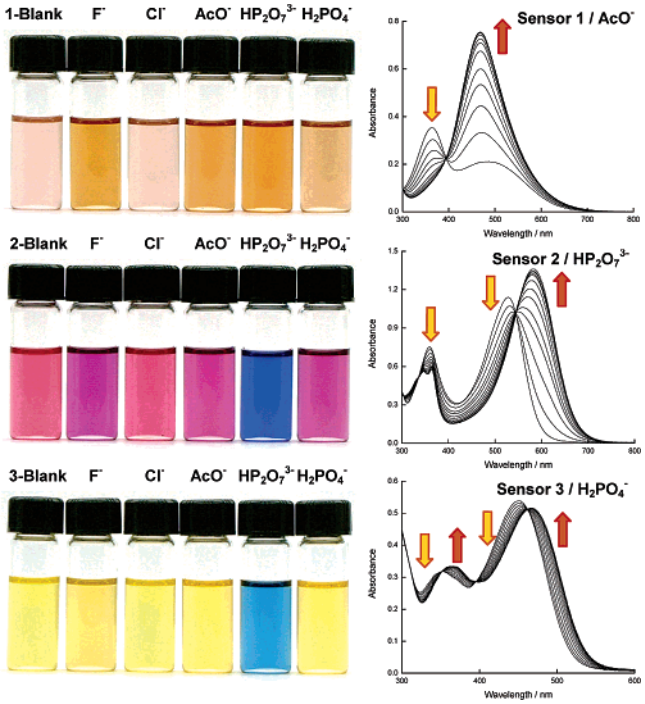


Figure 1. Left panels show sensors **1**−**3** (50 μM in DMSO) in the presence of anions in DMSO (10 equiv excess). Right panels show examples of changes in absorption spectra of **1**−**3** in the presence of selected anions.

interest (salicylate, ibuprofen, naproxen) were performed using a newly developed assay with sensors **1**−**3** embedded in polyurethane films.

Absorption spectroscopy titration experiments revealed large bathochromic shifts of spectra of sensors **1**−**3** upon addition of anions corresponding to changes in color. Such large red shifts can be attributed to a partial charge transfer resulting from the anion being bound to the NH proton of the pyrrole constituting the chromophore.[4a] The titration experiments provided the necessary quantitative insight into sensor−anion complexation. The respective binding constants for complexation of sensors **1**−**3** and various anions are shown in Table 1.

Table 1. Affinity Constants[a] for Sensors **1**, **2**, and **3** (M^{-1}) Calculated for Anionic Substrates in DMSO (0.5% of water) at 22 °C[7]

	Binding Constant K/M^{-1}		
anion	sensor **1**	sensor **2**	sensor **3**
F^-	$>10^{6b}$	$>10^6$	507000
Cl^-	1370	759	953
AcO^-	242000	22100	10400
$HP_2O_7^{3-}$[c]	584000	48200	$>10^{4b}$
$H_2PO_4^-$	5230	5560	4490

[a] All errors are $<\pm15\%$. [b] Binding isotherms show biphasic behavior. [c] K_a was calculated assuming that pyrophosphate forms a dimer in DMSO.[8]

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From Table 1, one can see that the sensors **1**−**3** strongly bind fluoride, acetate, pyrophosphate, and phosphate.[3b,c] Chloride, bromide, iodide, or nitrate showed weak or negligible binding. The strong anion binding is ascribed to the electron-withdrawing nature of dye moieties. These moieties increase the acidity of the pyrrole NH proton, which, in turn, enhances the availability of NHs for hydrogen bonding and affinity of sensors toward anions.[2b] The ^{1}H resonances of chromophore-modified pyrrole NHs in sensors **1**, **2**, and **3** appear at 7.61, 7.37, and 7.34 (δ scale in $CDCl_3$), respectively, which correlates with the trend in sensor−anion affinity, as reflected by the binding constants.

To prove that the observed changes in color are caused by anion binding, and not by deprotonation of the acidic NH proton in the dye pyrrole,[9] we performed ^{1}H NMR titrations. The observed downfield shifts of the pyrrole NH resonances are an indication of hydrogen bonding to an anion, as well as simplification of the pyrrole CH signals, a characteristic for transition from 1,3-alternate to a symmetrical conelike conformation.[10] Figure 2 shows ^{1}H NMR spectra of sensor **1** (a) and its complexes with anions (b−d), confirming that the color changes are the result of the anion−sensor association.

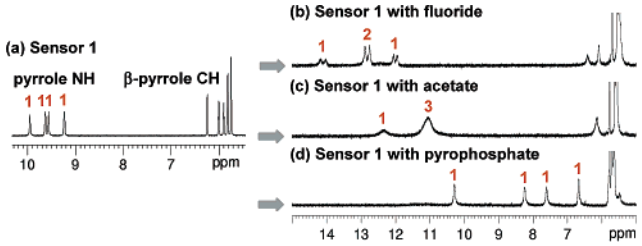

Figure 2. ^{1}H NMR spectra of sensor **1** (a) and complexes **1**/F^- (b), **1**/AcO^- (c), and **1**/$HP_2O_7^{3-}$ (d) recorded in DMSO-d_6 (0.5% water).

Strong selectivity of sensors **1**−**3** for carboxylates such as acetate and also for pyrophosphate compared to that of chloride and phosphate prompted us to investigate carboxylate sensing in the presence of chloride and/or phosphate. Such sensors may allow for sensing of carboxylates in blood plasma, which at physiological conditions, contains 0.1 M Cl^- and 2 mM HPO_4^{2-}.[1b] To explore the effect of water as a typical solvent for anions as well as the possible interference of competing anions, such as chloride, we performed the acetate sensing in a plasma-like aqueous solution (PLAS: 0.1 M Cl^-, 2 mM HPO_4^{2-}, 0.1 M Na^+, 4 mM K^+, pH = 7.4) and also PLAS containing bovine serum albumin (PLAS−BSA: 0.1 M Cl^-, 2 mM HPO_4^{2-}, 0.1 M Na^+, 4 mM K^+, 46 g/L BSA, pH = 7.4).[1b] Figure 3 (left panel) shows the color changes and absorbance traces recorded for sensor **1** (blank), sensor **1** upon addition of PLAS−BSA (A), and PLAS−BSA containing acetate (B).[11] These data show that sensor **1** can be used for detecting carboxylate anions added as an aqueous solution of ionic strength and pH corresponding to blood plasma.

Equally encouraging are the results of assays utilizing the sensors **1**−**3** embedded in polymer matrices.[12] Figure 3 (right panel) shows an example of a multi-well assay using polyurethane films with embedded sensor **2**. The polyurethane serves a dual purpose; it physically screens off the blood plasma protein carboxylates, such as C-termini, while its relatively hydrophobic nature precludes the hydrophilic anions (e.g., HCO_3^-) from penetrating the film and biasing the embedded sensors. Relatively lipophilic aromatic carboxylates seem to penetrate matrix and interact with the sensor, thus producing characteristic changes in polymer film color (Figure 3). The affinity order was as follows: naproxen ≈ ibuprofen ≥ salicylate[13] > laurate > acetate (solutions in PLAS), while no interaction was observed with PLAS alone, HCO_3^- in PLAS, PLAS−BSA, or blood plasma.

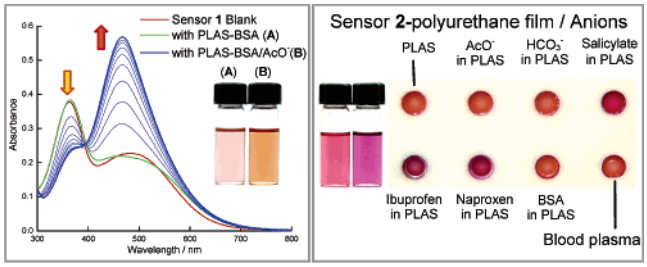

Figure 3. Left: Spectral changes of sensor **1** (50 μM in DMSO, 2 mL) upon addition of 10 μL of (A) PLAS−BSA and (B) PLAS−BSA containing acetate (4−40 mM), pH = 7.4. Inset: Color changes of sensor **1** upon addition of A and B. Right: Sensor **2** in polyurethane. PLAS solutions (25 μL) of anions (10 mM), BSA (46 g/L), all at pH = 7.4, and blood plasma were applied on polyurethane films.

In summary, we have demonstrated that calixpyrrole-based chromogenic sensors may be prepared via electrophilic aromatic substitution. The chromogenic OMCPs sense preferentially carboxylate and pyrophosphate anions with high affinity and selectivity, while showing dramatic change in color, even at high ionic strength (∼0.1 M NaCl). The preliminary experiments with polyurethane sensor films show a strong response to aqueous antipyretic carboxylates, such as naproxen, ibuprofen, or salicylate,[13] while not responding to chloride, bicarbonate, and carboxy termini in proteins of blood plasma. Further experiments toward sensing of carboxylates in biological fluids are underway.

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Supporting Information Available: Characterization of sensors **1**, **2**, and **3**, and experimental details on anion titrations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Hydrated tetrabutylammonium (TBA) salts of the anions were used in this study: fluoride ($X6H_2O$), chloride, bromide, iodide, phosphate ($X2H_2O$), and pyrophosphate ($X2H_2O$). The degree of hydration was estimated from elemental analyses. The fits are shown in Supporting Information.
(8) Titration experiments and Job plot indicated that the pyrophosphate−sensor stoichiometry was 2:1 due to pyrophosphate dimerization: Chu, F.; Flatt, L. S.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 4194−4204.
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(10) Deprotonation would be observed by disappearance of the corresponding NH resonance in the ^{1}H NMR spectrum.
(11) Binding constants for sensor **1** (in DMSO) with acetate in PLAS and PLAS−BSA are 211 000 and 26 400 M^{-1}, respectively.
(12) For more details, see Supporting Information.
(13) The upper therapeutic concentrations of antipyretic carboxylates, such as salicylate, are ∼2.5−3.0 mM in plasma. Clinical overdoses requiring treatment are in the excess of 4.0−5.1 mM in adults; see ref 1a, pp 343−345.

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