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Fluorescent Sensing of Pyrophosphate and Bis-carboxylates with Charge Neutral PET Chemosensors†

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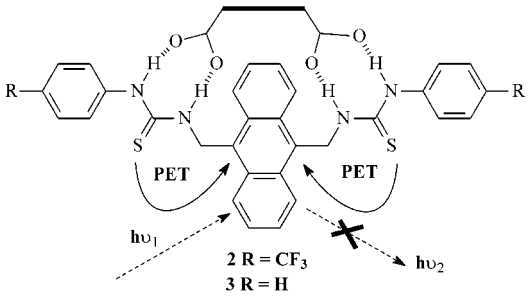
ABSTRACT



The fluorescent photoinduced electron transfer (PET) chemosensors 2 and 3 were designed for the recognition of anions possessing two binding sides such as dicarboxylates and pyrophosphate; the anion recognition in DMSO takes place through the two charge neutral thiourea receptor sites with concomitant PET quenching of the anthracene moiety. The anion binding of acetate, phosphate, and pyrophosphate to 2 and 3 was also evaluated by using ^{1}H NMR in DMSO-d_6.

Over the past few years, fluorescent and luminescent chemosensors for the detection of cations have been successfully developed.[1,2] Conversely, the development of optically based anion sensors has been less successful. Given the important role of anions in biology, clinical diagnostics, and environmental monitoring, the need for easily synthesized fluorescent anion chemosensors is of great importance.[3] A variety of anion receptors have been reported,[4,5] including many capable of luminescent sensing.[6] However, rather few have the simplicity and accessibility which is ideally required for practical devices.

We have been interested in the development of luminescent chemosensors for the detection of cations, anions, and neutral molecules.[7] We are currently particularly interested in developing luminescent anion chemosensors where the anion recognition takes place at charge neutral recognition

† Affectionately dedicated to Sigrúnar Ingibjargar Gísladóttur on the occasion of her 70th birthday.

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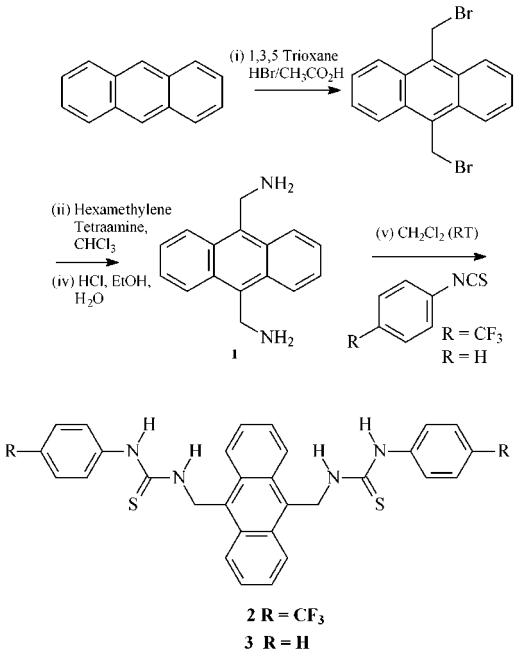
sites with concomitant changes in the photophysical properties of a lumophore by modulation of a Photoinduced Electron Transfer (PET) mechanism.[8] We have chosen to demonstrate such anion recognition of biologically relevant anions such as $H_2PO_4^-$ and AcO^- in DMSO by employing simple thiourea and urea recognition sites, connected to an anthracene fluorescent moiety by a covalent spacer. Here the anion recognition takes place through hydrogen bonding between the thiourea hydrogens and the anion.[9] Such chemosensors should in principle show *ideal* PET behavior upon anion recognition, i.e., only the quantum yield and the lifetime of the excited-state emission should be modulated upon anion recognition.[10] In this letter we demonstrate such PET fluorescent sensing of anions flanked with *two binding sites*.[11] Such fluorescence sensing is both exceptional and of great physiological relevance since many dicarboxylates are components of various metabolic processes, and pyrophosphate is the product of ATP hydrolysis under cellular conditions.[12] However, it has up to now been difficult to achieve without the use of structurally complicated hosts.[4,5] With this in mind we developed **2** and **3**, which have two thiourea moieties that can form hydrogen bonding complexes with bis-anions. To the best of our knowledge, these chemosensors are the first examples of charge neutral fluorescent PET sensors that show ideal PET behavior for bis-anions.

Sensors **2** and **3** (Scheme 1) can be described as being designed as "*receptor−spacer−fluorophore−spacer−receptor*" conjugates[13] where the anion recognition takes place at the two-thiourea moieties. They are easily synthesized, and simple modification to the thiourea moiety (by incorporating aromatic or aliphatic electron withdrawing groups) can be used to "*tune*" the anion sensitivity and selectivity, as the acidity of the thiourea hydrogens is modulated.[4] **2** and **3** were synthesized in good yield (Scheme 1) from 9,10-diaminomethylanthracene (**1**). The synthesis of this starting material has been described previously in the literature, using Gabriel synthesis.[14] However, due to the insolubility of the bis-phthalimide intermediate the yield of **1** was found to be extremely poor. With this in mind we synthesized **1** using an alternative method that involved the initial synthesis of 9,10-bis-bromomethylanthracene in one step[15] in 75% yield. Accordingly, **1** was synthesized from this bis-bromide intermediate with hexamethylenetetramine in anhydrous $CHCl_3$ under inert atmosphere.[16] This method gave **1** in 85% yield as a crude product that could be used without further purification. The two sensors **2** and **3** were subsequently

Scheme 1. The Synthesis of PET Anion Chemosensors **2** and **3**



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made by reacting **1** with phenyl and 4-(trifluoromethyl)-phenylisothiocyanate, respectively, in dry CH_2Cl_2 under argon at room temperature. The resulting light-yellow precipitate was collected by filtration, washed several times with cold CH_2Cl_2, and recrystallized from either hot CH_2Cl_2 or $CHCl_3$. All products were analyzed with conventional methods.[17] The ^{1}H NMR of **2** and **3** in DMSO-d_6 indicated the high symmetry of the sensors, with only two sets of aromatic signals and a single resonance for the $-CH_2-$ spacer. For **2**, the thiourea protons appeared as two resonances at 9.68 and 8.38 ppm, respectively.

The sensitivity and the selectivity of these sensors toward a series of mono- and bis-anions was evaluated by observing the changes in their fluorescence emission spectra in DMSO and in the ^{1}H NMR upon anion titration (with $(C_4H_9)_4N^+$ (TBA) salts) in DMSO-d_6. The fluorescence emission spectra of **2** consisted of three bands at 409, 430, and 455 nm when excited at 378 nm. Upon addition of monodentate anions such as AcO^- and $H_2PO_4^-$ the emission was ca. 70−95% "*switched off*" or quenched due to the formation of the anion−receptor complex. No other spectral changes were observed in the emission spectra, i.e., there was no evidence of either exciplex or excimer emissions.[10] Concurrently the changes in the absorption spectra (peaks at 358, 378, and 400 nm) of the anthracene moiety were only minor. Similar results were observed for **3**. This is a typical PET behavior since the receptors are separated from the fluorophore by the two $-CH_2-$ spacers; the only interaction between the two moieties is via electron transfer. Upon addition of spherical anions such as Cl^- and Br^- no significant quenching was observed, ruling out quenching by the heavy atom effect. However, F^- quenched the emission effectively (∼98%) due to its small size and high charge density. The addition of AcO^- to a solution of **2** with a 40 mM background concentration of Cl^- quenched the emission to the same degree as seen previously for AcO^-, indicating that the two receptors were selectively binding AcO^- over Cl^-. Plotting the changes of the fluorescence intensity at 430 nm as a function of p*A* (−log[anion]) gave, in all cases, sigmoidal profiles (see Figure 2 for AcO^-). However, for both **2** and **3**, these profiles changed over ca. 3−4 pA units. This can be regarded as an indication of a possible 2:1 binding. This was further confirmed by observing the changes in ^{1}H NMR of the thiourea protons upon titration.[5] With either $H_2PO_4^-$ or AcO^- (0 → 5−6 equiv of TBA salts), the thiourea resonances were gradually shifted downfield by >2.5 ppm, confirming the formation of anion−receptor complexes. Analysis of the changes in the "inner proton" (8.38 ppm) vs concentration showed 1:2 binding for both of these anions as seen for $H_2PO_4^-$ in Figure 3.

When **2** and **3** were titrated with TBA salts of the biologically important bis-anions such as glutarate, malonate, and pyrophosphate (with tris(tetrabutylammonium) hydrogen pyrophosphate), the emission spectra were also quenched. As before, no other significant spectral changes were observed in the emission spectra (Figure 1 for the titration of **2** with pyrophosphate). For the two organic anions, the fluorescence emission of **2** was "*switched off*" by ca. 70% and 86% for glutarate and malonate, respectively ([anion] = 40mM in all cases). Similar reduction was seen in the fluorescence emission of **3**. The changes in the quantum yields of fluorescence (Φ_F) of **2** and **3** upon anion sensing were measured in comparison with that of 9,10-dimethylanthracene (9,10-DMA). For **2**, these were measured to be

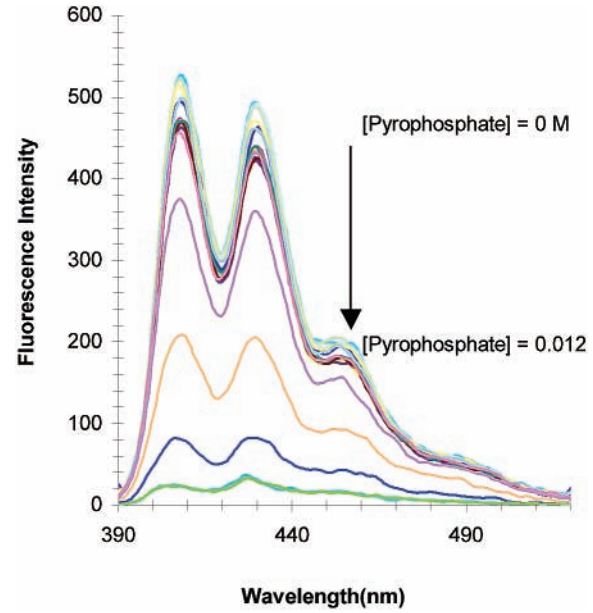

Figure 1. The changes in the fluorescence emission spectra of **2** upon addition of pyrophosphate.

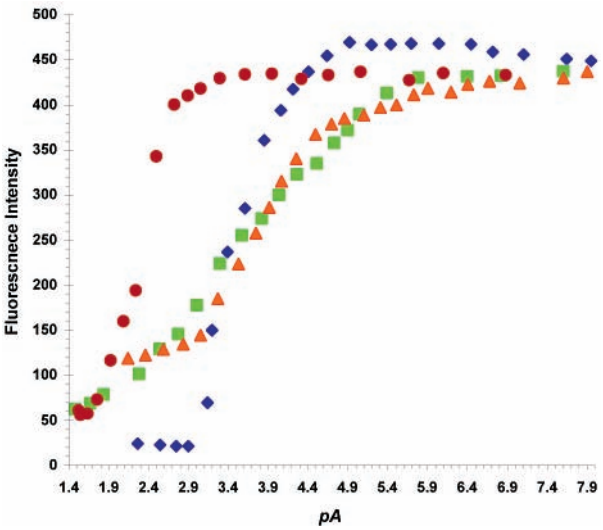

Figure 2. Fluorescence titration curve for **2** when measured at 430 nm (OD = 0.1) in DMSO: malonate (●), pyrophosphate (◆), glutarate (▲), and acetate (■) .

(17) Calculated for **2** ($C_{32}H_{24}N_4S_2F_6$): C, 59.80; H, 3.76; N, 8.72. Found: C, 59.82; H, 3.76; N, 8.70. Calculated for **3** ($C_{30}H_{26}N_4S_2$): C, 71.12; H, 5.17; N, 11.06. Found: C, 71.10; H, 5.17; N, 11.10. ^{1}H NMR (400 MH, DMSO-d_6)for **2** and **3** are available as Supporting Information.

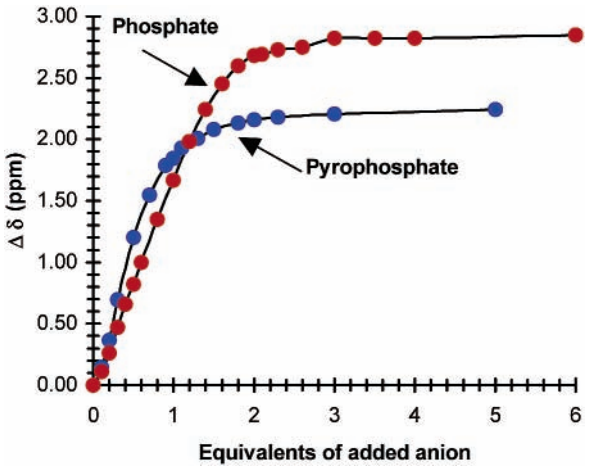

Figure 3. ^{1}H NMR titration of **2** with phosphate (red) and pyrophosphate (blue) in DMSO-d_6.

0.012 and 0.011 for glutarate and malonate, respectively.[18] We believe that this relatively high degree of fluorescence quenching is due to the increase in the reduction potential of the thiourea receptor moieties *after* anion recognition. This affects the rate of the electron transfer from the HOMO of the receptor to the excited state of the fluorophore, i.e., ΔG_{ET} becomes more negative upon anion recognition and hence more thermodynamically favorable. This causes the emission to be "*switched off*". We were unable to demonstrate this by measuring the changes in the redox potential for the receptor since the thiourea was irreversibly oxidized. However, Φ_F measurements of 9,10-DMA, which lacks the two receptor sites, gave a $\Phi_F = 0.87$ in DMSO, which is substantially larger than that of **2**, $\Phi_F = 0.047$, and **3**, $\Phi_F = 0.11$, in DMSO. This implies that PET is active prior to the anion recognition, but becomes even more so *after* anion recognition. The addition of 10 mM of AcO^- or pyrophosphate did not affect the Φ_F of 9,10-DMA. For pyrophosphate the quenching was even more efficient, being ca. 95% for **2** (Figure 2) ($\Phi_F = 0.001$) and 90% ($\Phi_F = 0.017$) for **3**. No other spectral changes were seen in the emission spectra. Plotting the emission changes at 430 nm vs pA gave sigmoidal curves for all the bis-anions (Figure 3). Importantly, the emission is "*switched off*" over two pA units, for pyrophosphate and malonate, indicating 1:1 binding and simple equilibrium. For the larger glutarate anion, the emission was "*switched off*" over ca. 3 pA units. From these changes (Figure 2) the binding constant logβ of 3.74($\pm$0.05), 2.34($\pm$0.05), and 3.40($\pm$0.05) was determined for glutarate, malonate, and pyrophosphate, respectively, for **2**. For **3**, these values were found to be 3.07 ($\pm$0.05), 3.15($\pm$0.05), and 2.02-($\pm$0.05) for pyrophosphate, glutarate, and malonate, respectively. To investigate these binding interactions in greater detail, we carried out a ^{1}H NMR titration on **2** using pyrophosphate. From these changes, Figure 3, a logβ of 3.81-($\pm$0.05) was determined, with a clear 1:1 binding, which is in good agreement with that seen above. For such recognition to take place, the anion would have to bridge the anthracene moiety. Although this did not affect the absorption spectra of **2** to any great extent (see Supporting Information), then upon closer examination an isosbestic point was observed at ca. 406 nm. However, the ^{1}H NMR spectra of **2** showed somewhat greater changes for the anthracene resonances upon titration with pyrophosphate than that seen in the ^{1}H NMR titration of $H_2PO_4^-$. We are currently investigating these anion-binding features in greater detail, and carrying out modifications on these chemosensors to further enhance the bis-ion selectivity and sensitivity.

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Supporting Information Available: Synthesis of **1**, **2**, and **3** and absorption spectrum of **2** upon titration with pyrophosphate. This material is available free of charge via the Internet at http://pubs.acs.org.

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