



Fluorescent detection of anions by dibenzophenazine-based sensors

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ABSTRACT

Binding studies of two sulfonamide-functionalized dibenzophenazine-based sensors are reported. Spectroscopic studies showed that both sensors are effective fluorescent turn-on sensors for several anions. Both sensors showed responses to acetate, benzoate, cyanide, and fluoride ions. NMR titrations confirmed the mode of binding of the sensors to be through H-bonding to the sulfonamide groups.

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The design of anion sensors is a fast emerging research field due to the importance of detecting anions in medicine, chemical industry, and the environment.^{1–7} Chloride and nitrate ions, for example, are tracers of water pollution⁸ while cyanide, a highly toxic anion, is a dangerous environmental contaminant associated with leakage, from electroplating and herbicide industries.^{9,10} Therefore, the development of fast, easy, and sensitive detection techniques for the continuous monitoring of anions is important. Such diagnostic tools can be developed through the utility of fluorescent sensors that can be tailored to have high selectivity or broad detection of ions.^{6,9} While the development of ion sensors that work efficiently in aqueous media is still challenging, non-aqueous systems can serve as useful model systems and may still have niche applications. The design of an effective fluorescent sensor relies mainly on an active recognition site for the anion, a fluorophore capable of exhibiting a high quantum yield, and an efficient signaling mechanism whereby anion binding affects the fluorophore. Typically, for an effective fluorescent sensor, it is desired that the binding of an anion to the active site triggers electronic changes in the fluorophore leading to a ‘turn ON’ of the fluorescence.

Intramolecular charge transfer (ICT) is one of the various signaling mechanisms^{11–13} by which the electronic transitions from the

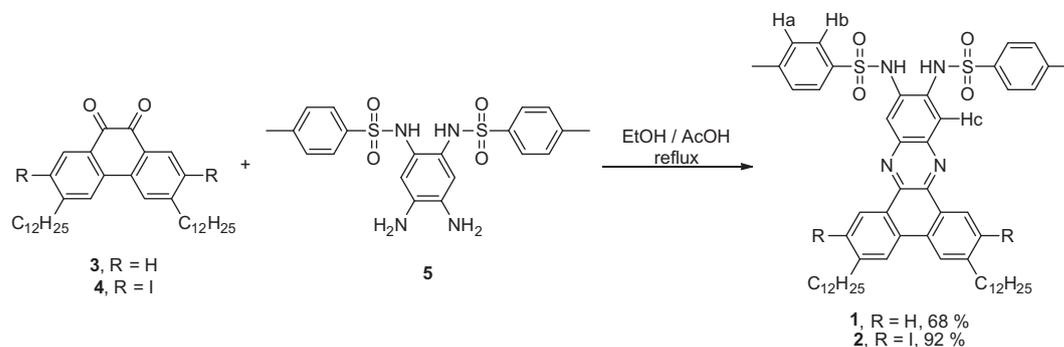
binding site to the fluorophore take place through a ‘push–pull’ mechanism, with binding affecting the donor/acceptor strength of the binding substituent^{12,13} and the resultant change in electronic structure leading to either emission enhancement or quenching. A variety of neutral receptors have been reported for selective anion recognition based on H-bonding owing to the strength and selectivity of this interaction.^{14,15} This has been conventionally achieved by employing N–H fragments of amides, ureas, thioureas, and pyrroles, which can bind to anions in a reversible manner and, therefore, allowing continuous monitoring.^{5,6}

In our ongoing efforts to develop fluorescent sensors, we have reported sensors for cations¹⁶ and anions¹⁷ employing quinoxalino-phenanthrophenazine (TQPP) and phenanthrophenazine (DPP) as fluorophores using crown ethers and sulfonamide groups as binding sites, respectively. In this Letter, we introduce two new fluorescent sensors **1** and **2** for anions using a dibenzo[*a,c*]phenazine fluorescent core functionalized with sulfonamide groups as the H-bonding site for anions. As shown in Scheme 1, both sensor molecules are functionalized with solubilizing dodecyl groups, while the structure of **2** differs from that of **1** by the presence of iodine substituents, the electronic effects of which are expected to result in different emission responses.

Compound **2** has previously been reported in the literature as an intermediate in the synthesis of a ‘nano-truck’¹⁸ and was synthesized from the diiododiketone **4** and the ditosylated tetraamine **5** following the previously reported procedure (Scheme 1). Diketone **3** is the immediate precursor to **4**¹⁸ and was also converted

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Scheme 1. Synthesis of sensors **1** and **2**.

into the corresponding dibenzo[*a,c*]phenazine **1**, which is a new compound, by the reaction with **5**.

Absorption, emission, and NMR spectroscopic studies were conducted on sensors **1** and **2** in the presence of anion solutions at different concentrations to evaluate the efficacy with which they detect various anions and the effect of the iodo substituents on the output signal. The absorption spectra of the two sensors were similar, exhibiting characteristic peaks at 280 nm, 325 nm, 400 nm,

and 420 nm (Fig. 1). The addition of anion solutions to the sensors resulted in similar spectral changes of the sensors with the same anions. For example, the addition of acetate, cyanide, and fluoride anions (1.0 mM in CHCl₃) to a solution (5.0 μM in CHCl₃) of the sensors resulted in major changes in the UV spectra (Fig. 1) including enhancement of absorbance at ~310 nm and ~455 nm with concurrent reduction in absorbance at 280 nm, 400 nm, and 420 nm. Isosbestic points at 300 nm, 365 nm, and 425 nm indicate a clean transformation of the free sensor into the sensor-anion complex. On the other hand, the addition of chloride, bromide, iodide, nitrate, and perchlorate anions had no significant effect on the absorption spectra of either sensor.

These spectral changes were attributed to strong hydrogen-bonding interaction between the N–H bonds of the sulfonamide groups and the anions in line with the similar changes seen for other sulfonamide-based systems.¹⁹ Proton-transfer between the sulfonamide and the anions was considered unlikely due to the low polarity of the solvent (previously reported related sulfonamide sensors were reported to form hydrogen bonds to anions in acetonitrile, but to undergo proton transfer to the anions in the more polar DMSO¹⁹). This reasoning was supported by the results obtained from the titration of the sensors **1** and **2** with TBA hydroxide, where the anion is more basic. Fig. 2 depicts the changes in the absorbance of **1** and **2** at 455 nm and 460 nm, respectively, as a function of the concentration of various anions, including the hydroxide anion. Both sensors exhibited ~10 times enhancement in the absorbance at these wavelengths after the addition of ~1 M equiv of carboxylate, cyanide, or fluoride anions. On the other hand, the addition of the hydroxide anion gives qualitatively different changes, resulting in a ~5 times increase in the absorbance of sensor **1** after more than two molar equivalents of the anion were added. Sensor **2** showed much less enhancement in its absorbance at 460 nm even after addition of more than five molar equivalents of the anion. These results indicated different modes of interaction for the hydroxide anion and the other anions with the sensors. This further favors the hydrogen-bonding interaction mode of the anions with sulfonamides rather than proton-transfer (vide infra NMR studies).

The binding isotherms of acetate, benzoate, cyanide, and fluoride anions were generated from the change in absorption of the sensors (Fig. 3). To calculate the binding constants of the sensors to these anions (Table 1), the binding isotherms were fitted to a 1:1 binding model as suggested by the Job plots. The results obtained were in accordance with previously observed and reported results on similar systems.^{17,19} Meanwhile, the spectral alterations effected by the addition of chloride, bromide, iodide, nitrate, and perchlorate anions were too insignificant to generate any meaningful binding isotherm.

The binding of anions to **1** and **2** resulted in drastic changes in their emission spectra, but to different extents for each sensor.

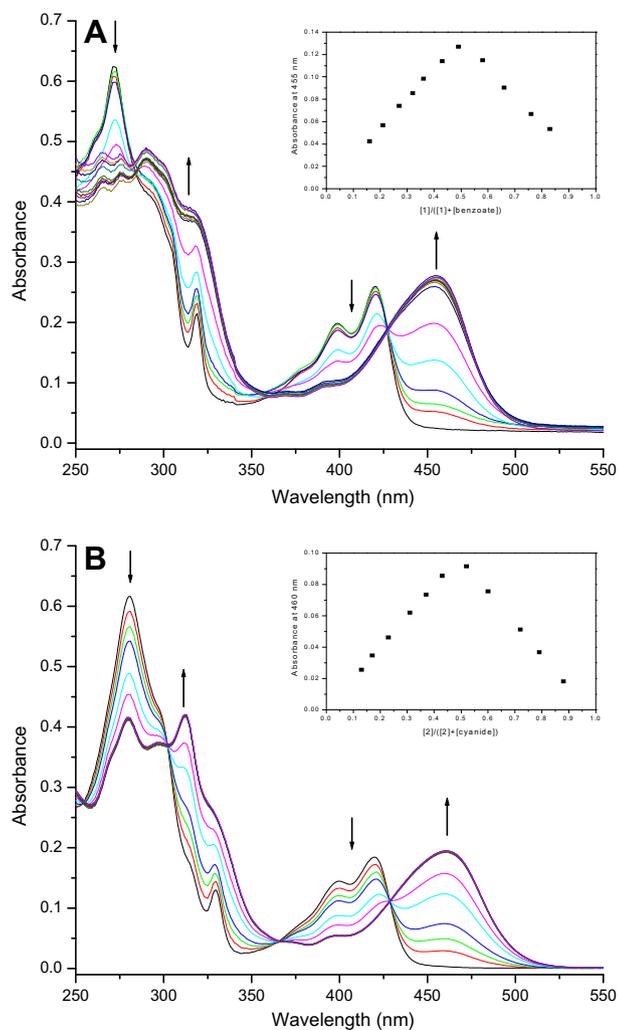


Figure 1. UV-vis spectra of **1** (A) and **2** (B) (5 μM) upon titrating with solutions (1.0 mM) of TBAOBn and TBACN, respectively, in CHCl₃. The insets are the Job plots for each anion.

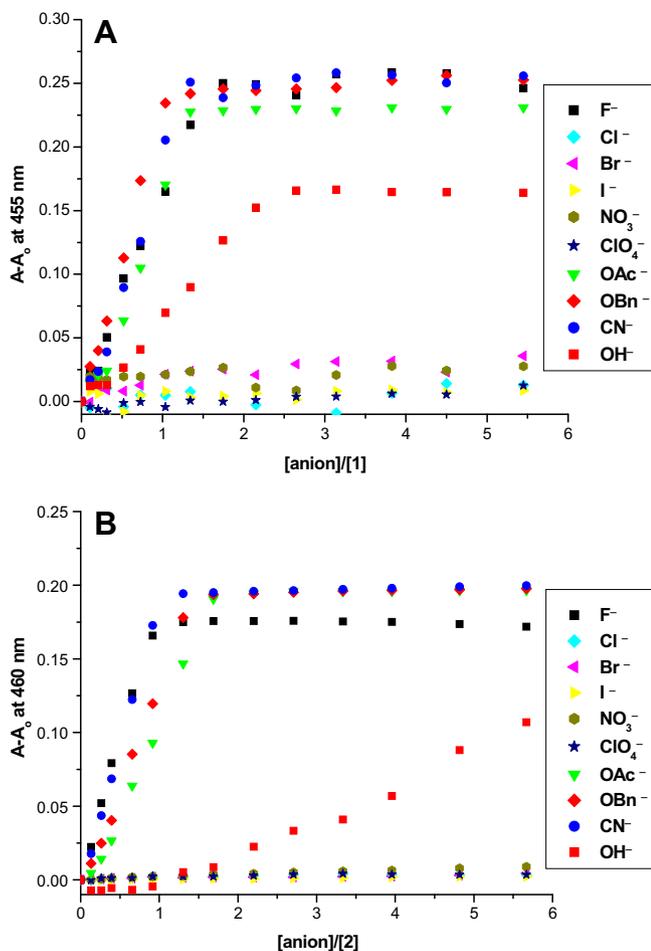


Figure 2. The change in the absorbance of **1** (A) and **2** (**5.0** μ M) at 455 nm and 460 nm, respectively, upon titrating with different anion solutions (1.0 mM) in $CHCl_3$.

Sensor **1** shows an emission maximum at 438 nm upon excitation at 350 nm in $CHCl_3$; the addition of acetate (Fig. 3), cyanide, or fluoride anions resulted in a substantial bathochromic shift of the emission band to 550 nm. Accordingly, the emission at 438 nm is quenched and there is a large (140–160 times) enhancement of

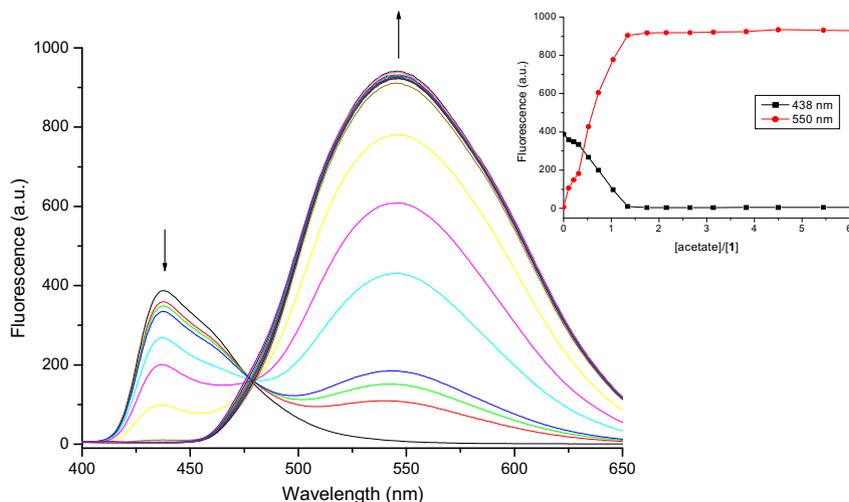


Figure 3. Emission spectra of **1** (5.0 μ M, $CHCl_3$) upon the addition of acetate anion solution. The inset depicts the change in the emission intensity at 438 nm and 550 nm as a function of molar equivalence of the anion relative to **1**.

Table 1

Binding constants of sensors **1** and **2** with several anions^a

Sensor	1	2
Anion	logK	logK
OAc^-	5.93	5.94
OBz^-	6.73	6.43
CN^-	6.01	7.73
F^-	5.72	5.95

^a K_a values were calculated from changes in the absorbance of the sensors at 455 nm for **1** and 460 nm for **2**. See Supplementary data for more details.

the emission at 550 nm. Meanwhile, the addition of the same ions (acetate, cyanide, or fluoride) to **2** resulted in a 700–800-fold enhancement of its emission at 555 nm (again with $\lambda_{ex} = 350$ nm) (Fig. 4). These differences can partly be attributed to the greater increase in the absorbance at 350 nm (the excitation wavelength) seen for **2** on binding (Fig. 1), but also depend on the relative quantum yields of bound and unbound forms of each sensor. The bathochromic shift of both absorption and emission seen on binding is consistent with binding leading to an increase in the π donor strength of the sulfonamides and so with the lowest energy states of the bound species having significant ICT character between the sulfonamide donors and the electron-poor heterocyclic acceptor. The slightly longer wavelength emission found for **2** is consistent with the expected weak electron-withdrawing effect of the iodine lowering the energy of such an ICT state. Finally, the addition of chloride, bromide, iodide, nitrate, and perchlorate anions had insignificant effect on the emission of the sensors (Fig. 4).

NMR titrations of **1** and **2** further supported the trend observed for anion binding in the photophysical studies. The chemical shifts of the N–H protons of the sulfonamide groups which are associated with the binding process disappeared from the proton NMR spectrum upon the first addition of anion solution due to chemical exchange with the residual water in the solvent. Thus, the chemical shifts of the neighboring C–H protons, H_c and H_b (Scheme 1), were monitored as aliquots of anion solution were added into solutions of the sensors in 2% $DMSO-d_6$ in $CDCl_3$. Fig. 5 shows partial proton NMR spectra of **1** where the signals of the aromatic protons are shown as the concentration of the fluoride anion was increased. The signals of H_c and H_a shifted upfield from 8.03 and 7.27 ppm to 7.83 and 7.17 ppm, respectively. This

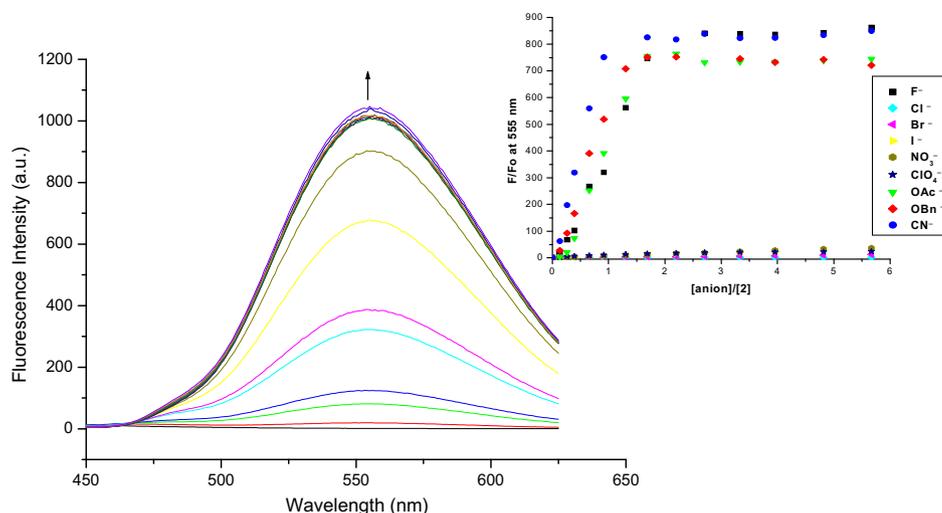


Figure 4. Emission spectra of **2** (5.0 μM , CHCl_3) upon the addition of the fluoride anion solution. The inset depicts the relative change in the emission of **2** (5.0 μM) at 555 nm upon titrating with different anion solutions (1.0 mM) in CHCl_3 .

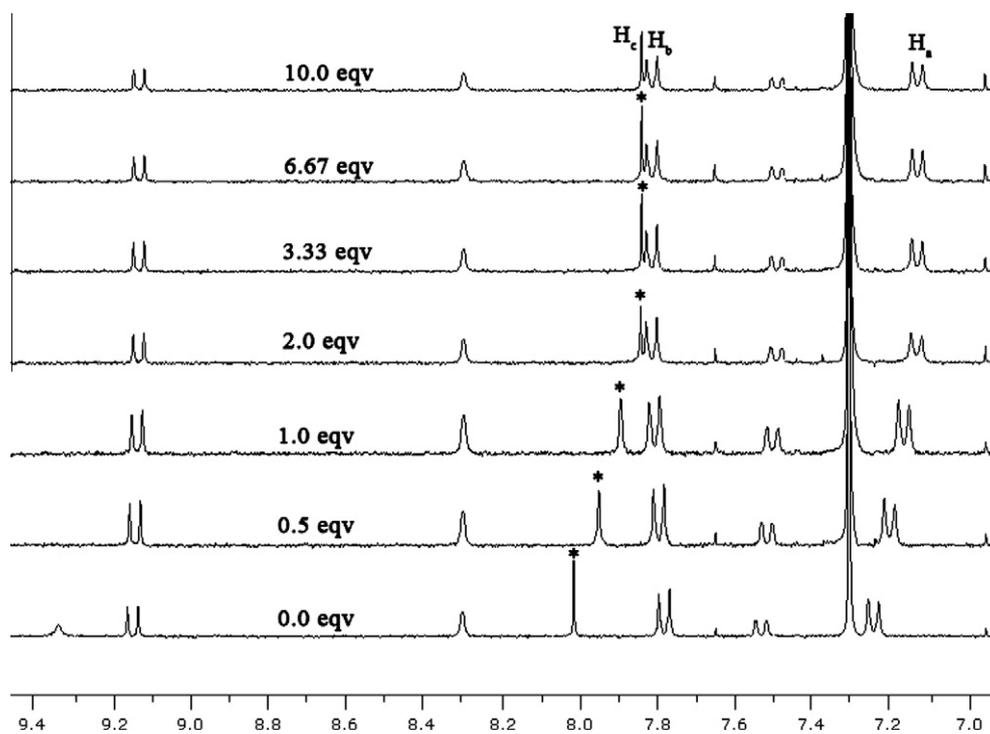


Figure 5. Partial ^1H NMR spectra of **1** (1.0 mM) upon titrating with TBAF solution (10 mM) in 2% $\text{DMSO}-d_6$ in CDCl_3 .

upfield shift can be attributed to the expected increase in the electronic density on the neighboring sulfonamide groups caused by H-bonding of the N–H protons to the anions. Meanwhile, the signal of H_b shifted downfield from 7.82 ppm to 7.85 ppm, presumably due to a through-space effect of the anion bound in close proximity to these protons. Effects on the chemical shift of other signals were only minor.

The effects on the NMR spectra of titration with hydroxide anions were insignificant compared to those of acetate, benzoate, cyanide, or fluoride anions. Fig. 6A depicts the change in the chemical shift of the H_c protons of **1** and **2** upon titrating with acetate or hydroxide anions. The chemical shift of H_c decreased (~ 0.2 ppm) upon the addition of acetate anions and it reached a constant value once one molar equivalent of the anion had been added indicating

strong binding with 1:1 stoichiometry (Fig. 6B). Meanwhile, the addition of more than five molar equivalents of hydroxide anions shifted the signals of the H_c protons by less than 0.04 ppm. These results further support our interpretation that the interaction of the anions with the sensor is mainly due to hydrogen-bonding rather than proton-transfer to the anions. Both sensors exhibited similar trends in the changes of chemical shifts of the aromatic protons (H_c and H_b) upon the addition of acetate, benzoate, cyanide, or fluoride anions.

In summary, two dibenzophenazine-based sulfonamide molecules have been synthesized and have been shown to be effective fluorescent turn-on sensors for several anions in chloroform. Both sensors showed responses to acetate, benzoate, cyanide, and fluoride ions. In particular, sensor **2** exhibited the strongest changes in

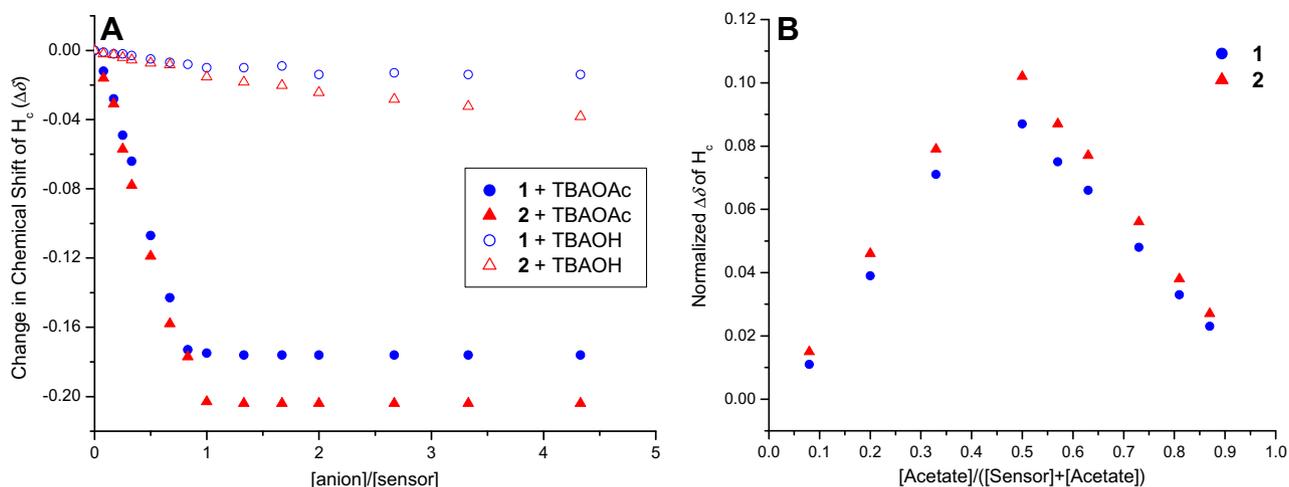


Figure 6. (A) The change in the chemical shifts of H_c for **1** and **2** (1 mM) upon titrating with TBAOAc and TBAOH solutions (10 mM) in 2% DMSO-*d*₆/CDCl₃; (B) Job plots of the acetate NMR titration of **1** and **2**.

its emission spectra upon the addition of the anions at different concentrations. NMR titrations suggested that the anions bind to the sensors through H-bonding to the sulfonamide groups.

Sensor **2** was synthesized as previously described from the diketone **4** and ditosylated tetraamine **5** as shown in Scheme 1.¹⁸ Sensor **1** is a new compound and was obtained in 68% yield in the same way, but using diketone **3**¹⁸ in place of **4**.

Data for **1**: ¹H NMR (400 MHz, CDCl₃): *d* 9.03 (d, *J* = 8.4 Hz, 2H), 8.23 (s, 2H), 7.79 (s, 2H), 7.70 (d, *J* = 8.4 Hz, 4H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.30 (s, 2H), 7.21 (d, *J* = 8.4 Hz, 4H), 2.85 (t, *J* = 7.6 Hz, 4H), 2.34 (s, 6H), 1.75 (m, 4H), 1.39 (m, 4H), 1.23 (m, 32H), 0.85 (t, *J* = 6.8 Hz, 6H) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): *d* 145.67, 144.52, 142.72, 139.93, 135.00, 131.97, 131.87, 129.83, 128.40, 127.71, 127.61, 126.17, 123.55, 122.31, 32.60, 31.89, 31.62, 29.67, 29.63, 29.58, 29.48, 29.34, 22.66, 21.58, 14.10 (one alkyl resonance not observed, presumably due to overlap). HRMS (MALDI): Calcd for C₅₈H₇₅N₄O₄S₂ (MH⁺): 955.5230. Found: 955.2223. Anal. Calcd for C₅₈H₇₄N₄O₄S₂: C, 72.92; H, 7.81; N, 5.86. Found: C, 73.22; H, 7.97; N, 5.91.

Spectroscopic titration: A solution of the sensor (5 mM, 2 mL) in CHCl₃ placed into a 1 cm. 1 cm cuvette was titrated with a solution of a tetra-*n*-butylammonium salt of the anion (1.0 mM, CHCl₃) that contained the sensor (5 mM). Aliquots of the anion solution were added to the cuvette via a syringe until a total of 10 or more equivalents of the anion had been added (the number of additions was around 20 with an increase in the amount of anion solution added). The UV–vis spectrum and emission spectrum (*I*_{ex} = 350 nm) were recorded after each addition.

¹H NMR titration: A solution of the sensor (1 mM, 600 mL) in 2% DMSO-*d*₆/CDCl₃ placed in an NMR tube was titrated with a solution of the anion (10 mM). Aliquots of the anion solution were added to the NMR tube via a syringe until a total of 10 equiv of the anion had been added (the number of additions was around 15 with an increase in the amount of anion solution added). A ¹H NMR spectrum was collected after each addition and the chemical shifts of the aromatic protons were recorded. The collected data was analyzed using a non-linear least-squares regression program to fit the data to a theoretical model with a 1:1 binding stoichiometry.

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Supplementary data

Supplementary data (calculations of binding constants, changes in the absorption of **1** and **2** upon the addition of different anions, ¹H NMR spectra of **2** upon titrating with TBAF, and the change in the chemical shift of H_c in **1** and **2** upon titration with different anions) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.11.121.

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