Dipyrrolyl quinoxalines with extended chromophores are efficient fluorimetric sensors for pyrophosphate†

Dmitry Aldakov and Pavel Anzenbacher, Jr*

Department of Chemistry, Center for Photochemical Sciences, Bowling Green State University, Bowling Green, Ohio, 43402, USA. E-mail: pavel@bgnet.bgsu.edu; Fax: 419-372-9809; Tel: 419-372-2080

2,3-Di(pyrrole-2-yl)quinoxaline anion sensors with extended chromophores display dramatic enhancement in fluorescence intensity (sensing output), increased affinity for inorganic anions as well as strong selectivity for pyrophosphate over phosphate anion.

Inorganic anions such as chloride, phosphate, pyrophosphate, cyanide or fluoride play an important role in energy transduction in organisms, and control metabolic processes *via* participation in enzymatic reactions.[1] Inorganic anions are also used in industry, for example as agricultural fertilizers and raw materials in numerous industrial processes.[2]

There are several reasons why reliable sensing of anions is a challenging area of research.[3] Compared to cations, anions are larger, display a wide range of geometries and significant charge delocalization.[4] All these factors result in a low surface charge concentration and make binding of anions less effective. It is not surprising that sensitive and selective sensors for anions are still rare.[4] In general, the performance of chemosensors can be improved either by (i) increasing its selectivity through preorganization of the receptor, or (ii) through the improved signal transduction (read-out) that leads to a reliable substrate-specific response. Given the sensitivity of fluorescence as a method, we believe that the latter can be achieved by using sensors with a strong fluorescent (instead of or in addition to colorimetric) response.[5,6] Although both strategies for sensor development are equally useful, a majority of the research efforts are focused on the improvements of the receptor moieties. Our results suggest that the improvements in a signal transduction, a road significantly less travelled in the anion sensing field, may result in a dramatic improvement of the overall sensor performance.

In order to illustrate this point, we have selected 2,3-di-(pyrrole-2-yl)quinoxaline (DPQ),[6] a compound that binds anions *via* hydrogen bonding to the pyrrole NH-moieties. DPQ was recently recognized as a potential colorimetric and fluorescent sensor for anions.[6a] Previous efforts were oriented towards improving the receptor *via* tuning the electronic properties, cryptate effect, *etc*.[6] In this study we focus on improving the signal transduction process in the DPQ-derived anion sensors (Fig. 1).
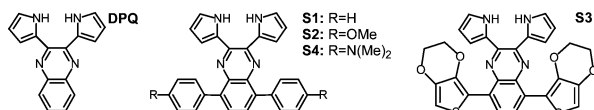

Fig. 1 Structures of 2,3-di(pyrrole-2′-yl)quinoxaline (DPQ), 5,8-diphenyl-2,3-di(2-pyrrolyl)quinoxaline (**S1**), 5,8-di(4-methoxyphenyl)-2,3-di(2-pyrrolyl)quinoxaline (**S2**), 5,8-di(EDOT)-2,3-di(2-pyrrolyl)-quinoxaline (**S3**) and 5,8-di(4,4′-*N*,*N*′-dimethylaminophenyl)-2,3-di(2-pyrrolyl)quinoxaline (**S4**).

In order to enhance and tune the fluorescence output from the DPQ sensor, aryl moieties were attached to the 5- and 8-positions of the quinoxaline ring. The 5,8-aryl substituents serve to enhance the sensor emissivity by expanding the

conjugated chromophore, and tune the emission wavelength of the sensor through substituent effects. This electronic tuning of absorption and emission properties is considered particularly useful as it may be used to avoid potential interference from fluorescent impurities present in the anion samples.

The synthesis of sensors **S1**–**S4** is accomplished by condensation of 3,6-dibromo-1,2-phenylenediamine (**1**)[7] with 1,2-dipyrrolyl-2′-ylethanedione (**2**)[6a,b] to give **3**. The Stille coupling[8] of **3** with stannylated arenes[9] yields sensors **S1**–**S4** in reasonably high yields, 22, 38, 41 and 48%, respectively (Fig. 2).
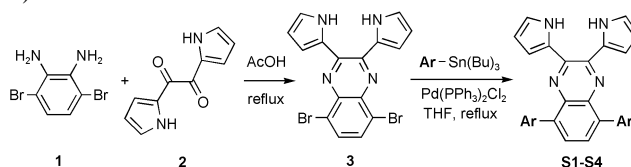
S1: Ar = -Ph, **S2**: Ar = -Ph-OMe, **S3**: Ar = - EDOT, **S4**: Ar = -Ph-N(Me)₂

Fig. 2 Synthesis of sensors **S1**–**S4**.

As expected, the presence of 5,8-aryl substituents in sensors **S1**–**S4** results in both a dramatic increase of emission intensity and shifted emission maximum compared to the parent DPQ (λ_f = 490 nm, Φ_f = 0.155). Thus **S1** displays the emission maximum at 500 nm (Φ_f = 0.199)‡, **S2** – 502 nm (Φ_f = 0.237), **S3** – 590 nm (Φ_f = 0.222), and **S4** – 610 nm (Φ_f = 0.256). Both properties may be easily observed by the naked eye (Fig. 3).
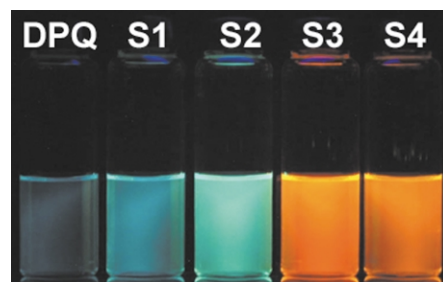

Fig. 3 The strong fluorescence emission can be observed by the naked eye. A photograph is taken upon illumination with a UV-lamp (360 nm). The solutions of sensors were 1.0 μM in CH₂Cl₂.

The qualitative estimation of the anion binding affinity of sensors **S1**–**S4** was performed visually (Fig. 4). The observed changes in fluorescence intensity in the sample without any anion and in the presence of the tested anion allow for the prediction of the sensor-anion binding affinities. Sensors **S1**–**S4** responded to the presence of fluoride (hexahydrate) and pyrophosphate (dihydrate) anions through a dramatic decrease in the sample emission. Such quenching is not observed with chloride (dihydrate), bromide, dihydrogenphosphate (dihydrate) or hydrogensulfate (dihydrate).§ The fact that the presence of water solvates does not interfere with the sensing process is encouraging considering the demand for sensors capable of anion sensing in the presence of water. Sensors **S1**–**S4** are capable of anion sensing in acetonitrile ($< 0.3\%$ of water) and DMSO ($< 0.5\%$ of water). Dichloromethane for titration experiments was selected to generate data comparable with the rest of the literature on DPQ-related sensors.[6]

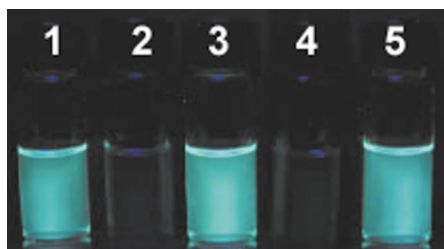† Electronic supplementary information (ESI) available: experimental data. See http://www.rsc.org/suppdata/cc/b3/b301362f/

Fig. 4 The photograph under UV light of the 1.0 μM in CH₂Cl₂ solutions of the sensor **S1** (1) in the presence of the following anions (100 eq.): fluoride (2), chloride (3), pyrophosphate (4), and phosphate (5).

Further insight and quantitative evaluation of the sensing capability of sensors **S1**–**S4** was obtained from UV-vis and fluorescence quenching experiments. Representative titration curves are shown in Fig. 5. In general, the addition of fluoride and pyrophosphate anions to the solution of sensors **S1**–**S4** in all cases results in the decrease of the absorption intensity in the 400–450 nm region together with the appearance of a strong band centered at 500–550 nm, which is responsible for the red color of the solution. The addition of fluoride and pyrophosphate resulted in a significant quenching ($>95\%$) of the fluorescence emission intensity.
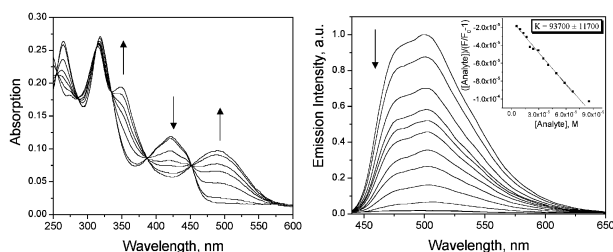

Fig. 5 Left panel: changes in UV-vis spectra of **S1** (10 μM in CH₂Cl₂) upon addition of pyrophosphate TBA salt (0–1.0 mM). Right panel: decrease in fluorescence emission intensity of **S1** (2 μM in CH₂Cl₂) upon the addition of pyrophosphate (0–86 μM); ($\lambda_{exc} = 446$ nm) Inset: linearized binding isotherm used for a binding constant determination.

The emission quenching data was used to calculate the respective binding constants summarized in Table 1. One can clearly see that DPQ-sensors with extended chromophores, namely **S1** and to a lesser extent also **S2**–**S4**, show substantially increased affinity (sensitivity) for fluoride and pyrophosphate compared to the parent DPQ sensor. More importantly, all sensors **S1**–**S4** show a reversed fluoride/pyrophosphate selectivity ($K_{PP} \approx 2K_F$) compared to DPQ ($K_{PP} \geq K_F$) (Table 1).

Table 1 Anion binding constants (K_a)^a (M^{-1}) obtained from titrations of the sensors with anions

	Anion^b			
	F$^-$	Cl$^-$	HP$_2$O$_7{}^{3-}$	H$_2$PO$_4{}^-$
Molar Ratio Water-Anion	6:1	2:1	2:1	2:1
DPQ^{6a}	18200	<50	14300	<100
S1^c	51300	<100	93700	<200
S2	24700	<100	58900	<100
S3	25600	<100	57300	<100
S4	27500	<50	39000	<50

^{a} All errors are ± 15%. All binding constants are reported as the average of 3 trials. ^{b} Anions were used as TBA salts. ^{c} K_a were determined by fluorescence quenching in CH₂Cl₂. Fitting was carried out according to Connors.[10] The 1:1 stoichiometry of the complexes was determined from Job-plots[3] carried out at different sensor concentrations.

While the strong affinity for fluoride is not unexpected given its small size and resulting high surface charge density, the high binding constants for the pyrophosphate anion are particularly interesting in light of a potential application in the sensing of nucleotide di- and triphosphates. Such remarkable selectivity may be explained by a different binding mode. We propose that the pyrophosphate may be capable of effective ditopic hydrogen bonding to the two pyrrole moieties of the sensor. Such ditopic binding could account for the strong complexation of pyrophosphate. This feature is a subject of continuing studies. Figure 6 shows schematic representation of the complex (energy minimized using MM+, HyperChem 5.1).
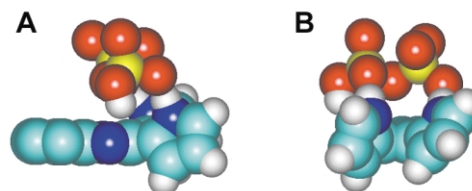

Fig. 6 Proposed structure of the pyrophosphate–DPQ complex (A: side view, B: front view).

In conclusion, we demonstrated that the chromophore extension in simple fluorescence-based anion sensors such as DPQ may result in dramatic improvement of sensor performance as judged by the enhanced fluorescence output and improved affinity for anionic substrates. The fact that simple sensors such as DPQ can selectively bind pyrophosphate is scientifically noteworthy since such sensors may serve as a departing platform for the development of sensors for other biologically important anions. Efforts toward the synthesis of materials capable of anion sensing in the presence of water are under way.

Notes and references

‡ Fluorescent quantum yields (Φ_f) referenced to quinine sulfate in 0.1 N H₂SO₄ ($\Phi_f = 0.545$)[11] as a standard.
§ The anions used were in the form of tetrabutylammonium salts (TBA) hydrates. The degree of hydration was determined from elemental analyses of the respective salts.

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