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A squaramide fluorescent ensemble for monitoring sulfate in water

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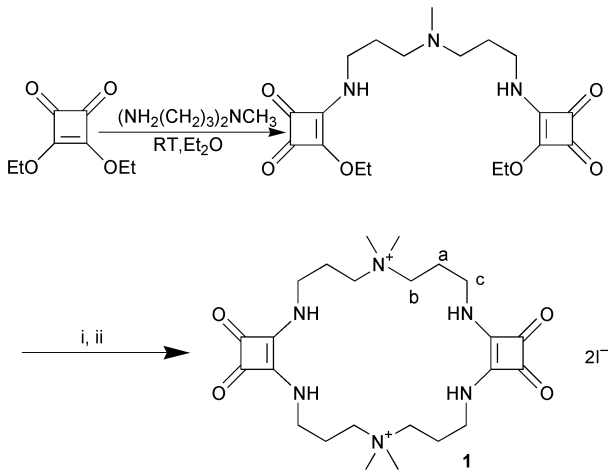
A simple molecular sensor that uses the exclusive quenching and binding abilities of two squaramide units included within an anionic recognition site is proposed for monitoring sulfate in water.

Squaramides are ideal components for molecular sensing devices due to their quenching and binding abilities. We recently described a fluorescent molecular probe,[1] showing the classical covalent arrangement of a fluorophore close to the recognition site that takes advantage of the quenching ability of squaramides. In addition, squaramides feature two hydrogen bond donor atoms that, in extended conformation, bind carboxylate anions tighly.[2] Furthermore, it is possible to achieve strong and selective binding of oxoanions by combining electrostatics together with more directional hydrogen bonding interactions, as in a related streptavidin-2-iminobiotin sulfate complex.[3] On this basis, using the hydrogen bond donor capabilities of squaramides, we describe herein a competitive strategy for sensing sulfate based on a simple macrocyclic charge-assisted squaramide receptor.

In contrast to the usual high dilution conditions required for macrocyclization, receptor **1** was obtained on a gram scale, in 60% overall yield after three steps (Scheme 1). Despite its apparent flexibility, the rigidifying effect provided by the two squaramide rings and a charge repulsion avoid the collapse of **1** and, at the same time, generates a concave cavity lined with two quaternary ammonium groups and four hydrogen bond donors useful for binding. The resulting bowl-shaped receptor is well suited for recognising tetrahedral anions as it matches the spatial and charge requirements of a target guest like sulfate placed at the centre of the cavity.

The formation of a **1**–SO_4^{2-} association complex was evidenced from the observation of characteristic association-induced shifts (CIS) on both, the squaramide and cyclophane hydrogens of **1**. Additional support was obtained from HOESY experiments performed in methanol on a related **1**–$^{13}C_2O_4^{2-}$ complex. In this case, diagnostic ^1H to ^{13}C NOE intermolecular contacts between the oxalate carboxylate at 176.8 ppm and H_a, H_b hydrogens in **1**, were observed, in accordance with the proposed localisation of the anion in **1**.



Scheme 1 Synthesis of **1**, (i) 3,3′-diamino-*N*-methyldipropylamine (1.0 equiv.) EtOH, 7 h, rt; (ii) MeI (7 equiv.), DMF, 70 °C, 12 h.

Host–guest association was fully characterized by isothermal titration calorimetry (ITC).[4] This technique is a valuable resource for investigating the energetics of host–guest processes[5] as it allows the determination of association constants larger than 10^5 M^{-1} that are clearly out of the possibilities of the standard NMR direct titration methods. In the present case, for the complexes with SO_4^{2-}, $PhOPO_3^{2-}$, $C_2O_4^{2-}$ and **1**, respectively, the value of the experimental stoichiometry parameter "n" was always in the range $0.95 < n < 1.05$ in good agreement with a 1 : 1 mode of binding.

The results obtained with selected oxodianions, show that the association is endothermic and entropically driven in all cases. The binding model emerging from the calorimetric data is consistent with an exchange equilibrium between **1** and the anionic guests. Upon formation of the complex both, **1** and the guest, must break their bonds with methanol and the release of solvent bound molecules to the bulk solvent would outweigh the entropic cost of the association.[6] Remarkably, the binding of monoanions such as halides, nitrate, acetate, and others is negligible owing to the electroselectivity imposed by the tetraalkylammonium groups. On the other hand, the preference for tetrahedral sulfate is accounted for by the structural constraints of the hydrogen bond donor array of the squaramide units in **1**, favouring the binding of sulfate over other divalent anions.

Addition of **1** to a solution of fluorescein disodium salt (FNa_2)† in MeOH–H_2O mixtures (9 : 1 v/v) produced a non-fluorescent self-assembled ensemble as a consequence of the effective quenching of the fluorescein emission band in the complex. The observed fluorescence quenching is probably due to photo-induced electron transfer (PET) from the donor squaramide rings of **1** to the fluorescein excited singlet state. This effect, namely an increase of fluorescence on binding the anion due to the suppression of PET that occur when the **1**–FNa_2 complex dissociates, is formally equivalent to the well-known cation promoted suppression of PET widely used for cation recognition. In the present case, the system is not a fluorescent chemosensor, defined as an integrated fluorophore–receptor species,[7] but merely a mixture of a receptor and a fluorophore in which the latter competes with the target ion in complexation.

Table 1 Thermodynamic parameters for the interaction of **1** with key dianions[a]

Guest	K_a/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}
$C_2O_4^{2-}$	$(3.2 \pm 0.3) \times 10^5$	−31.0	+12.2	+43.2
$PhOPO_3^{2-}$	$(1.5 \pm 0.2) \times 10^4$	−23.5	+12.5	+36.0
SO_4^{2-}	$(4.6 \pm 1.0) \times 10^6$	−37.5	+11.3	+48.7

[a] Titration conditions: 30–40 injections (5 μL each) of a 5–10 mM solution of the anion, as sodium or tetramethylammonium salt, were introduced into a sample cell at 294 K containing 1.5 mL of a 0.8 mM solution of **1** in methanol. The heats of dilution were subtracted prior to data analysis by Origin MicroCal software. In all cases the c parameter,[4] defined as $c = K_a$ [**1**]$_t n$, was kept between 10 and 1000. Errors were calculated at a confidence level of 95%.

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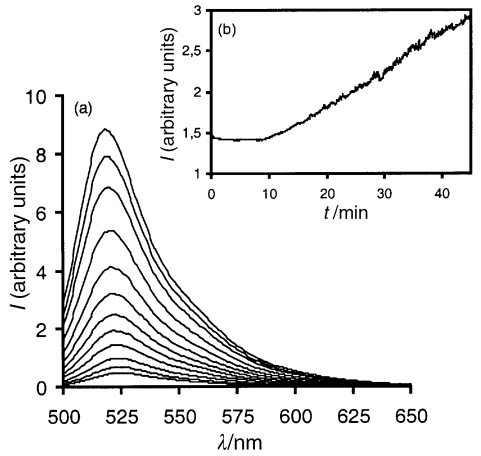

Fig. 1 (a) Fluorescence emission band ($\lambda_{exc.}$ 490 nm) of fluorescein, [FNa_2] = 1.4×10^{-5} M before (upper band) and after addition of **1**, [**1**] = 2.0×10^{-4} M (lowest band). Fluorogenic emission response with several concentrations of sulfate, [SO_4^{2-}] = 4.6, 6.5, 8.4, 10.0, 11.7, 13.3, 14.8, 15.9×10^{-5} M. The subset reflects the response of the **1**–FNa_2 ensemble to sulfate addition. (b) In this experiment, after an approximate 10 min period, the sulfate concentration of a water solution was continuously increased from 0 to 200 ppm and mixed at 0.10 mL min^{-1} together with a stream of [**1**] = 2.6×10^{-4} M and [FNa_2] = 7.4×10^{-4} M in MeOH–H_2O (90 : 10 v/v) at a flow rate 0.90 mL min^{-1}. Fluorescence was measured at 2 s intervals.

The association constant of the measuring **1**–FNa_2 ensemble (K_{ass} = $4.7 \pm 0.6 \times 10^4$ M^{-1}) was obtained by measuring the change in the fluorescence intensity of FNa_2 in the presence of an increasing amount of **1**.[8] In the same solvent system, the association constant for SO_4^{2-} (K_{ass} = $5.2 \pm 1.2 \times 10^6$ M^{-1}) estimated by competitive spectrophotometry[9] was clearly higher. In accordance, upon competitive addition of SO_4^{2-}, fluorescein was displaced[10] restoring the original fluorescence of FNa_2 (Fig. 1) and effectively signalling the presence of sulfate anion. This ensemble is also adequate for real time on-line determination of sulfate in water (pH ≥ 10). To this end, a stock solution of the sensing ensemble **1**–FNa_2 is mixed together at a 9 : 1 v/v ratio with an incoming water stream that contains an increasing concentration of sulfate. The result, shown in Fig. 1(b), shows a linear response within a wide range of sulfate concentration.

In conclusion, we have developed a squaramide-based receptor useful for sensing sulfate. In this ensemble the effective fluorescence quenching of the squaramides combined with an adequate hydrogen bond pattern are valuable resources that will be exploited in the future for sensing other targets.

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Notes and references

† The IUPAC name for fluorescein is 9-(2-carboxyphenyl)-6-hydroxy-3*H*-xanthen-3-one.

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