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Evaluation of anion selectivity in protic media by squaramide–Cresol Red ensembles

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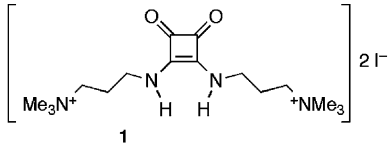
Abstract—A new series of positively charged squaramide-ammonium compounds **1**–**6** bind sulfate and hydrogen phosphate in ethanol–water mixtures by a combination of electrostatic and hydrogen bonding interactions. A sensing ensemble composed of Cresol Red and an squaramide receptor signals the association events of these anions. Competitive experiments in ethanol–water mixtures (9:1) reveal a moderate selectivity for sulfate over hydrogen phosphate and suggest that squaramide **4**, featuring an intermediate distance between squaramide subunits, is better suited for complexation of sulfate.

There is an increasing interest in the development of host molecules for sensing anions in protic solvents and/or in water, the natural solvent for most anions.[1] Despite this interest there are only a few selective hosts for sensing anions in aqueous media due to the high solvating ability of water.[2] This is especially true for anions of similar charge and shape such as sulfate[3] and hydrogen phosphate,[4] two closely related anions. In water and water–ethanol mixtures these oxyanions are highly solvated and their effective binding require the participation of Lewis acid centers[5] and/or positively charged groups such as quaternary nitrogen atoms.[6]

In our approach to the problem of selective recognition of anions in protic media, we became interested in studying the effect on the selectivity of binding when introducing certain structural variations, including among these a variable number of hydrogen bonding interactions, while keeping constant two electropositive centers as a main force of binding. The final goal of our work is to unravel the factors that control the selective recognition of oxyanions in water. In fact, a combination of electrostatic and hydrogen bonding forces is responsible of the specificity of sulfate or phosphate binding proteins.[7]

Inspired in the same principle of design we report a new set of pH-independent squaramido-ammonium receptors **1**–**6** capable of binding oxyanions in water–ethanol mixtures.



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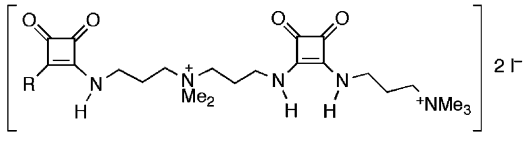
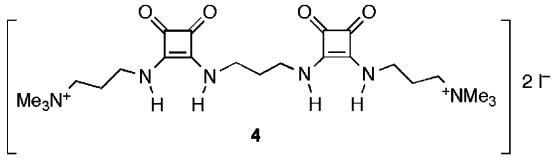


2 R = OEt
3 R = $NH(CH_2)_3NMe_2$



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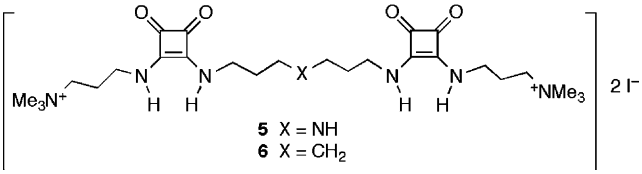

5 X = NH
6 X = CH_2

Keywords: Molecular sensor; Squaramides; Anion recognition; Colorimetric ensemble; Cresol Red.
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We also set out a colorimetric ensemble[8] for evaluating the relative selectivity of sulfate (SO_4^{2-}) over hydrogen phosphate (HPO_4^{2-}). In our measuring ensemble we took advantage of the complexation-induced displacement of the acid–base equilibrium of Cresol Red, a common pH indicator. Squaramides **1**–**6** were obtained in good yield from commercially available materials.[9] All these compounds have two electropositive tetraalkyl ammonium groups but differ in their hydrogen bonding abilities and the distances between charges. Thus, the distance between the two electropositive centers in **1**–**3** is shorter when compared to **4** and **5**, **6**. Also important, squaramides **1**–**6** do not absorb below 350 nm in ethanol and they are soluble in ethanol or water–ethanol mixtures.

Cresol Red was selected as signaling agent for assembling a colorimetric sensing device. In solution the color of this indicator change from yellow to purple by deprotonation of a phenolic monoanion to give a dianionic semiquinone with extended conjugation that is recognized by the squaramide receptors. The UV–vis spectrum of Cresol Red (4×10^{-5} M) registered in a Tris (10^{-2} M, pH = 9) buffered solution, at a pH close to the pK_2 of Cresol Red (8.46), still displays the yellow band of the monoanion at $\lambda_{max} = 428$ nm. However the spectrum of a solution containing Cresol Red and each one of the squaramides **1**–**6** exhibit also a band at $\lambda_{max} = 580$ nm, as depicted in Figure 1A. Visually, the observed color change from yellow to purple is accounted for by complexation-induced shift of the acid–base equilibrium in Cresol Red[10] due to the preferential complexation of the dianionic over the monoionic form. The stoichiometry and the association constants between Cresol Red and squaramides **1**–**6** were evaluated at $\lambda_{max} = 580$ nm, by nonlinear curve fitting assuming a 1:1 equilibrium (Table 1).[11] This band, assigned to the dianionic form of the indicator, gives us a direct measure of the concentration of Cresol Red in a complexed state with squaramide receptors.

The colored complex formed by association of Cresol Red and squaramides **1**–**6** is reversible and the band at $\lambda_{max} = 428$ nm, corresponding to uncomplexed Cresol Red, is completely recovered after addition of SO_4^{2-} or HPO_4^{2-} oxyanions. As a consequence, the displacement of receptor-bound Cresol Red by these anions is signaled visually (see Graphical abstract) and this effect allows the visible spectrophotometric determination of both anions.

Table 1. Association constants (K_a) for the binding of Cresol Red to squaramides **1**–**6** in an ethanol–water mixture (9:1) and relative selectivity of sulfate versus hydrogen phosphate determined by competition assays

Compound	K_a (M^{-1})	SO_4^{2}/HPO_4^{2-}
1	2.2×10^4	1.06
2	9.5×10^3	1.04
3	7.0×10^3	1.22
4	7.6×10^3	2.28
5	4.0×10^3	1.13
6	6.0×10^3	1.49

Remarkably, all squaramides studied so far display lower affinity for Cresol Red than for sulfate or hydrogen phosphate as is evidenced by the change in color when solutions of these two anions are slowly added to a solution containing an squaramide receptor–Cresol Red sensing pair. Taking advantage of the above observations, we studied the relative selectivity of sulfate versus hydrogen phosphate by preparing different signaling ensembles composed of Cresol Red and each one of the squaramide receptors **1**–**6**. The competition that takes place between Cresol Red and sulfate anion is illustrated in Scheme 1.

In all cases, the sensing ensemble was prepared by mixing Cresol Red and compounds **1**–**6** in a 1:5 molar ratio in an EtOH–H_2O (9:1) solution of Tris buffer (10^{-2} M). When the solution containing the ensemble was titrated with a solution of sulfate or hydrogen phosphate the color changed visually from purple (580 nm) to yellow (428 nm). In the UV–vis spectrum the absorption at 580 nm decreases while the band at 428 nm increases upon addition of the above anions. Figure 2 shows the decrease of the absorption at 580 nm observed by addition of both anions.

The relative affinities of sulfate and hydrogen phosphate were determined by competitive colorimetric titration. In the present case, the initial slope of a plot obtained by representing the change in absorbance versus anion concentration was used to estimate their relative binding

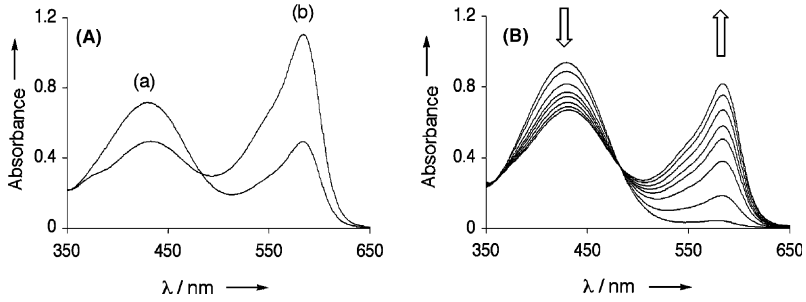

Figure 1. UV–vis spectra of (A) a solution of Cresol Red (4.2×10^{-5} M) (a) before and (b) after the addition of compound **6** (9.2×10^{-4} M) at 21 °C. (B) Changes in the absorbance produced by increasing the concentration of **6** until a final concentration 10 times higher relative to that of Cresol Red. The traces show the absorbance at 0; 4.1×10^{-5}; 9.1×10^{-5}; 1.3×10^{-4}; 1.7×10^{-4}; 1.3×10^{-4}; 2.3×10^{-4}; 4.1×10^{-4} M, respectively.

Scheme 1. Schematic representation of the competitive equilibria between Cresol Red and sulfate for squaramide receptor **2**.

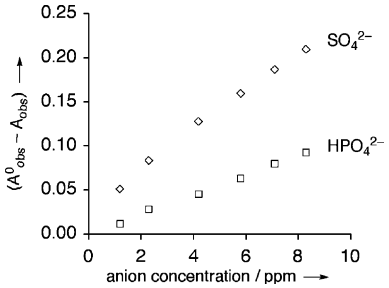

Figure 2. Change in absorbance at 580 nm of an ensemble composed of Cresol Red and squaramide **4** upon titration with sulfate (○) and hydrogen phosphate (□).

(Table 1). The possible competition of the target anions with other potentially interfering anions was also investigated. In a preliminary study, the UV spectra of the ensemble remains essentially unaltered by addition of 25 ppm of each one of the following anions as sodium salts: carbonate, nitrate, nitrite, fluoride, chloride, bromide, iodide.

The above results illustrated a slight selectivity of SO_4^{2-} over HPO_4^{2-}. In terms of energy a maximum difference of only 0.4 kcal mol^{-1} observed for **4** is clearly insufficient for selectivity purposes. However, it suggests a route for the synthesis of more selective receptors. In all these cases, molecular modeling shows that squaramides **1**–**6** are complexed to sulfate or hydrogen phosphate by using only two of the four oxygen atoms available for binding whereas a significant portion of the oxyanion is still exposed to solvent. If the observed energy gap reflects a true difference in binding between anions it can be deduced that the structural pattern of squaramide **4** is better suited than other structures studied so far. It is also conceivable that selectivity will improve with receptors capable of interacting simultaneously with all oxygen atoms of these isomorphic tetrahedral oxyanions.

In summary, we demonstrate the use of competitive colorimetric titrations, to evaluate rapidly a number of partial structures designed for binding oxydianions. We demonstrated that the charged squaramide compounds alter the acid–base equilibrium of Cresol Red by complexation with the dianionic form of the indicator. We also studied, for the first time, the use of this indicator in a sensing device for signaling the presence of sulfate or hydrogen phosphate. Currently this technique is applied as a tool to evaluate structural modifications directed to the synthesis of selective receptors in protic solvents.

Acknowledgements

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References and notes

1. (a) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486–516; (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
2. Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302–308.
3. Sancenon, F.; Descalzo, A. B.; Martinez-Manez, R.; Miranda, M. A.; Soto, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2640–2643.
4. Kubik, S.; Goddard, R.; Kirchner, R.; Nolting, D.; Seidel, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2648–2651.
5. Tobey, S. L.; Jones, B. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 4026–4027.
6. (a) Galbraith, S. G.; Plieger, P. G.; Tasker, P. A. *Chem. Commun.* **2002**, 2662–2663; (b) Kobiro, K.; Inoue, Y. *J. Am. Chem. Soc.* **2003**, *125*, 421–427.
7. (a) Shrestha, S.; Salins, L. L. E.; Ensor, C. M.; Daunert, S. *Biotechnol. Bioeng.* **2002**, *78*, 517–526; (b) Ledvina, P. S.; Tsai, A.; Wang, Z. G.; Koehl, E.; Quiocho, F. A. *Protein Sci.* **1998**, *7*, 2550–2559.
8. (a) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666–3669; (b) Niikura, K.; Bisson, A. P.; Anslyn, E. V. *J. Chem. Soc., Perkin 2* **1999**, 1111–1114; (c) Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 10109–10110.
9. In a representative procedure, squaramide **2** was synthesized by condensation between *N*,*N*-bis-(3-aminopropyl)-methylamine and diethyl squarate in diethyl ether at room temperature. The bis-squaramide ester intermediate was first condensed with 3-(dimethylamino)-1-propylamine in ethanol and the resulting product was then treated with

excess iodomethane in DMF–acetone. Finally, crude **2** was purified by crystallization from ethanol, yielding an off-white solid in 41% overall yield starting from diethyl squarate. Selected spectroscopic data of new compounds: **1** (82%); mp 240–244 °C; ^{1}H NMR (DMSO-d_6): δ 7.62 (br s, NH); 3.68 (b t, 4H); 3.17 (s, 18H); 2.07 (m, 4H) ppm; IR(KBr): 1809 cm^{-1}; HRMS ESI(+) calcd for $C_{16}H_{32}N_4O_2I$: 439.1570, found: 439.1561. Compound **2** (41%); mp 124–128 °C; ^{1}H NMR (DMSO-d_6): δ 8.96 (s, NH); 8.72 (s, NH); 7.62 (br s, NH); 4.75 (q, 2H); 3.64 (t, 2H); 3.13 (s, 15H); 2.05 (m, 6H); 1.47 (t, 3H) ppm; IR(KBr): 1801cm^{-1}; HRMS ESI(+) calcd for $C_{24}H_{41}N_5O_5I$: 606.2152, found: 606.2174. Compound **3** (30%); mp 124–128 °C; ^{1}H NMR (DMSO-d_6) δ: 8.96 (s, NH); 8.72 (s, NH); 7.62 (br s, NH); 4.75 (q, 2H); 3.64 (t, 2H); 3.13 (s, 15H); 2.05 (m, 6H); 1.47 (t, 3H) ppm; IR(KBr): 1801 cm^{-1}; HRMS ESI(+) calcd for $C_{27}H_{49}N_7O_4I$: 662.2891, found: 662.2920. Compound **4** (56%); mp 218–222 °C; ^{1}H NMR (DMSO-d_6): δ 7.46 (br s, NH); 3.74 (t, 4H); 3.67 (t, 4H); 3.19 (s, 18H); 2.08 (m, 4H); 1.91 (m, 2H) ppm; IR(KBr): 1792 cm^{-1}; HRMS ESI(+) m/z calcd for $C_{23}H_{40}N_6O_4I$: 591.2156: found: 591.2147. Compound **5** (63%); mp 101–104 °C; ^{1}H NMR (DMSO-d_6): δ 7.62 (br s, NH); 3.66 (t, 8H); 3.19 (s, 18H); 2.68 (t, 4H); 2.07 (m, 4H); 1.94 (m, 4H); 1.77 (m, 4H) ppm; IR(KBr): 1800 cm^{-1}; HRMS ESI(+) calcd for $C_{26}H_{47}N_7O_4I$: 648.2734, found: 648.2722. **6** (54%); mp 155–157 °C. ^{1}H NMR (DMSO-d_6) δ: 7.54 (br s, NH); 3.65 (t, 8H); 3.15 (s, 18H); 2.07 (t, 4H); 1.61 (m, 4H); 1.39 (m, 10H) ppm; IR(KBr): 1799 cm^{-1}; HRMS ESI(+) calcd for $C_{29}H_{50}N_3O_5I$: 647.2795, found: 647.2796.

10. Schneider, H.-J.; Yatsimirsky, A. K. *Principles and Methods in Supramolecular Chemistry*; Wiley: Chichester, 2000.
11. In a typical run, a Tris (10^{-3} M) buffered solution of Cresol Red (4×10^{-5} M) in an ethanol–water mixture (9:1) was titrated with the squaramide (8×10^{-4} M) dissolved in the same solution. The absorption at 580 nm were fit to the equation: $A_{obs} = Q(I_0 + L_0 + 1/K_a - ((I_0 + L_0 + 1/K_a)^2 - 4L_0I_0)^{1/2})/2$, where Q is a constant that include ε_I.