Evidence of anion-induced dimerization of a squaramide-based host in protic solvents†

M. Neus Piña,[a] Carmen Rotger,*[a] Bartomeu Soberats,[a] Pablo Ballester,[b] Pere M. Deyà[a] and Antoni Costa*[a]

Received (in Cambridge, UK) 29th September 2006, Accepted 21st November 2006
First published as an Advance Article on the web 8th December 2006
DOI: 10.1039/b614172b

The combination of squaramide units with tetraalkylammonium groups leads two hosts that bind distinctively dianions in water–ethanol mixtures. The formation of complexes of 2 : 1 stoichiometry with host 2 was supported by ITC, fluorescence, and ^{1}H NMR data.

The important role of anions in environmental and biochemical processes together with recent advances in anion-templated syntheses and directed self-assembly have stimulated the rise of numerous hosts for these species, many of them incorporated into sensing devices.[1] In aprotic solvents a number of molecular hosts have been shown to bind oxoanions by weak interactions such as hydrogen bonding.[2] In contrast, the binding in polar protic solvents, or in water, the natural solvent for anions, is difficult due to strong solvation. In these solvents, anion recognition is usually achieved *via* metal complexes[3] or *via* nitrogen charged groups involved in salt bridges with anions.[4]

In protic media it still remains a challenge to build hosts that display both high affinity and selectivity for their targets. In this contribution, we focus on two isomeric squaramide-based hosts to investigate the influence in the complexation produced by changing the relative position of a squaramide unit with that of an ammonium group. The hosts are composed of two tetraalkylammonium groups joined through covalent bonds that incorporate two squaramide modules (Chart 1). The squaramides not only offer two efficient hydrogen bond donors as well as two hydrogen bond acceptors, but also impart some rigidity to the hosts. Thus, the quasi-flexible squaramide-based hosts **1** and **2** are intended for binding oxodianions in an adaptive way by establishing multiple attractive interactions to satisfy the double valence concept recently introduced by Bowman-James.[5]

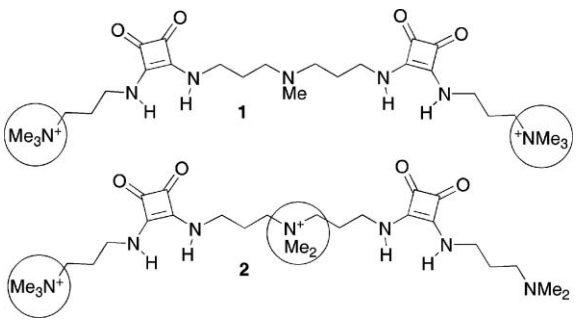

Chart 1 Structures of squaramide-based hosts **1** and **2**.

Squaramide hosts **1** and **2** were obtained in good yields as the diiodide salts by a sequence of condensation and methylation reactions as shown in Schemes S1 and S2 (ESI†). The structure of **1** and **2** also includes a tertiary amine that is important for solubility considerations.[6] They are soluble in DMSO, MeOH, EtOH, H_2O and in hydroalcoholic mixtures. Based upon literature precedents, ion pairing with the iodide contra-ions in protic solutions is considered negligible in the range of concentrations used in this work (10^{-6}–10^{-3} M).[7]

The binding properties of the diiodide salts of **1** and **2** with tetramethylammonium sulfate $(TMA)_2(SO_4^{2-})$ and with the sodium salt of Erythrosin B (ErB) were investigated by isothermal titration calorimetry (ITC), and by fluorescence and ^{1}H NMR spectroscopy, when possible under the same experimental conditions. Under these circumstances a comparison of the results for **1** and **2** must give insightful information of the influence of their structural differences in the complexation events.

Inspection of Table 1 shows that for all equilibria involving hosts **1** or **2**, ΔH° values are close to zero and ΔS° are positive. These data emphasized the importance of the solvent in the formation and organization of the ion-paired species. In all cases, the small magnitude of ΔH° and the favourable ΔS° suggests the formation of more or less structured complexes incorporating a variable number of solvent molecules. At the same time, a significant proportion of solvent molecules are released into bulk solution resulting in a reorganization of the solvent which is in agreement with the observed favourable entropic term.[8] The strength of the binding was estimated from the ITC data with some difficulty due to the low thermicities observed with these equilibriums. Thus, while integrated heat data of **1** with

Table 1 Apparent association constants and thermodynamic parameters[a] from ITC titrations for complexation of squaramide hosts **1** and **2** with sulfate and Erythrosin B in EtOH–H_2O at 293 K[b]

Complex	K/M^{-1}	ΔH°	$T\Delta S^\circ$	ΔG°	n
1·(SO_4^{2-})	$1.0 \pm 0.2 \times 10^5$	−0.1	+6.6	−6.7	1.0
1·ErB	$7.4 \pm 0.1 \times 10^4$	+1.0	+7.4	−6.5	0.9
2·(SO_4^{2-})	$7.0 \pm 2.4 \times 10^6$	0.0	+9.2	−9.2	1.0
$\mathbf{2}_2$·(SO_4^{2-})	$3.9 \pm 0.5 \times 10^4$	−0.1	+6.6	−6.7	0.9
2·ErB	$2.4 \pm 0.3 \times 10^4$	+0.3	+6.2	−5.9	0.9
$\mathbf{2}_2$·ErB	$4.8 \pm 1.1 \times 10^5$	+1.0	+8.6	−7.6	0.9

[a] In kcal mol^{-1}. [b] (96%) EtOH–H_2O (9 : 1 v/v), pH 8.9, TRIS buffer 10^{-2} M.

[a]*Department of Chemistry, Universitat de les Illes Balears, 07122 Palma de Mallorca, Spain. E-mail: antoni.costa@uib.es; Fax: (+34) 971 173 426; Tel: (+34) 971 173 266*
[b]*Institute of Chemical Research of Catalonia (ICIQ), Avgda, Països Catalans, s/n, 43007, Tarragona, Spain*

† Electronic supplementary information (ESI) available: Synthesis and characterization data of **1** and **2**, chemical shift changes observed by the addition of $(TMA)_2SO_4$ to **2**, and ITC titrations. See DOI: 10.1039/b614172b

$(TMA)_2SO_4$ or ErB were fitted to 1 : 1 models, those of **2** could only be satisfactorily fitted taking into account the existence of 2 : 1 stoichiometries.[9] These results are noticeable because the formation of a high order complex involves the addition of a second molecule of **2** on an already existing ion pair. This consecutive equilibrium, although not totally discarded, has not been observed with host **1**.

To gain more insight into the mode of complexation of **1** and **2** we carried out additional titrations with Erythrosin B by fluorescence in the same solvent mixture. ErB is a well known dye that is pink-red in the dianionic state. The ErB absorption is linearly dependent on concentration over the range from 0.1×10^{-5} to 5×10^{-5} M, the conditions of this study, thereby excluding self-aggregation effects.

The decrease in the intensity of the fluorescence emission band of ErB recorded at 552 nm (λ_{exc} = 535 nm) was used to determine ligand association (Fig. 1). Note that at the end of a titration the squaramide hosts are in excess and will tend to shift the equilibrium towards the formation of higher aggregates of type $\mathbf{2}_n$·ErB ($n > 1$). Analysis of the fluorescent titration data of ErB with **1** and **2** to both 1 : 1and 2 : 1 models[10] was performed by non-linear curve fitting.[11] In this way, the association constant of **1** with ErB calculated with a 1 : 1 complexation model is 7.4×10^4 M^{-1}, while those with host **2** with a 2 : 1 model are 1.1×10^4 M^{-1} and 6.2×10^5 M^{-1} for **2**·ErB and $\mathbf{2}_2$·ErB, respectively. It is relevant to note that these association constants compare well with those obtained by ITC at a different range of concentrations and that **2** shows 2 : 1 stoichiometry as above.

The complexation of **1** and **2** with dianions was also investigated by ^{1}H NMR in MeOH-d_4. Although in MeOH-d_4 the NH squaramide resonances are obscured due to fast exchange, the ^{1}H NMR spectra of **1** and **2** still show distinctive features. Upon titration with $(TMA)_2SO_4$ the chemical shift changes of both compounds were analyzed by the molar ratio method,[12] Fig. 2. In host **1**, the resonances of NMe and NCH_2 initially at 2.30 and 2.56 ppm move gradually downfield, Fig. 2(a). In a parallel experiment, host **2** shows initially two sets of observable signals centered at around 2.39 (NMe) and 2.57 ppm (NCH_2), consistent with the presence of *anti-syn* conformers typical of squaramides

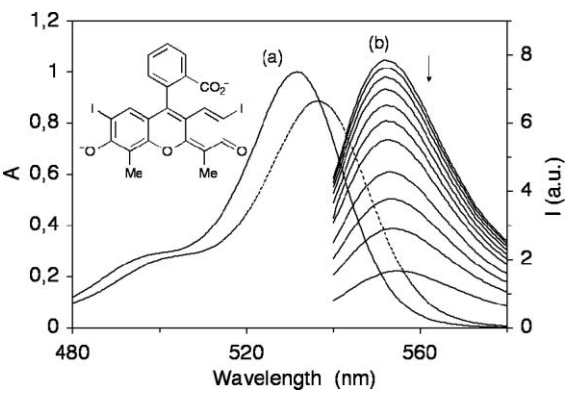

Fig. 1 (a) Left *y*-axis, absorption spectra of ErB (3.60×10^{-6} M) in EtOH–H_2O (9 : 1 v/v) before and after addition of 10 equiv. of **2** (broken line). (b) Right *y*-axis, from the top down, fluorescence emission spectra of ErB (λ_{exc} = 535 nm) and the effect produced by the addition of **2**: 0, 0.195; 0.378; 0.550; 0.712; 1.01; 1.27; 1.73; 2.27; 3.46; 69.1 ($\times 10^{-5}$) M, respectively.

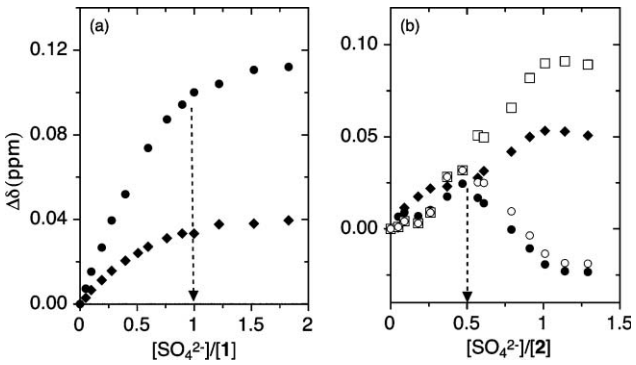

Fig. 2 Chemical shift changes observed for **1** (a) and **2** (b) (*ca.* 4.5×10^{-3} M) in MeOH-d_4: (a) NMe (◆), NCH_2 (●); (b) NMe(1) (◆), NMe(2) (●), NCH_2(1) (□), NCH_2(2) (○).

substituted with dimethylaminopropyl groups.[6] Upon addition of $(TMA)_2SO_4$ a pair of NMe and NCH_2 signals moved downfield by 0.04 and 0.09 ppm, respectively, while a second pair moved initially downfield around 0.03 ppm and then, at 0.5 equiv. of sulfate shifted upfield by 0.03 ppm, Fig. 2(b). The observation of two inflections at ratios $[SO_4^{2-}]$/[**2**] around 0.5 and 1, respectively, indicates formation of $\mathbf{2}_2$·(SO_4^{2-}) and **2**·(SO_4^{2-}) complexes. Furthermore, this also indicates that the association constants K_1 and K_2 are larger compared to $1/[\mathbf{2}]_t$ and, with a small ratio between constants ($K_2/K_1 < 0.1$).[13] In both compounds, the resonances of NMe_3 initially at 3.24 ppm moved upfield 0.02 ppm as a consequence of the anion binding.[14]

The formation of the complexes was confirmed by electrospray mass spectra (ESMS). A solution of host **1** (100–200 μM) in MeOH gives ion signals at *m*/*z* 520.7 and 662.3 (base peak) that are assigned to $[\mathbf{1} - 2I^- - H^+]^+$and $[\mathbf{1} - I^-]^+$, respectively. A sample of **1** containing $(TMA)_2SO_4$ exhibits new ion signals for $[\mathbf{1} - 2I^- + SO_4^{2-} + H^+]^+$ and $[\mathbf{1} - 2I^- + SO_4^{2-} + TMA^+]^+$ at *m*/*z* 632.3 and 705.4, respectively, which are indicative of the presence of a 1 : 1 complex in solution. On the other hand, the ESMS of **2** besides ion peaks at 632.3 and 705.4 ppm, shows a weak ion signal assigned to $[\mathbf{2}_2 - IMe - 3I^- + SO_4^{2-}]^+$ at *m*/*z* 1151.7 that supports the formation of a 2 : 1 complex.

Considering the charged nature of hosts **1** and **2**, the observation of dimeric complexes in solutions of **2** (10^{-3}–10^{-6} M) with dianions of different sizes and shapes is quite remarkable. It implies that dimerization proceeds due to interactions on an already ion-paired neutral complex. If one recalls that the formation of both 1 : 1 and 2 : 1 complexes is characterized by a favourable entropic term, the most probable driving force of dimerization arises from the entropically favoured partial desolvation of a second molecule of **2** that interacts with the already existing ion pair. This consecutive process involves an added bonus to the favourable overall energy of a system heavily dominated by electrostatic interactions. However, electrostatic interactions *per se* are unable to explain the observed anion-induced dimerization of host **2**, because parallel experiments performed with **1** lead only to the detection of 1 : 1 complexes.

Taken together our results confirm that the interaction of solvent with hosts and guests as well as solvent reorganization plays a crucial role in the complexation events. Also, observation of a uniform response of the two anions investigated, sulfate and ErB, which have very different geometric requirements, suggests

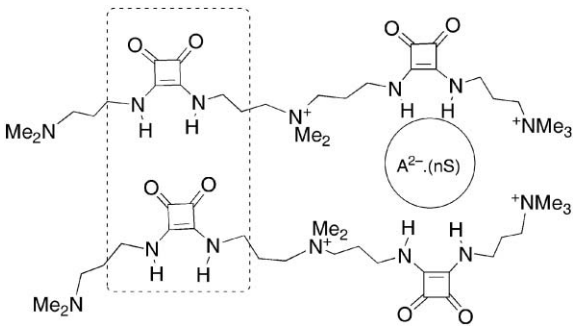

Chart 2 Schematic representation of an anion-induced dimer of **2**. In this illustration $A^{2-}\cdot(n\mathrm{S})$ represents a generic, partially solvated guest, although the host actually is also partially solvated.

that the actual size and the area of contact between hosts and guests are significantly bigger than assumed. A situation that is probable is if complexation takes place by interaction of the host with an anionic guest included in a solvation cluster.[15] In that case, a variable number of solvent molecules of the first solvation shells also participate in the interaction event, thus modifying the actual size and shape of host and guest. From the thermodynamic side, numerous populated states of similar energies are likely to exist in solution for such a system.[8] In this scenario, a plausible explanation for the anion-induced dimerization of **2** (Chart 2) involves the formation of an initial 2 : 1 complex held by electrostatic interactions with the tetraalkylammonium groups and two squaramides, which trigger new host–host favourable C=O to NH interactions through the squaramide ends. This event liberates more solvent molecules from the solvation cage to the bulk, thus providing an extra stabilization to the dimeric complex. Under these circumstances the dimeric state becomes populated and can be experimentally detected.

In summary, in this work, we have demonstrated that subtle changes in the structural arrangement of flexible hosts can lead to unexpected changes in the stoichiometry of the complexes in protic solvents. Anion-induced dimerization modifies the affinity and/or the selectivity of a host for a given guest and can be used advantageously in the design of more effective hosts. Further studies with other squaramide-based hosts and with anions such as oxalate and phosphate are currently being investigated in our laboratories.

This work is supported by a MEC grant ref. CTQ2005-08989-C02-01 and a CAIB grant ref. PCTIB-2005GC3-08. C. R. thanks the MCyT for a "Ramón y Cajal" contract. M. N. P. thanks the DGR + D + I (Govern Balear) for a predoctoral fellowship.

Notes and references

1 R. Martínez-Mañez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419; P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486; J. J. Lavigne and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 2001, **40**, 3118; For a number of representative examples, see also: Tetrahedron Symposium-in-Print, '*Synthetic receptors as sensors*', ed. E. V. Anslyn, *Tetrahedron*, 2004, **60**(49), 11 041–11 315; Special issue, '*Chemical Sensors*', ed. A. B. Ellis and D. R. Walt, *Chem. Rev.*, 2000, **100**(7), 2477–2738; For an application of squaramides in anion sensing, see: R. Prohens, G. Martorell, P. Ballester and A. Costa, *Chem. Commun.*, 2001, 1456; M. N. Piña, M. C. Rotger, A. Costa, P. Ballester and P. M. Deyà, *Tetrahedron Lett.*, 2004, **45**, 3749.

2 A. Bianchi, K. Bowman-James and E. García-España, *Supramolecular Chemistry of Anions*, Wiley-VCH, New York, 1997; P. Gale, *Acc. Chem. Res.*, 2006, **39**, 465; M. M. G. Antonisse and D. N. Reinhoudt, *Chem. Commun.*, 1998, 443; M. A. Hossain, J. M. Llinares, D. Powell and K. Bowman-James, *Inorg. Chem.*, 2001, **40**, 2936; R. Varghese, S. J. George and A. Ajayaghosh, *Chem. Commun.*, 2005, 593; K. Choi and A. D. Hamilton, *Coord. Chem. Rev.*, 2003, **240**, 101; L. J. Kuo, J. H. Liao, C. T. Chen, C. H. Huang, C. S. Chen and J. M. Fang, *Org. Lett.*, 2003, **5**, 1821.

3 P. D. Beer and E. J. Hayes, *Coord. Chem. Rev.*, 2003, **240**, 167; V. Amendola, L. Fabrizzi, C. Mangano, P. Pallavicini, A. Poggi and A. Taglieti, *Coord. Chem. Rev.*, 2001, **219**, 821; S. Mizukami, T. Nagano, Y. Urano, A. Odani and A. Kikuchi, *J. Am. Chem. Soc.*, 2002, **124**, 3920; R. S. Dickins, T. Gunnlaugsson, D. Parker and R. D. Peacock, *Chem. Commun.*, 1998, 511; I. Tsagkatakis, N. Chaniotakis, R. Altman, K. Jurkschat, R. Willem, J. C. Martins, Y. Qin and E. Bakker, *Helv. Chim. Acta*, 2001, **84**, 1952; S. L. Tobey and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 14 807, and refs cited therein. For an example of anion binding *via* hydrogen bonding in polar protic solvents, see: T. Gunnlaugsson, P. E. Kruger, P. Jensen, J. Tierney, H. D. P. Ali and G. M. Hussey, *J. Org. Chem.*, 2005, **70**, 10 875.

4 F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609; S. Aoki and E. Kimura, *Rev. Mol. Biotechnol.*, 2002, **90**, 129; X. Salvatella, M. W. Peczuh, M. Gairí, R. K. Jain, J. Sanchez-Quesada, J. de Mendoza, A. Hamilton and A. E. Giralt, *Chem. Commun.*, 2000, 1399; J. Chin, S. Chung and D. H. Kim, *J. Am. Chem. Soc.*, 2002, **124**, 10 948.

5 K. Bowman-James, *Acc. Chem. Res.*, 2005, **38**, 671.

6 M. C. Rotger, M. N. Piña, A. Frontera, G. Martorell, P. Ballester, P. M. Deyà and A. Costa, *J. Org. Chem.*, 2004, **69**, 2302.

7 Y. Geng and L. S. Romsted, *J. Phys. Chem. B*, 2005, **109**, 23 629; In aprotic solvents the affinities for a given anion are strongly dependent on the solvent and the counterion, see: J. L. Sessler, D. E. Gross, W. Cho, V. M. Lynch, F. P. Schmidtchen, G. W. Bates, M. E. Light and P. A. Gale, *J. Am. Chem. Soc.*, 2006, **128**, 12 281.

8 F. P. Schmidtchen, *Top. Curr. Chem.*, 2005, **255**, 1.

9 For related examples of 2 : 1 aggregation in protic media, see: M. Rekharsky, Y. Inoue, S. Tobey, A. Metzger and E. Anslyn, *J. Am. Chem. Soc.*, 2002, **124**, 14 959; V. Rüdiger, H.-J. Schneider, V. P. Solov'ev, V. P. Kazachenko and O. A. Raevsky, *Eur. J. Org. Chem.*, 1999, 1847; S. Kubik, R. Kircner, D. Nolting and J. Seidel, *J. Am. Chem. Soc.*, 2002, **124**, 12 752.

10 I. M. Klotz, *Ligand–Receptor Energetics*, John Wiley & Sons, New York, 1997; A. K. Connors, *Binding Constants*, John Wiley & Sons, New York, 1987.

11 A. P. Bisson, C. A. Hunter, J. C. Morales and K. Young, *Chem.–Eur. J.*, 1998, **4**, 845; We are indebted to Professor C. A. Hunter for making these programs available to us.

12 H. Tsukube, H. Furuta, A. Odani, Y. Takeda, Y. Kudo, Y. Inoue, Y. Liu, H. Sakamoto and K. Kimura, *Comprehensive Supramolecular Chemistry*, ed. J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vogtle and J.-M. Lehn, Elsevier, New York, 1996, vol. 8, p. 425.

13 K. Choi and A. D. Hamilton, *J. Am. Chem. Soc.*, 2003, **125**, 10 241.

14 For more details, see the ESI†.

15 X. Wang, J. B. Nicholas and L. Wang, *J. Chem. Phys.*, 2000, **113**, 10 837; B. Gao and Z. Liu, *J. Chem. Phys.*, 2004, **121**, 8299.