Supplementary Material (ESI) for Chemical Communications

Supporting Information

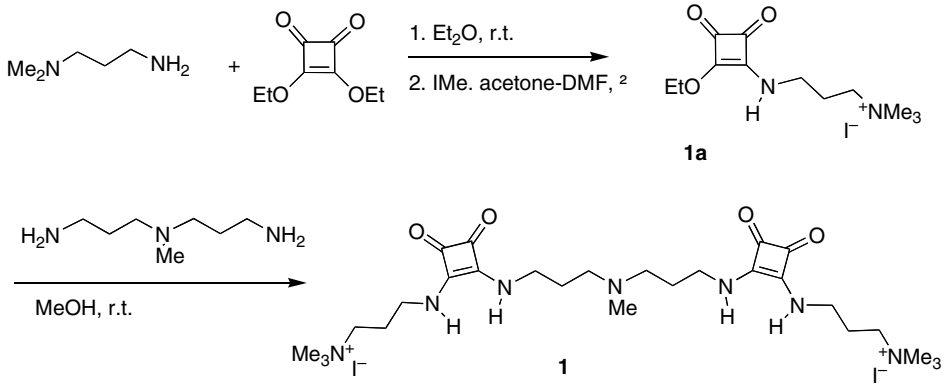
Evidence of anion induced dimerization of an squaramide-based host in protic solvents

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

Instruments and synthetic procedures

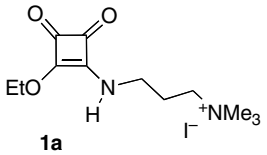
General: All commercially available reagents were used without further purification unless otherwise stated. Diethyl ether and ethanol used for synthesis were distilled from calcium hydride immediately before use. MeOH-d_4, DMSO-d_6 were used from freshly opened ampoules, and $CDCl_3$(99.8% D) was stored on molecular sieves (3 Å). $(TMA)_2SO_4$ (Aldrich) was dried in a drying pistol (< 0.1 mm Hg; refluxing toluene) for 12 h over sodium pentoxide before use. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance at 300 and 75 MHz at 23 ºC. Chemical shifts are reported as parts per million (δ) referenced to the residual hydrogen signal of deuterated solvents. Electrospray mass spectra (ESMS) were recorded with a Micromass, Autospec3000 spectrometer provided with an electrospray module. The ITC experiments were carried out on a Microcal, ultramicrocalorimeter MCS-ITC. The ITC instrument was periodically calibrated using an internal electric heater, following the procedures recommended by the manufacturer. The UV-Vis absorption spectra were registered with Varian Cary50-Bio and the fluorescence titrations with an Aminco-Bowman series2 spectrophotomers.

Synthesis of host 1



Scheme S1

Preparation of 3-(trimethylamoniumpropylamino)-4-ethoxycyclobut-3-ene-1,2-dione

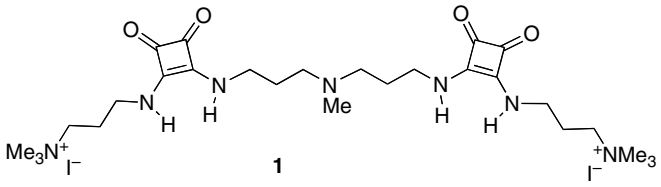


1a

A solution of N,N-dimethylpropylendiamine (1g, 9.8 mmol) in diethyl ether (10 mL) was added dropwise over 1 h to a stirred solution of diethyl squarate (2g, 11.8 mmol) in diethyl ether (10 mL). The mixture was stirred overnight at room temperature in an atmosphere of argon. After cooling at 0°C, the mixture was diluted with *n*-pentane (20 mL). The resulting white solid was isolated by centrifugation after decanting the supernatant and purified by washing with *n*-pentane-diethyl ether (9:1 v/v, 10 mL x 3), to give the monosquaramide ester[1] (2.2 g, 98 %) δ_H(300 Mhz; DMSO-d_6) 8.98 (br s, 0.6 × NH_{syn}), 8.65 (br s, 0.4 × NH_{anti}), 4.74 (br q, J = 7.0 Hz, 2H), 3.60 (br m, 0.4 × $2H_{syn}$), 3.41(br m, 0.6 × $2H_{anti}$), 2.33 (t, J = 6.9 Hz, 2H), 2.21 (s, 6H), 1.73 (quintet, J = 7.0 Hz, 2H) and 1.46 (t, J = 7.0 Hz, 3H); δ_C(75 Mhz; DMSO-d_6) 189.3, 182.2, 176.5, 172.5, 68.7, 55.9, 44.9, 40.3, 28.2, 27.8 and 15.7; m/z HRMS-ES (+) 249.1216 ($M + Na^+$. $C_{11}H_{18}N_2O_3Na$ requires 249.1215).

Methyl iodide (0.91 mL, 14.6 mmol) was added via syringe over 30 min to a stirred solution of the monosquaramide ester (2.17 g, 9.6 mmol) in acetone (60 mL). The mixture was heated under reflux in an atmosphere of argon for 12 h, after which it was cooled at room temperature and diluted with *n*-pentane (20 mL). The tetramethylammonium salt was obtained as a yellow solid that precipitates upon cooling at 0°C. The solvent was removed with a Pasteur pipette, and the solid was purified by washing with cold acetone-*n*-pentane (8:2 v/v, 20 mL x 3). The yellow product was dried in vacuo to give the tetramethylammonium salt **1a** (3.53 g, 90 %). ν_{max} (KBr)/cm^{-1}: 3515, 3191, 1796, and 1695; δ_H(300 Mhz; DMSO-d_6) 8.89 (br s, 0.55 × NH_{syn}), 8.72 (br s, 0.45 × NH_{anti}), 4.76 (br q, J = 6.9 Hz, 2H), 3.64 (m, 0.4 × $2H_{syn}$), 3.42 (m, 0.6 × $2H_{anti}$), 3.15 (s, 9H), 2.05 (m, 2H), 1.47 (t, J = 6.9 Hz, 3H); δ_C(75 Mhz; DMSO-d_6) 189.3, 182.4, 177.2, 172.7, 68.9, 62.7, 52.2; 40.8, 23.6 and 15.7; m/z HRMS-ES (+) 241.1552 ($M^+ - I^-$, $C_{12}H_{21}N_2O_3$ requires 241.1558).

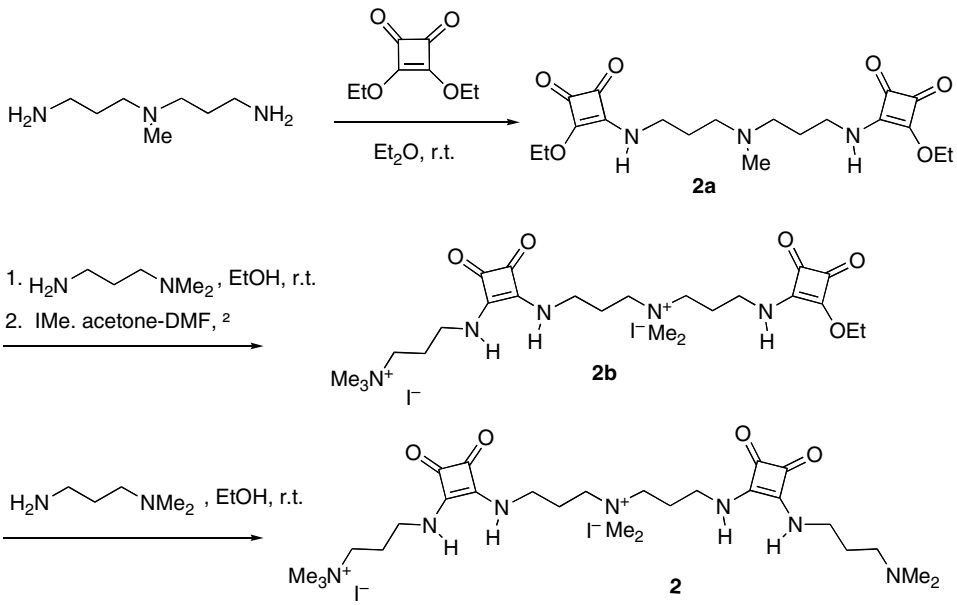
Preparation of N,N-bis-[3-[2-(trimethylammoniumpropylamino)-3,4-dioxo-1-cyclobutenyl]aminopropyl]methylamine diiodide (1)



1

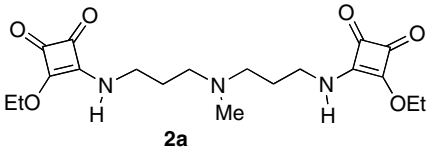
A solution of N,N-bis(3-aminopropyl)methylamine (248 µL, 1.5 mmol) in methanol (25 mL) was added dropwise over 1 h to a stirred solution of the monosquaramide-ammonium salt **1a** (1 g, 2.72 mmol) in methanol (5 mL). The mixture was stirred overnight at room temperature in an atmosphere of argon. After this period, a yellow solid had formed in the botom of the flask. The reaction was diluted with pentane (20 cm^3), cooled to 0°C and centrifugated. The resultant yellow solid was washed with *n*-pentane-methanol (9:1 v/v, 10 mL x 3) and dried under high vacuum to give **1** as the diiodide salt (903 mg, 77%). ν_{max} (KBr)/cm^{-1}: 3453, 1802, 1661, 1597 and 1543; δ_H(300 Mhz; DMSO-d_6) 7.51 (br s, NH), 3.65 (br t , 8H, NH-CH_2), 3.43 (m,4H, Me_3N^+-CH_2), 3.18 (s, 18H, Me_3N^+-), 2.43 (br t, J = 6.6 Hz, 4H, N-CH_2), 2.22 (s, 3H, N-Me), 2.08 (m, 4H, $N^+CH_2\underline{CH_2}$), 2.08 (br t, J = 6.6 Hz, 4H, $NCH_2\underline{CH_2}$); δ_C(75 Mhz; DMSO-d_6) 182.6, 182.1, 167.9, 167.6, 62.9, 54.1, 52.3; 41.7, 28.5 and 24.5; m/z HRMS-ES(+) 662.2892 ($M^+ - I^-$, $C_{27}H_{49}N_7O_4I$ requires 662.2891).

Synthesis of host 2



Scheme S2

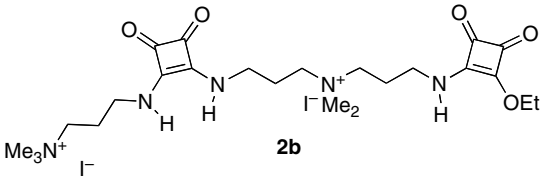
Preparation of N,N-bis-[3-(2-ethoxy-3,4-dioxo-1-cyclobutenyl)-aminopropyl)methylamine[1]



2a

A solution of N,N-bis(3-aminopropyl)methylamine (464 μL, 5.58 mmol) in ether (60 mL) was added dropwise to diethyl squarate (1.5 g, 8.8 mmol) in eter (5 mL) under Ar atmosphere. After the addition, the mixture was stirred at room temperature overnight. During this period, a yellow precipitated was formed. The reaction was diluted with *n*-pentane (20 mL), cooled at 0ºC and the yellow solid was collected by centrifugation, washed with *n*-pentane-ether (9:1 v/v, 10 mL x 3) and dried under high vacuum for 24 h to provide the bis-squaramide ester as an amorphous yellow solid **2a** (878 mg, 74%). ν_{max} (KBr)/cm^{-1}: 3448, 1801, 1653, 1600, 1540 and 1457; δ_H(^{1}H-RMN, DMSO-d$_6$) 8.88 (br s, 0.6 × 2H, NH$_{syn}$), 8.67 (br s, 0.4 × 2H, NH$_{anti}$), 4.74 (q, J = 6.9 Hz, 4H), 3.60 (m, 0.4 × 4H$_{syn}$), 3.39 (m, 0.6 × 4H$_{anti}$), 2.39 (m, 4H), 2.20 (s, 3H), 1.73 (quintet, J = 6.9 Hz, 4H) and 1.45 (t, J = 7.0 Hz, 6H); δ_C(75 Mhz; CDCl$_3$) 190.2, 183.5, 177.9, 173.1, 70.2, 56.2, 44.3, 42.0, 27.8 and 16.4; m/z HRMS-ES(+) 416.1778 (M + Na$^+$. $C_{19}H_{27}N_3O_6Na$ requires 416.1798).

Preparation of N-[3-(2-ethoxy-3,4-dioxo-1-cyclobutenyl)-aminopropyl]-N-[3-[2-(trimethylammoniumpropylamino)-3,4-dioxo-1-cyclobutenyl]aminopropyl]-N,N-dimethylammonium diodide.

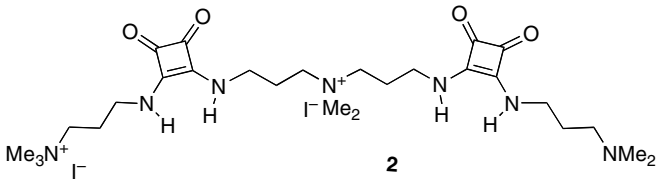


2b

A solution of N,N-dimethylpropane-1,3-diamine (130 mg, 1.27 mmol) in ethanol (45 mL) was added dropwise over 24 h to a solution of the bis-monosquaramideester (1 g, 2.5 mmol) in ethanol (50 mL) under Ar atmosphere. After the addition, the reaction was stirred at room temperature for an additional period of 12h. After this time, EtOH was evaporated at vacuo. The residue was treated with acetonitrile (30 mL) and the resulting solid was collected by filtration. The crude solid was redissolved in THF (4 mL) and re-precipitated by addition of toluene (30 mL). After stirring for a short period, the mixture was set aside for 15 min., and then the solid was decanted. This process was repeated for three times. The resulting solid was redissolved in ethanol (20 mL) and evaporated again to dryness at vacuo. Finally, The solid was rinsed with ether (10 mL) and dried under reduced pressure to afford the mixed bis-squaramide ester as an amorphous yellow powder (60-80 %, depending on the rate of the initial addition). δ_H(300

Mhz; $CDCl_3$) 4.75 (m, 3H); 3.72 + 3.58 (two m, 6H); 2.55 + 2.48 (two m, 6H), 2.37 (s, 6H), 2.24 (s, 3H), 1.82 (m, 6H), 1.47 (t, 3H).
Methyl iodide (1.94 g, 13.6 mmol) was added via syringe over 30 min to a stirred solution of the mixed squaramide ester (2.0 g, 4.45 mmol) in a minimum volume of anhydrous DMF (30 mL) and acetone (50 mL). The mixture was heated under reflux in an atmosphere of argon for 12 h. After cooling at room temperature, a yellow solid was collected by centrifugation followed by removal of the supernatant. The solid was purified by washing with ethanol (2 x 10 mL) and dried under reduced pressure to provide the title compound **2b** as an amorphous pale yellow solid (2.3 g, 71 %). ν_{max} (KBr)/cm^{-1}: 3448; 1801; 1653; 1600; 1540 and 1457; δ_H(300 Mhz; DMSO-d_6) 8.89 (br s, NH), 8.72 (br s, NH), 7.62 (br s, NH), 4.65 (br q, J = 6.9 Hz, 2H), 3.64 (m, 4H), 3.16 (s, 9H), 3.10 (br s, 6H), 1.95 (m, 6H), 1.36 (t, J = 6.9 Hz, 3H); δ_C(75 Mhz; DMSO-d_6) 186.8, 185.5, 182.5, 174.9, 167.8, 68.9, 62.8, 60.7, 60.4, 50.4, 50.1, 40.3, 24.1, 23.7 and 15.6; m/z HRMS-ES (+) 607.0336 ($M^+ - I^-$, $C_{24}H_{41}N_5O_5I$ requires 607.0319).

Preparation of N-[2-(3-dimethylamino-1-propylamino)-3,4-dioxo-1-cyclobutenyl]-3-aminopropyl]-N-[(2-(3-trimethylammonio-1-propylamino)-3,4-dioxo-1-cyclobutenyl)-3-aminopropyl]-N,N-dimethylammonium diodide. (2)



2

A solution of N,N-dimethylpropylendiamine (418 mg, 4,09 mmol) in methanol (20 mL) was added dropwise over 1 h to a stirred solution of **2b** (2.3 g, 3.13 mmol) in methanol (200 mL). The mixture was stirred overnight at room temperature in an atmosphere of argon. After this time, the solvent was removed at vacuo. The resultting yellow solid was purified by washing with acetone (3 x 20 mL) and finally dried under reduced pressure to afford **2** (2.1 g, 85%). ν_{max} (KBr)/cm^{-1}: 3452, 1799, 1646, 1583, 1457 and 1361; δ_H(300 Mhz; DMSO-d_6) 7.51 (br s, NH), 3.7-3.4 (m, 8H, NH-CH_2)(m, 6H, N^+-CH_2), 3.17 (s, 9H, Me_3N^+-), 3.12 (s, 6H, Me_2N^+), 2.47 (br t, J = 6.9 Hz, 2H, N-CH_2), 2.25 (s, 6H, NMe), 2.07 (m, 6H, $N^+CH_2\underline{CH_2}$), 1.77 (br t, J = 7.2 Hz, 4H, $NCH_2\underline{CH_2}$); δ_C(75 Mhz; DMSO-d_6) 182.5, 182.3, 167.5 (br), 64.5, 60.7, 55.8, 52.4, 50.1, 44.9; 41.6; 40.5; 28.5; 24.1(br); m/z HRMS-ES(+) 662.2920 ($M^+ - I^-$, $C_{27}H_{49}N_7O_4I$ requires 662.2891).

Determination of association constants by isothermal titration calorimetry

In a typical titration, 20-45 injections (5-10 µl per injection) of a buffered solution of **1** or **2** ($4.5x10^{-3}$M) in 96 % ethanol-water (9:1 v/v),[3] TRIS buffer (10^{-2}M), were injected into the sample cell containing 1.3 ml of a solution of $(TMA)_2SO_4$ or ErB ($5x10^{-4}$ M) containing also TRIS buffer (10^{-2}M) in the same solvent mixture. In all cases "c" parameter was > 40. The heats of dilution of de component in the syringe were subtracted prior to data analysis. The titration data were analyzed by curve-fitting software implemented (ORIGIN), which gave a number of sites (n), apparent binding affinity (K), and the standard enthalpy change (ΔH^0). The standard deviation based on the scatter of the data points in a single titration was also calculated.

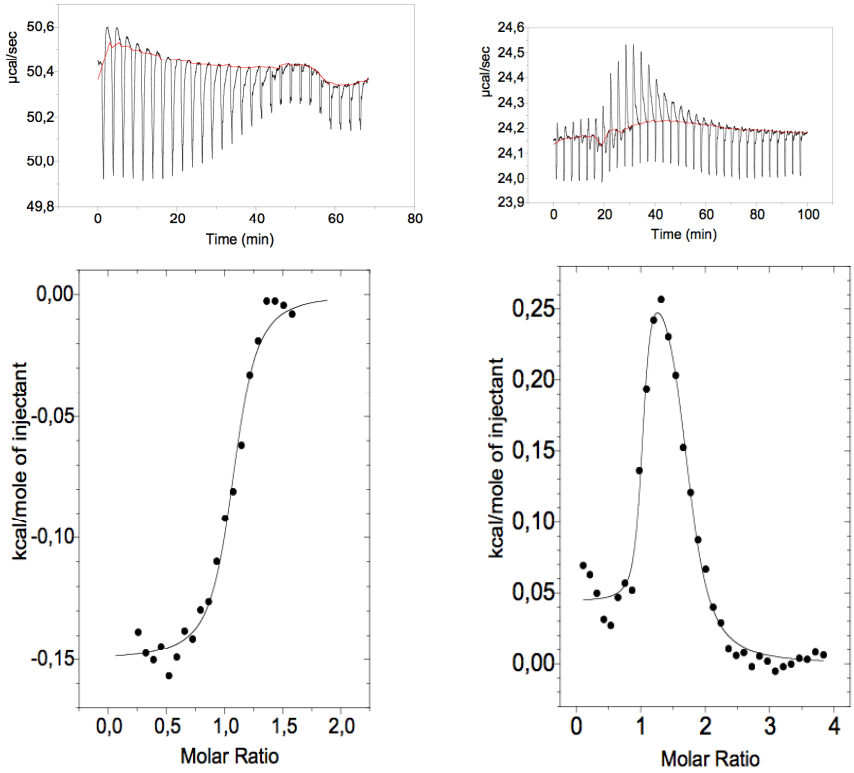


Fig. S1. ITC data of **1** (1.0×10^{-3} M) *vs* $(TMA)_2SO_4$ (8.5×10^{-3} M) (left side), of $(TMA)_2SO_4$ (5.0×10^{-4} M) *vs* **2** (7.5×10^{-3} M) (right side), data in 96%EtOH-H_2O (TRIS buffer, 10^{-2}M) at 293 K. See Table 1 for the corresponding thermodynamic parameters.

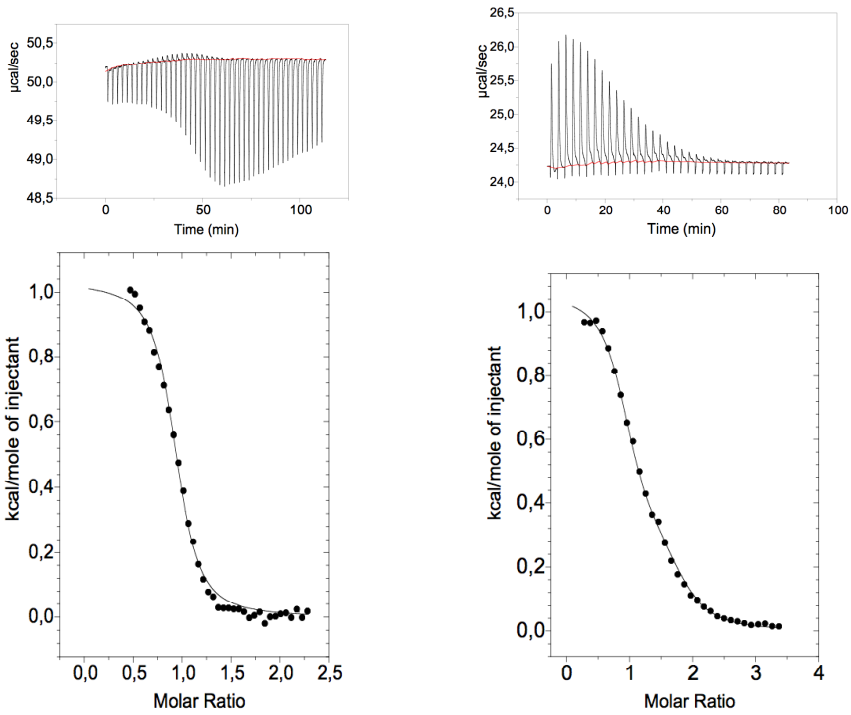
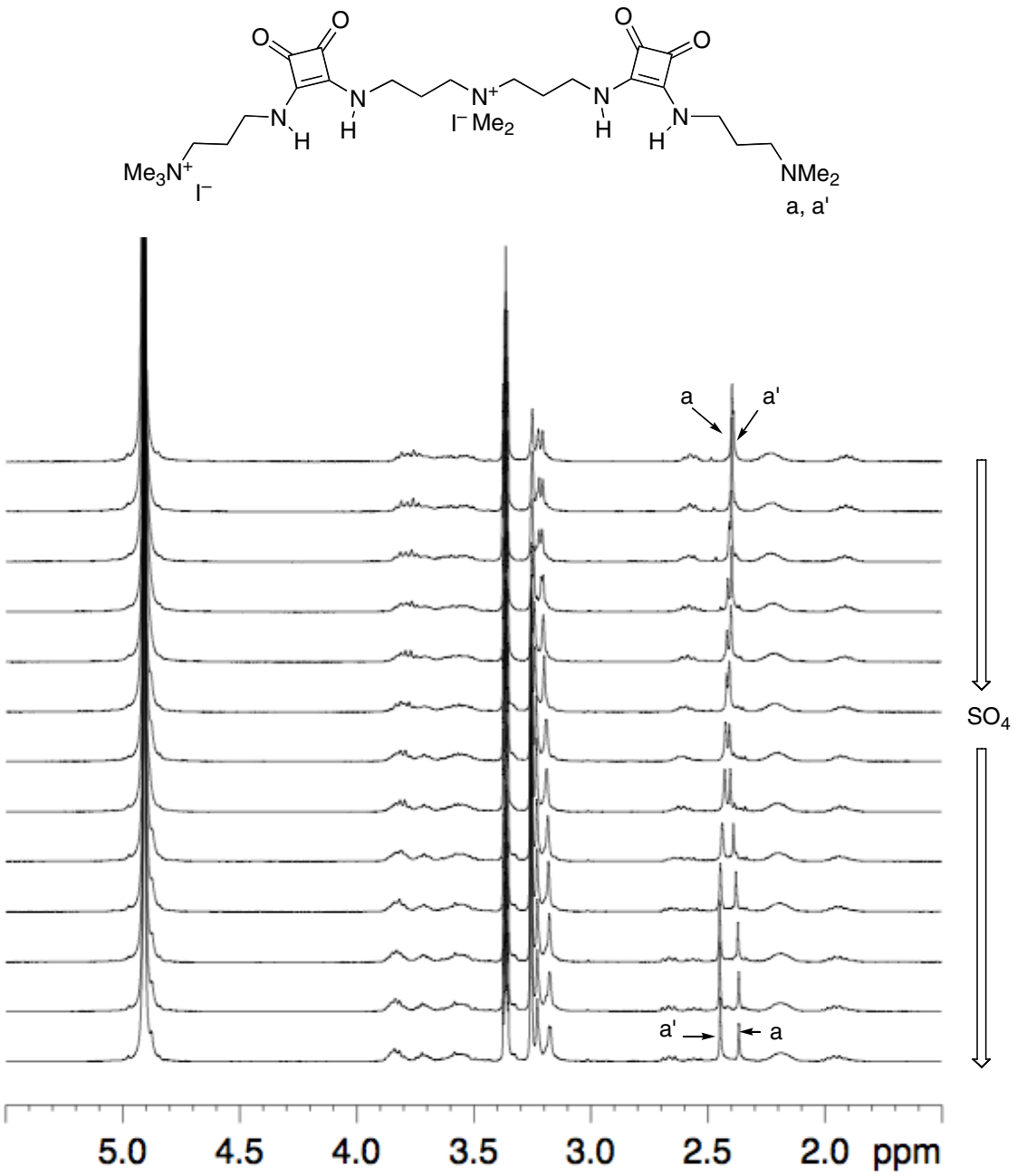


Fig. S2. ITC data of **1** (1.0×10^{-3} M) *vs* Erythrosin B (8.5×10^{-3} M) (left side), of Erythrosin B (5.0×10^{-4} M) *vs* **2** (7.5×10^{-3} M) (right side), data in 96%EtOH-H_2O (TRIS buffer, 10^{-2}M) at 293 K. See Table 1 for the corresponding thermodynamic parameters.

Changes in the ^{1}H NMR of 2 upon addition of $(TMA)_2SO_4$ in MeOH-d_4



[2] = 4.86×10^{-3} M in MeOH-d4

The graphical representation in **Fig. 2b** originated from a set of ^{1}H NMR spectra such as those shown here for host **2**.

The tetramethyl ammonium groups resonance of hosts **1** and **2** initially set at 3.24 ppm move 0.02 ppm upfield upon titration with tetramethyl ammonium sulphate addition. This is consistent with the involvement of these groups with the anion-binding event as is expected. However, they are not easily fallen through the titration experiment. Broadening of the signals together with the close proximity of other resonances make these groups useless for fitting purposes.

References:

1 All mono-squaramide esters exist as a mixture of *syn/anti* rotamers that are clearly observed in the ^{1}H NMR spectra. See, M. C. Rotger, M. Neus Piña, A. Frontera, G. Martorell, P. Ballester, P. M. Deyà, A. Costa, *J. Org. Chem.*, 2004, ***69***, 2302.

2 ITC data were obtained in a mixture of 96 % ethanol: water (9:1 v/v) thus, the actual percentage of water in this mixture is around 14 %.

N,N-dimethylpropylendiamine