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# Thermodynamic characterization of the squaramide–carboxylate interaction in squaramide receptors

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**Abstract**—The thermodynamic characterization of squaramides is described. This fundamental information shows that the association in chloroform or DMSO is mainly exothermic. In contrast, in MeOH the equilibrium is endothermic and is entropically driven. The data shows the influence of a squaramide ring alone, modified or combined with tetraalkylammonium groups as the binding subunit for molecular recognition. © 2001 Elsevier Science Ltd. All rights reserved.

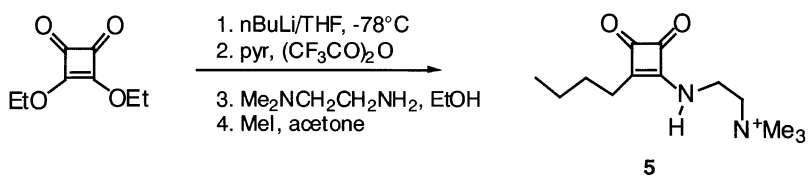
Squaramides are valuable compounds for molecular recognition due to a structurally rigid square-shaped framework featuring two hydrogen bond donors (NH) and two carbonyl acceptors (C=O). Squaramides have been successfully applied to the recognition of cationic ammonium salts,[1] carboxylates,[2] and for self-association.[3] In this regard, squaramides are especially effective for the recognition of carboxylates by forming strong 1:1 association complexes in polar solvents. In this role, they are similar to other well-known binding units for carboxylates, such as urea, thiourea and guanidium salts.[4] In connection with our ongoing interest in developing new receptors involving squaramides, we used isothermal titration calorimetry (ITC)[5] to quantify the thermodynamic parameters of the association between model squaramides and carboxylates. Two fundamental questions will be addressed. First, the thermodynamic origin of the binding energy and, second, the possibility of modulating this interaction by modifying the squaramide ring.

To this end, squaramides **1**–**4** and **6**–**8** were synthesized by sequential condensation procedures starting from the corresponding amines and diethyl squarate[6] followed by exhaustive methylation with methyl iodide when necessary.[7] Contrary to most disecondary squaramides **1** was soluble in chloroform probably due to the formation of internal hydrogen bonding that prevents its aggregation. Squaramide **5** was prepared by the addition of *n*-butyllithium followed by elimination as is outlined in Scheme 1.[8]

**CAUTION**: It is known that diethyl squarate can be an irritant agent for certain individuals. In fact, one member of our group suffered a strong allergic reaction. Consequently, this compound must be handled and disposed of with care in a well-ventilated hood.

Thermodynamic data were obtained by calorimetric titration of squaramides **1**–**6** and tetrabutylammonium *p*-nitrobenzoate (TBA, $p$-$NO_2Bzt^-$). Tetramethylammonium acetate (TMA, $AcO^-$) was used in one case for comparison.[9]

The results summarized in Table 1, show that in DMSO all equilibria are exothermic. The thermody-




**5**

**Scheme 1.**

*Keywords*: carboxylate receptors; isothermal titration calorimetry (ITC); squaramides.

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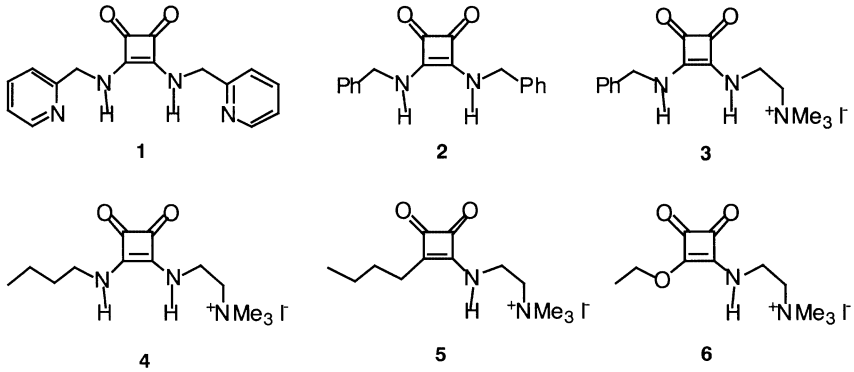
**Table 1.** Representative thermodynamic data obtained in DMSO from squaramides **1**–**6**[a]



| Comp. | Guest | $K_{ass}$ ($M^{-1}$) | $\Delta G$ (kcal $mol^{-1}$) | $\Delta H$ (kcal $mol^{-1}$) | $T\Delta S$ (kcal $mol^{-1}$) |
|---|---|---|---|---|---|
| **1**[b] | (TBA) pNBzt$^-$ | 780±120 | −3.9 | −2.7 | +1.2 |
| **2** | (TBA) pNBzt$^-$ | 160±5 | −2.9 | −2.0 | +0.9 |
| **3** | (TBA) pNBzt$^-$ | 877±41 | −4.0 | −2.1 | +1.9 |
| **4** | (TMA) AcO$^-$ | 14000±500 | −5.6 | −3.1 | +2.5 |
| **4** | (TBA) pNBzt$^-$ | 498±22 | −3.6 | −3.1 | +0.5 |
| **5** | (TBA) pNBzt$^-$ | 98±4 | −2.7 | −1.6 | +1.1 |
| **6** | (TBA) pNBzt$^-$ | 75±3 | −2.5 | −0.8 | +1.7 |

[a] $K_{ass}$, $\Delta H$ and $\Delta S$, were obtained at 294 K by curve fitting using Origin 5.0 software as implemented by MicroCal™.
[b] In chloroform.

namic analysis reveals that the association is enthalpically and entropically favored, with the former term predominating. On the other hand, when a tetraalkylammonium group is combined with the squaramide ring, as in squaramides **3** and **4**, the association strength increases in comparison with **2**. This effect is consistent with a cumulative favorable electrostatic interaction that is added to the squaramide hydrogen bond interaction.

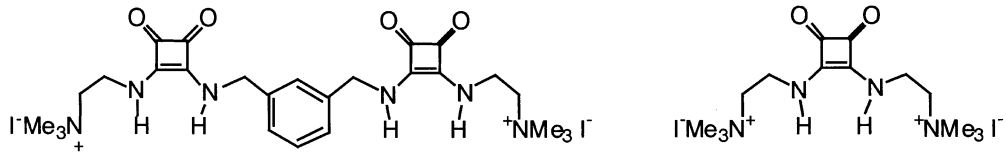
The modulating ability of the squaramide ring was examined from squaramides **4**–**6**. As can be seen in Table 1, squaramides **5** and **6** lacking a N–H hydrogen donor give $K_{ass}$ that are one order of magnitude lower in comparison with **4**, but still showing the correct stoichiometry of a 1:1 complex. This observation is very important in terms of modulation of the strength of binding. Moreover, the replacement of an attracting NH by a repulsive O atom can also be important in terms of selectivity. In this regard, it is worth noting to remember that the affinity of glycopeptide antibiotics for bacterial cell-wall precursor analogues terminating in D-alanine-D-lactate is reduced drastically compared to that terminating in D-alanine-D-alanine.[10] In the same way the NH to O replacement could be used for controlling the selectivity towards selected targets in squaramide based receptors.

The cumulative effect of combining one or two charged ammonium residues with one or two squaramide rings can be observed from the association of **7** and **8** in front of several dicarboxylates (Table 2). Thus, with tetramethylammonium isophtalate (TMA) iPht$^{2-}$ the association strength is raised significantly compared to the value obtained with **3** in front of (TBA) pNBzt$^-$, which would be the structurally closest monosquaramide complex. The data for the doubly charged bis-squaramides **7** and **8** allowed the estimation of the favorable effect due to the presence of an extra squaramide ring. Thus, compound **8**, featuring two tetraalkylammonium groups but only one squaramide ring, shows an association weaker than that of **7**. As above, in all these cases the association in DMSO was always enthalpically and entropically favored.

In general, the above observations can be accounted for through the formation of a bidentate hydrogen bond between the carboxylate and the squaramide NH system combined, in squaramides **3**–**8**, with electrostatic interactions. Both types of binding forces are essentially of enthalpic origin and their effects cannot be compensated by the entropy loss associated with the desolvation of a carboxylate which is poorly solvated in DMSO. This scenario could be acceptable if one assumes that the thermodynamic change associated with the exchange of tetraalkylammonium groups, as well as the cost of the conformational changes that take place during the association, have a minor influence on the magnitude of the thermodynamic parameters.

In order to gain insight into the association in more polar and protic media[11] squaramides **7** and **8** were titrated in MeOH in front of bis-triethylammonium oxalate (TEA, Oxl$^2$), and bis-triethylammonium squarate (TEA, Sq$^{2-}$). The results presented in Table 2 show that $K_{ass}$ values are still high in MeOH, but the thermodynamic origin of the binding is different. In this solvent, the association is always endothermic (Fig. 1) and the host–guest equilibria are driven under the influence of a strongly favorable entropic term.[12] As above, the stoichiometry parameter '$n$' was in all cases in the range 0.9–1.1 corresponding to the formation of

**Table 2.** Thermodynamic data obtained by ITC[a] from squaramides **7** and **8**



**7** **8**

| Comp. | Guest | Solvent | $K_{ass}$ ($M^{-1}$) | $\Delta G$ (kcal $mol^{-1}$) | $\Delta H$ (kcal $mol^{-1}$) | $T\Delta S$ (kcal $mol^{-1}$) |
|---|---|---|---|---|---|---|
| **7** | (TMA) $iPht^{2-}$ | DMSO | $75000 \pm 3000$ | $-6.5$ | $-4.0$ | $+2.5$ |
| **7**[b] | (TEA) $Oxl^{2-}$ | DMSO | $31000 \pm 4000$ | $-6.1$ | $-5.6$ | $+0.5$ |
| **8** | (TMA) $iPht^{2-}$ | DMSO | $15000 \pm 1000$ | $-5.7$ | $-2.3$ | $+3.4$ |
| **8** | (TEA) $Oxl^{2-}$ | DMSO | $19000 \pm 1000$ | $-5.7$ | $-4.7$ | $+1.0$ |
| **8** | (TEA) $Squ^{2-}$ | DMSO | $250000 \pm 20000$ | $-7.3$ | $-1.6$ | $+5.7$ |
| **7** | (TEA) $Oxl^{2-}$ | MeOH | $300000 \pm 10000$ | $-7.3$ | $+5.0$ | $+12.3$ |
| **7** | (TEA) $Squ^{2-}$ | MeOH | $84000 \pm 3000$ | $-6.6$ | $+4.1$ | $+10.7$ |
| **8** | (TEA) $Oxl^{2-}$ | MeOH | $39000 \pm 3000$ | $-6.2$ | $+4.5$ | $+10.7$ |
| **8** | (TEA) $Squ^{2-}$ | MeOH | $26000 \pm 11000$ | $-6.0$ | $+2.4$ | $+8.4$ |

[a] As in Table 1.
[b] In this particular case $n=1$, 2.

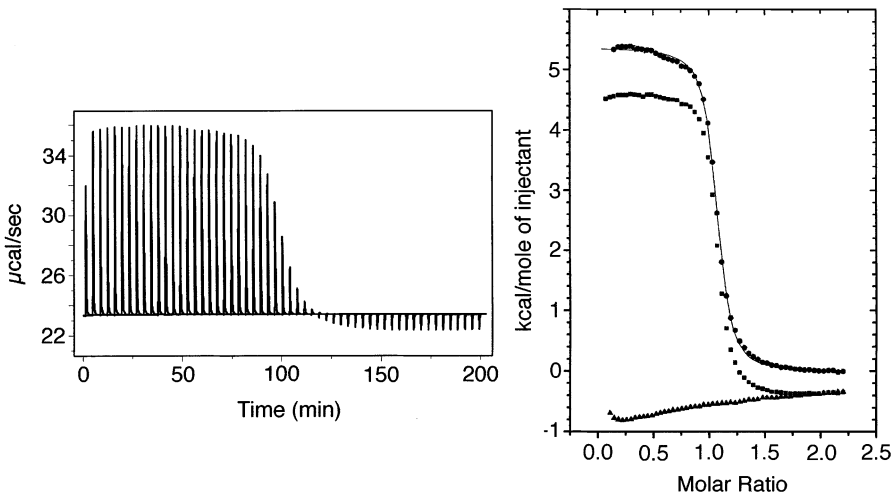



**Figure 1.** ITC calorimetric plot and enthalpogram for titration of bis-triethylammonium oxalate to **7** in MeOH. [**7**] $=1.26\times10^{-3}$ M; [TEA, $Oxl^{2-}$] $=1.23\times10^{-2}$ M. Raw data (■); heat of dilution (▲) and calculated difference (●).

1:1 complexes. In consequence the different thermodynamic behavior of the squaramides in MeOH compared to DMSO has to be assigned to a more active participation of the solvent in the binding event.

The above observations are significant for the design of receptors in polar protic solvents. In non interacting solvents, usually apolar solvents, the thermodynamic contribution of the solvent is comparatively small. In these cases, from a thermodynamic point of view, the design of receptors is mainly focused on electronic factors in order to increase specific host–guest interactions, and/or in geometric and structural factors such as preorganization, to minimize the entropic loss due to the association. In contrast, in MeOH the solvent experienced a reorganization[13] that implies the breakage of specific solvent interactions and the release of solvent molecules to the bulk. The increase of the disorder of the solvent upon host–guest association is due to the implication of highly solvated polar groups in the binding site. In other words, the proximity of the squaramide NH groups to the positive ammonium group of the receptor is a key structural feature, responsible for the effective binding of the squaramide-based receptors.

In summary, we have demonstrated that the binding strength of squaramides can be modulated by NH to O replacement. We have also demonstrated that the thermodynamic origin of the binding energy in chloroform and DMSO is different to that observed in MeOH. While in the former the association is based on specific host–guest interactions, in MeOH the reorganization of the solvent is the major factor. At this point, we have also demonstrated that squaramides alone or combined with tetraalkylammonium groups could serve as binding units for carboxylates and other anions forming part of a receptor.

## Acknowledgements

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7. Spectroscopic data of new compounds: **1**: mp 168–170°C; $^1$H NMR ($CDCl_3$): $\delta$ 8.49 (d, $J=2.7$ Hz, 2H), 7.70 (m, 2H), 7.38 (d, $J=7.8$ Hz, 2H), 7.22 (m, 2H), 4.87 (br s, 4H) ppm; FABMS (NOBA) $m/z$ (%): 295 ($MH^+$, 100). Anal. calcd for $C_{16}N_4O_2H_{14}$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.05; H, 4.77; N, 18.91. **3**: mp 256–257°C; $^1$H NMR (DMSO-$d_6$): $\delta$ 8.05 (s, NH), 7.43 (m, 5H), 4.81 (br s, 2H), 4.05 (m, 2H), 3.61 (t, $J=6.6$ Hz, 2H), 3.2 (s, 9H) ppm; FABMS (NOBA) $m/z$ (%): 288 ($M-I^+$, 100). Anal. calcd for $C_{16}N_3O_2H_{22}I$: C, 46.28; H, 5.34; N, 10.12. Found: C, 45.28; H, 5.23; N, 9.83. **4**: mp 209–210°C; $^1$H NMR (DMSO-$d_6$): $\delta$ 7.68 (br s, NH), 7.55 (br s, NH), 4.03 (d, $J=6.0$ Hz, 2H), 3.61 (t, $J=6.7$ Hz, 4H), 3.22 (s, 9H), 1.58 (m, 2H), 1.41 (m, 2H), 0.99 (t, $J=7.3$ Hz, 3H) ppm; FABMS (NOBA) $m/z$ (%): 254 ($M-I^+$, 100). HRMS-ESI$^+$, calcd for $C_{13}H_{24}N_3O_2$: 254.1869. Found 254.1857 (–4.5 ppm). **5**: mp 115–118°C; $^1$H NMR (MeOH-$d_4$): $\delta$ 8.92 (br s, NH), 4.36 (t, $J=7.0$ Hz, 2H), 3.97 (t, $J=7.1$ Hz, 2H), 3.55 (s, 9H), 2.80 (t, $J=7.5$ Hz, 2H), 1.85 (m, 2H), 1.59 (m, 2H), 1.14 (t, $J=7.3$ Hz, 3H) ppm; FABMS (NOBA) $m/z$ (%): 239 ($M-I^+$, 100). HRMS-ESI$^+$, calcd for $C_{13}H_{23}N_2O_2$: 239.1760. Found 239.1766 (2.7 ppm). **6**: mp 188–190°C; $^1$H NMR (DMSO-$d_6$): $\delta$ 9.03 (br s, NH), 8.85 (br s, NH), 4.77 (q, $J=7.0$ Hz, 2H), 4.02 (br s, 1H), 3.85 (br s, 1H), 3.62 (t, $J=6.4$ Hz, 2H), 3.21 (s, 9H), 1.48 (t, $J=7.1$ Hz, 3H) ppm. FABMS (NOBA) $m/z$ (%): 227 ($M-I^+$, 100). HRMS-ESI$^+$, calcd for $C_{11}H_{20}N_2O_3$: 228.1474, 228.1476 (0.9 ppm). **7**: mp 105–110°C; $^1$H NMR (DMSO-$d_6$): $\delta$ 8.11 (br s, NH), 7.49–7.35 (m, 6H), 4.82 (br s, 4H), 4.05 (br s, 4H), 3.62 (t, 4H), 3.22 (s, 18H) ppm; FABMS (NOBA) $m/z$ (%): 497 ($M-2I-H^+$, 10); 625 ($M-I^+$, 5). **8**: mp 280–281°C; $^1$H NMR (DMSO-$d_6$): $\delta$ 7.78 (br s, NH); 4.06 (d, $J=5.8$ Hz, 4H), 3.62 (t, $J=6.6$ Hz, 4H), 3.23 (s, 18H) ppm; FABMS (NOBA) $m/z$ (%): 283 ($M-2I-H^+$, 100); 411 ($M-I^+$, 86). Anal. calcd for $C_{14}N_4O_2H_{28}I_2$: C, 31.24; H, 5.24; N, 10.41. Found: C, 31.03; H, 5.25; N, 10.20. EM (FAB$^+$, matrix NOBA) $m/z$ (%): 283 ($M-2I-H^+$, 100); 411 ($M-I^+$, 86).
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