Thermodynamic Aspects of Dicarboxylate Recognition by Simple Artificial Receptors

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Received April 23, 2001

Recognition of dicarboxylates by bis-functional hydrogen-bonding receptors displays divergent thermodynamics in different solvent systems. NMR titration and isothermal titration calorimetry indicated that neutral bis-urea and bis-thiourea receptors form exothermic complexes with dicarboxylates in DMSO, with a near zero entropic contribution to binding. The increased binding strength of bis-guanidinium receptors precluded quantitative measurement of binding constants in DMSO, but titration calorimetry offered a qualitative picture of the association. Formation of these 1:1 complexes was also exothermic, but additional endothermic events occurred at both lower and higher host−guest ratios. These events indicated multiple binding equilibria but did not always occur at a discrete 2:1 or 1:2 host−guest molar ratio, suggesting higher aggregates. With increasing amounts of methanol as solvent, bis-guanidinium receptors form more endothermic complexes with dicarboxylates, with a favorable entropy of association. This switch from association driven by enthalpy to one driven by entropy may reflect a change from complexation involving the formation of hydrogen bonds to that promoted by solvent liberation from binding sites.

Introduction

One approach to understanding the controlled bimolecular association that is fundamental to most aspects of biological function relies on the design and synthesis of small-molecule receptors that are capable of selectively recognizing a substrate under physiological conditions. A traditional approach would position hydrogen-bonding, electrostatic, or hydrophobic functionality on a receptor complementary to the desired guest.[1] Often not considered, but of seminal importance, is the role that the solvent plays in this association. Solvent participation was explicitly neglected in the earliest small-molecule receptors binding in nonpolar organic solvents, but increases in importance as host−guest design progresses into more competitive solvents, such as water.[2]

An indication of solvent participation can be gleaned from the enthalpic and entropic contributions to association. The decrease in rotational and translational degrees of freedom that occurs with bimolecular assembly is an entropically unfavorable event, present in all receptor−substrate interactions. The favorable entropy often observed with hydrophobic binding is largely associated with the liberation of solvent from lipophilic surfaces, compensating for the immobilization of the substrates, although enthalpic effects can still be the driving force.[3] Enthalpic effects are balanced between solvation of the substrates, interactions within bulk solvent, and the attraction between host and guest. These effects have been documented in lipophilic cyclophane hosts,[3] but remain to be established with receptors that rely on hydrogen bonding.

An analysis of solvent effects on hydrogen-bonded complexes is complicated by the dissipation in binding affinity that occurs in highly polar solvents, such as alcohols or water. Competition with solvent for binding sites makes association more difficult. Any investigation must be able to quantify both the high binding affinity observed in less polar solvents and the low binding affinity observed in highly polar media. One approach is to create a series of synthetic receptors that provide the same presentation of hydrogen-bonding sites but differ vastly in their binding strength. In our continuing exploration of the recognition of biologically relevant substrates, we have designed simple artificial receptors that complex dicarboxylate derivatives.[4] This paper focuses on association in increasingly competitive solvents, from dimethyl sulfoxide (DMSO) to water, and attempts to illustrate the thermodynamic consequences

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(1) Several others have designed receptors capable of substrate recognition in water using electrostatic and hydrogen-bonding forces. Torneiro, M.; Still, W. C. *Tetrahedron* **1997**, *53*, 8739−8750. Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208−11209. Rotello, V. M.; Viani, E. A.; Deslongchamps, G.; Murray, B. A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 797−798.

(2) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678−680.

(3) Smithrud, D. B.; Wyman, T. B.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5420−5426.

(4) Fan, E.; VanArman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369.

10.1021/jo010413y CCC: $20.00 © 2001 American Chemical Society
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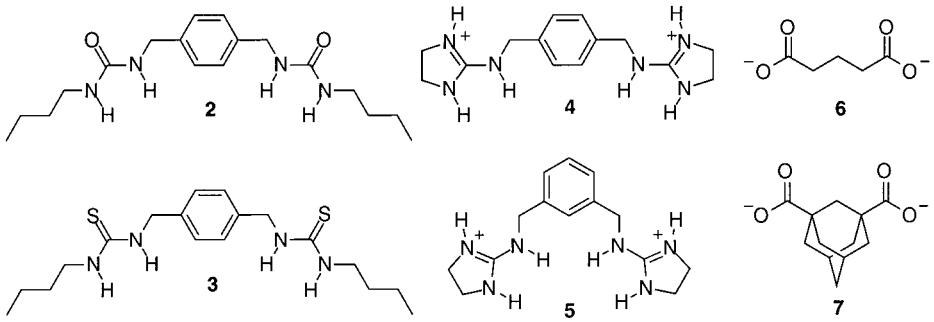
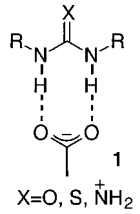
Chart 1



of binding in these various solvent systems. All receptors bind exothermically in DMSO, but become more endothermic with added methanol. In addition, calorimetric analysis demonstrated multiple binding equilibria which were not detectable using NMR titration.

Results and Discussion

Successful evaluation of the thermodynamic effects of solvent on dicarboxylate recognition requires a series of receptors that can form similar complexes but with varying binding strengths. This arises from the difficulty in the quantitative measurement of association for a single receptor in a variety of solvents, because the complexation is either too strong in less polar solvents or too weak in more polar solvents. Previous results from our group[4−6] and others[7−9] have shown that bidentate hydrogen-bonding groups are complementary to a variety of oxyanions.[10] This paper focuses on three binding moieties, ureas (**1**, X = O), thioureas (**1**, X = S), and guanidiniums (**1**, X = NH_2^+), that all show binding to



carboxylate, with the association strength increasing with hydrogen bond donor acidity.[4] Attachment of two of these bidentate binding groups onto a semirigid spacer unit creates synthetic receptors that can complex dicarboxylates via four hydrogen bonds. In this way, we have linked the hydrogen-bonding groups to a xylylene spacer to form bis-urea **2**, bis-thiourea **3**, and bis-guanidiniums **4** and **5** (see Chart 1). This series of artificial receptors permits an exploration of dicarboxylate recognition in competitive solvents such as DMSO, methanol, and water. Although these receptors successfully complex a variety of dicarboxylates, two representative substrates were chosen for this study: glutarate **6** and a more rigid analogue adamantane-1,3-dicarboxylate **7**. Both dicarboxylates were employed as their bis-tetrabutylammonium (TBA) salts. The fidelities of these ditopic interactions were supported by several techniques. Job's analysis verified the 1:1 stoichiometry for all three complexes.[11] Positioning within the host−guest complex was indicated by detection of intermolecular NOEs. For example, irradiation of the central aromatic protons of bis-guanidinium **4** in complex with both glutarate and 5-nitroisophthalate produced enhancement of the guest proton signals, as shown in Figure 1. Figure 1c shows the aromatic region of the NMR spectrum of the complex (Figure 1b) along with the difference spectrum produced from irradiation of the receptor aromatic signals (at right). Molecular modeling[12] indicated energetic minima with the anticipated positioning of each carboxylate interacting with one binding group.[13] Attempts to grow crystals suitable for X-ray analysis using these receptors produced only polymeric complexes with bidentate hydrogen bonding between binding group and carboxylate, similar to previous urea−carboxylate structures.[14] No crystals comprised of a distinct 1:1 complex were obtained. Further evidence for the presence of hydrogen bonding upon complexation was the large (>1 ppm) changes in the chemical shifts of both urea and thiourea NH protons with added guest; guanidinium NH protons were broadened under these conditions. NMR titration

(5) Linton, B. R.; Hamilton, A. D. *Tetrahedron* **1999**, *55*, 6027−6038.
(6) Salvatella, X.; Peczuh, M. W.; Gairi, M.; Jain, R. K.; Sanchez-Quesada, J.; deMendoza, J.; Hamilton, A. D.; Giralt, E. *Chem. Commun.* **2000**, 1399−1400.
(7) Sebo, L.; Schweizer, B.; Diederich, F. *Helv. Chim. Acta* **2000**, *83*, 80−92.
(8) Berger, M.; Schmidtchen, F. P. *J. Am. Chem. Soc.* **1999**, *121*, 9986−9993. Berger, M.; Schmitchen, F. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 2694−2695. Czekalla, M.; Stephan, H.; Habermann, B.; Trepte, J.; Gloe, K.; Schmidtchen, F. P. *Thermochim. Acta* **1998**, *313*, 137−144.
(9) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542−543. Anzenbacher, P., Jr.; Try, A. C.; Miyaji, H.; Jursikova, K.; Lynch, V. M.; Marquez, M.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 10268−10272. Sasaki, S.; Mizuno, M.; Naemura, K.; Tobe, Y. *J. Org. Chem.* **2000**, *65*, 275−283. Snellink-Ruel, B. H. M.; Antonisse, M. M. G.; Engbersen, J. F. J.; Timmerman, P.; Reinhoudt, D. N. *Eur. J. Org. Chem.* **2000**, *1*, 165−170. Xie, H.; Yi, S.; Yang, X.; Wu, S. *New J. Chem.* **1999**, *23*, 1105−1110. Davis, A. P.; Lawless, L. J. *Chem. Commun.* **1999**, 9−10. Metzger, A.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1998**, *37*, 649−651. Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072. Gross, R.; Durner, G.; Gobel, M. G. *Liebigs Ann. Chem.* **1994**, 49−54. Hamann, B. C.; Branda, N. R.; Rebek, J., Jr. *Tetrahedron Lett.* **1993**, *34*, 6837−6840. Smith, P. J.; Reddington, M. V.; Wilcox, C. S. *Tetrahedron Lett.* **1992**, *33*, 6085−6088.
(10) For comprehensive reviews of anion recognition, see the following: Bianchi, A.; Bowman-James, K.; Garcia-España, E. *Supramolecular Chemistry of Anions*; Wiley-VCH: New York, 1997. Schmitchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609−1646. Seel, C.; Galán, A.; deMendoza, J. *Top. Curr. Chem.* **1995**, *175*, 101−132. Snowden, T. S.; Anslyn, E. V. *Curr. Opin. Chem. Biol.* **1999**, *3*, 740−746. Beer, P. D.; Schmitt, P. *Curr. Opin. Chem. Biol.* **1997**, *1*, 475−482.
(11) Connors, K. A. *Binding Constants*; John Wiley and Sons: New York, 1987.
(12) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467. Molecular modeling was carried out using the Amber force field.
(13) Mitchell, J. B. O.; Thornton, J. M.; Singh, J. *J. Mol. Biol.* **1992**, *226*, 251−262. This provides a survey of guanidinum−carboxylate complexes through calculation and a survey of protein crystal structures.
(14) Zafar, A.; Geib, S. J.; Hamuro, Y.; Hamilton, A. D. *New J. Chem.* **1998**, *22*, 137−141.

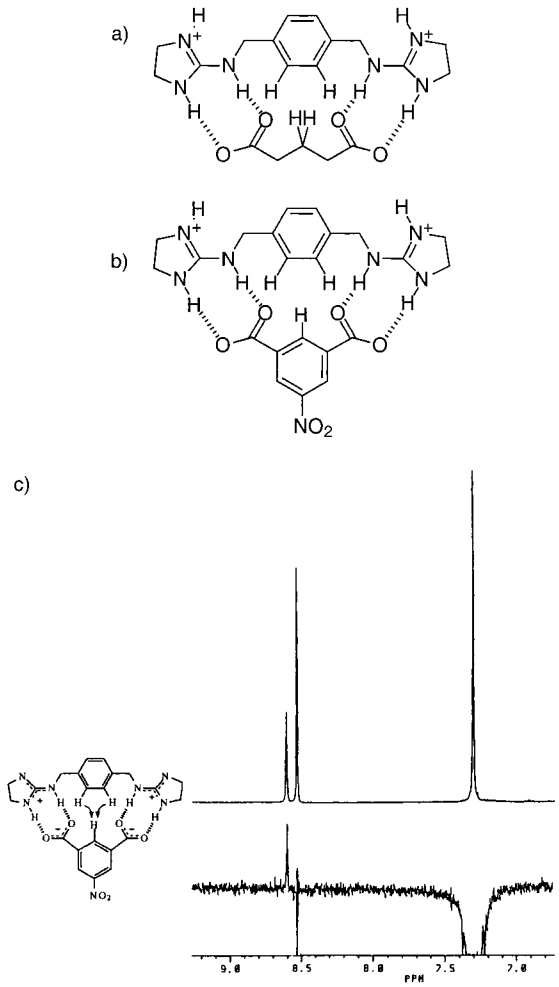


Figure 1. Irradiation of the central aromatic protons of **4** produced NOEs with the indicated protons of (a) glutarate and (b) 5-nitroisophthalate. (c) NOE difference spectrum for the complex shown in (b).

analysis also showed shifts in the benzylic methylene protons of all three receptors upon addition of glutarate, as well as shifts in the methylene protons of glutarate during reverse titrations. These results suggest the formation of chelate complexes, such as those shown in Figure 1, where each binding unit forms hydrogen bonds with the respective carboxylate.

Association with Dicarboxylates. Two techniques were employed to gauge the thermodynamics of dicarboxylate recognition: nuclear magnetic resonance titration and isothermal titration calorimetry (ITC). NMR titration follows the changes in chemical shift that occur in several signals upon complexation: a process repeated at a variety of temperatures to complete a van't Hoff analysis. ITC measures the heat evolved or absorbed from the controlled mixing of host and guest over a series of injections. The resulting binding isotherm was analyzed using a single-site binding model to determine binding affinity, association enthalpy, and stoichiometry from a single ITC experiment.[15] While widely applied to biological complexation,[16] calorimetry has only seen recent exploitation in the evaluation of weakly binding small-molecule systems.[3,6−8,17,18]

(15) Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131−137.

Table 1. Association Constants for Dicarboxylates in D_2O/MeOD Mixtures[a]

host	guest	10% D_2O	20% D_2O	25% D_2O	50% D_2O	75% D_2O
4	**6**	2800	850	720	230	
	7	3300	1300	780	300	130
5	**6**	3100	1100	840	290	
	7	5000	1500	1160	440	320

[a] Measured by NMR titration at 20 °C. K_a are listed in M^{-1}. Errors in K_a <10%.

NMR titration was utilized to determine association constants for the receptors **2**−**5** with glutarate in DMSO. As observed for monotopic receptors, binding was lowest for bis-urea **2** ($K_a = 640\ M^{-1}$) and increased significantly for bis-thiourea **3** ($K_a = 10\ 000\ M^{-1}$).[4] Association between glutarate and bis-guanidiniums **4** and **5** was too strong to be measured accurately under the conditions of the NMR titration in DMSO ($K_a > 50\ 000\ M^{-1}$), but addition of more competitive solvents reduced the association strength to a measurable level due to favorable solvation of both host and guest. In an effort to gauge the effect of solvent composition on association, binding titrations were performed in a variety of aqueous methanol mixtures, and the association constants for both bis-guanidiniums binding to both dicarboxylates are shown in Table 1. Suprisingly, little selectivity is observed between either receptor or either dicarboxylate, with both bis-guanidiniums being flexible enough to match the spacing of the dicarboxylates. The slightly tighter binding of the more rigid adamantane-dicarboxylate is probably a reflection of its increased preorganization The decline in association strength with added water reinforces the presumption that a more strongly hydrogen bond donating solvent will reduce binding affinity through increased solvation of both host and guest, as compared to methanol. Association was still observed, however, in high percentages of water suggesting that recognition in aqueous media can be achieved with the accumulation of multiple charged hydrogen-bonding groups.

Thermodynamics of Dicarboxylate Association. The thermodynamic contributions to dicarboxylate binding for all four receptors (**2**−**5**) were investigated by both van't Hoff analysis of NMR titrations as well as ITC. NMR titration data from 10 to 50 °C are shown in Table 2, in two solvent systems. The data for bis-urea **8** and bis-thiourea **9** were determined using d_6-DMSO as the solvent, but due to the increased strength of the bis-guanidinium-dicarboxylate association, data for derivatives **4** and **5** were determined in 25% D_2O/MeOD. Van't Hoff analysis of the variable temperature data permitted determination of the enthalpic and entropic contributions

(16) Breslauer, K. J.; Freire, E.; Straume, M. *Methods Enzymol.* **1992**, *211*, 533−567. Freire, E.; Mayorga, O. L.; Straume, M. *Anal. Chem.* **1990**, *62*, 950A−959A. Kamiya, M.; Torigoe, H.; Shindo, H.; Sarai, A. *J. Am. Chem. Soc.* **1996**, *118*, 4532−4538. Berger, C.; Jelesarov, I.; Bosshard, H. R. *Biochemistry* **1996**, *35*, 14984−14991. Livingstone, J. *Nature* **1996**, *384*, 491−492. Ladbury, J. E. *Structure* **1995**, *3*, 635−639. Ladbury, J. E.; Wright, J. G.; Sturtevant, J. M.; Sigler, P. B. *J. Mol. Biol.* **1994**, *238*, 669−681. Cooper, A.; McAlpine, A.; Stockley, P. G. *FEBS Lett.* **1994**, *348*, 41−45. Ream, J. E.; Yuen, H. K.; Frazier, R. B.; Sikorski, J. A. *Biochemistry* **1992**, *31*, 5528−5534.

(17) Arena, G.; Casnati, A.; Contino, A.; Gulino, F. G.; Sciotto, D.; Ungaro, R. *J. Chem. Soc., Perkin Trans. 2* **2000**, 419−423.

(18) Hünenberger, P. H.; Granwehr, J. K.; Aebischer, J.-N.; Ghoneim, N.; Haselbach, E.; vanGunsteren, W. F. *J. Am. Chem. Soc.* **1997**, *119*, 7533−7544. Zimmerman, S. C.; Kwan, W.-S. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2404−2406. Inoue, Y.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 10637−10644. Cromwell, W. C.; Bystrom, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326−332.

Table 2. Association Constants at Various Temperatures[a]

host	guest	10 °C	20 °C	30 °C	40 °C	50 °C	ΔH	ΔS
				d_6-DMSO				
2	**6**		790	620	510	420	−4.0	+0.3
	7		1950	1560	1150	910	−4.9	−1.5
3	**6**		11 000	8500	6300	5200	−4.8	+2.0
	7		22 000	19 800	16 100	12 900	−3.4	+8.3
				25% D_2O/MeOD				
4	**6**	600	720		1010	1430	+3.8	+26
	7	580	780		1550	2250	+6.2	+34
5	**6**	720	840		1070	1280	+2.5	+22
	7	1000	1160		1570	2200	+3.5	+26

[a] Measured by NMR titration with bis-TBA salts of listed dicarboxylates. Errors in K_a <10%. K_a are listed in M^{-1}, ΔH are listed in kcal mol^{-1}, and ΔS are listed in cal mol^{-1} K^{-1}.

to the association of all four receptors employed above, which are also shown in Table 2.

A dichotomy became evident between the association behavior of neutral receptors **2** and **3** in DMSO and the behavior of bis-guanidinium receptors **4** and **5** in water/methanol. The binding constants for the bis-urea systems decreased as temperature increased, as may be expected from the additional rotational and translational energy imparted to the complex at elevated temperatures. On the other hand, the bis-guanidinium complexes exhibited stronger binding as temperature increased, an effect consistent for all bis-guanidinium−dicarboxylate systems investigated. As suggested by the differing temperature effects, the thermodynamic data for complexation by bis-ureas and bis-thioureas were quite different from those of bis-guanidiniums. Association by bis-urea and bis-thiourea receptors was characterized by negative enthalpies and near zero entropies of association. The bis-guanidinium complexes demonstrated positive enthalpies as well as large positive entropies of association.[19]

Similar association and thermodynamic results were obtained using calorimetry, employing DMSO and methanol. These binding curves can be seen in Figure 2, and the association data obtained from a one-site model are listed in Table 3. The thermodynamic distinction between methanol and DMSO was again obvious from the positive or negative nature of the heat event at the beginning of each titration. Heat absorption is reflected as a positive ΔH while a negative ΔH indicates heat being produced upon association. Complexes with neutral receptors demonstrated exothermic association while guanidinium association in methanol is endothermic. This latter case is accompanied by a significantly favorable entropy of association.

Several suppositions can be drawn from our investigations of the thermodynamics of association. The negative enthalpy observed in the uncharged bis-urea and bis-thiourea receptors suggests that the complexes are stabilized by strong hydrogen bonding between host and guest. While DMSO can engage in hydrogen bonding itself, the exothermic nature of the association indicates that the hydrogen bonds that are formed upon host−guest complexation are stronger than those initially formed to solvent (DMSO). The association is also facilitated by a near zero entropy contribution, suggesting that the energy gained by the release of solvent from the binding sites is nearly equivalent to the energy lost through bimolecular association and the freezing of bond rotations that occur upon formation of the complex.

(19) For earlier examples of entropically driven complexation see refs 5−8.

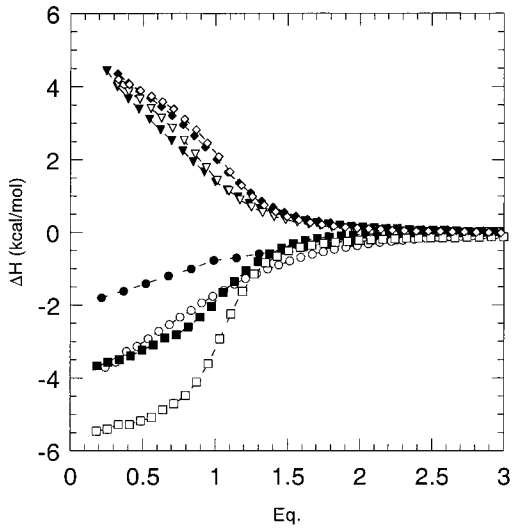


Figure 2. ITC binding curves for dicarboxylate complexes at 25 °C: ● = **2**/**6**; ○ = **2**/**7**; ■ = **3**/**6**; □ = **3**/**7**; ▼ = **4**/**6**; ▽ = **4**/**7**; ◆ = **5**/**6**; ◇ = **5**/**7**.

Table 3. Association Data for Dicarboxylates via Titration Calorimetry[a]

receptor	guest	K_a	ΔH	ΔS
		DMSO		
2	**6**	1300	−2.5	+5.9
	7	2000	−4.5	+0.1
3	**6**	8400	−4.1	+4.3
	7	15 000	−5.9	−0.6
		MeOH		
4	**6**	2700	+3.7	+28
	7	9500	+4.0	+32
5	**6**	7500	+4.0	+31
	7	12 100	+4.4	+34

[a] Measured by ITC at 25 °C. Errors in K_a <10%. ΔH are listed in kcal mol^{-1} and ΔS are listed in cal mol^{-1} K^{-1}.

This situation is reversed in complexes with bis-guanidiniums in more competitive solvents. The positive enthalpy term indicates that the hydrogen bonds formed between host and guest are weaker than the sum of the hydrogen bonds needed to be broken for bimolecular association. Presumably, these are comprised of solvent interactions with the binding sites of both host and guest. This association is accomplished due to the entropic gain that results from the liberation of solvent molecules from both host and guest.

The above analysis is complicated by the changes in both receptor and solvent. A direct comparison of all receptors in the same solvent would be beneficial but is difficult due to the different association strengths of the various receptors. Association with bis-guanidiniums is too strong to measure in DMSO, and neutral receptors form weak complexes in aqueous methanol mixtures. A qualitative description, however, can be gathered using titration calorimetry.

ITC analysis of the addition of glutarate **6** to bis-thiourea receptor **3** in methanol indicated extremely weak ($K_a \cong 30$ M^{-1}), exothermic ($\Delta H \cong -1.0$ kcal mol^{-1}) association. Similar analysis with adamantane-dicarboxylate produced a flat curve with no heat being absorbed or evolved as a result of association. This can indicate that there is either no association or binding that

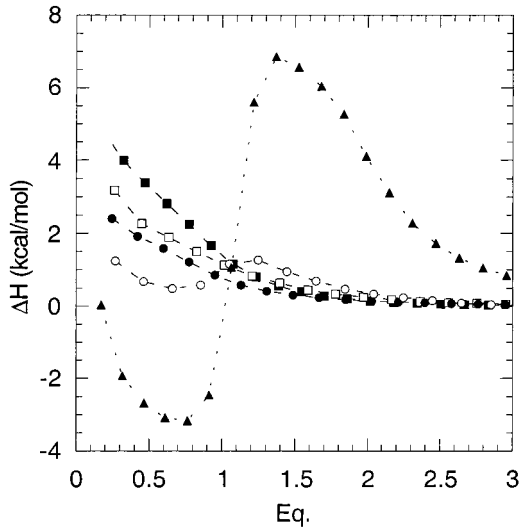


Figure 3. ITC titrations for **4** and **6** in methanol−DMSO mixtures: ■ = 100% MeOH; □ = 50% MeOH; ● = 30% MeOH; ○ = 10% MeOH; ▲ = 100% DMSO.

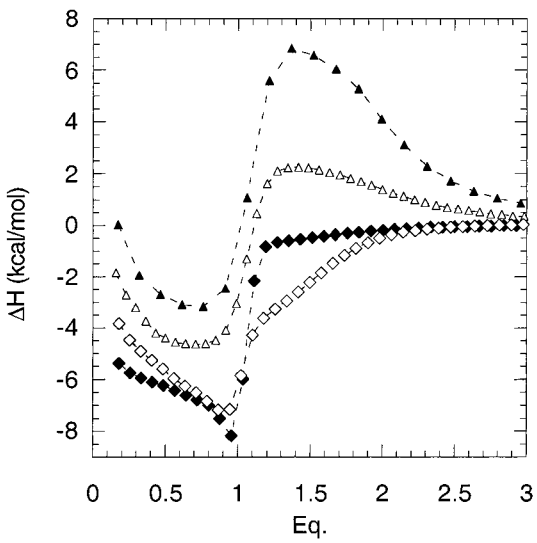


Figure 4. Comparison of bis-guanidinium−dicarboxylate interactions in DMSO: ▲ = **4**/**6**; △ = **4**/**7**; ◆ = **5**/**6**; ◇ = **5**/**7**.

is enthalpically neutral. It seems unlikely that the association between the bis-thiourea **3** and adamantane-dicarboxylate **7** is weaker than association to glutarate, since the adamantane derivative shows slightly stronger association in every previous case. Presumably, binding exists but is enthalpically neutral, producing the same flat curve as no binding. Any association must be promoted by entropic effects, presumably solvent liberation. No signs of association were observed in the ITC titration between bis-urea **2** and dicarboxylates in methanol.

Even though the association of bis-guanidiniums **4** and **5** to dicarboxylates in DMSO was too strong to be measured accurately, the calorimetric binding curves can provide a qualitative analysis of these interactions (Figure 3). Earlier NMR titrations indicated that solvents of different polarity changed not only the binding affinity but also the thermodynamic character of the association. Calorimetric titration also indicates the presence of other types of complexation. While the binding curve obtained in methanol (■, Figure 3) displays only endothermic 1:1 association, the same experiment in DMSO (▲, Figure 3) displays an exothermic transition at 1 equiv (1:1 complex) with additional endothermic events occurring at both low and high concentrations. Presumably, these are due to multiple binding equilibria such as the 2:1 and 1:2 complexes, but the absence of transitions at distinct molar equivalents (see below) suggests nondiscrete aggregation rather than formation of a distinct complex.

Titrations performed in mixtures of methanol/DMSO illustrated the change in the thermodynamics of association that occurred with differing solvent composition. Titrations performed in 50 (□, Figure 3) and 30% methanol (●, Figure 3) showed a reduction in the heat absorbed, suggesting either reduced binding affinity or association that is less endothermic. Since greater amounts of DMSO result in increased binding affinity, these curves must indicate a reduction in the endothermic nature of the association. At a mixture of 10% methanol/90% DMSO (○, Figure 3), multiple binding equilibria are again observed. In this experiment, the 1:1 association is exothermic, as indicated by the negative slope of the binding curve at 1 equiv. It is unclear if the additional binding events observed in DMSO become too weak to be seen at higher methanol concentrations or if higher order associations become enthalpically neutral. Curve fitting is not thought to be reliable with the possibilities of multiple binding equilibria, so only a qualitative picture is possible. It is apparent that the 1:1 association changes from endothermic to exothermic as the solvent polarity decreases with additional DMSO.

Calorimetric titrations were performed for the other bis-guanidinum−dicarboxylate combinations as well, and all four binding curves are shown in Figure 4. None of these titrations resembled the simple 1:1 stoichiometry observed for the neutral receptors above but rather displayed various multiple binding equilibria that were present throughout the titration. Central to all four titrations was the strong, exothermic 1:1 association discernible from the negative signal immediately before the transition at 1 equiv. A second binding event was visible from the portion of the curve directly after 1 equiv. Presumably, this was a result of 1:2 complexation and other analogous aggregates, but since the saturation did not show a transition at 2 equiv, it was impossible to assign this interaction to a discrete 1:2 complex.[20] This higher order aggregation appears to be endothermic for complexes with glutarate and exothermic for complexes with adamantane-dicarboxylate. Early in all four titrations, an additional contribution from 2:1 complexation or similar aggregates could be seen. The prevalence of multiple binding equilibria as well of the lack of calorimetric events at distinct integral equivalents precluded accurate curve fitting.

The multiple binding equilibria that are observed in the calorimetric titrations in DMSO suggest that a similar event occurs with all dicarboxylate recognition, even if not experimentally observed. Complexation in methanol showed reduced 1:1 binding affinity, so it follows that the 1:2 complexation is also reduced to a point where none is visible. Calorimetry is more suited to observe these multiple binding equilibria due to the distinct nature of the alternate complexes. Each complex has a distinct enthalpy of association. If the enthalpies

(20) A discrete 1:2 complex is observed in the titration of TBA acetate with either **4** or **5**.

are coincident, then the two events are indistinguishable, as was observed in the titration of TBA acetate and receptors **4** and **5** (data not shown). If the association enthalpies are different, as in Figure 4, then the two events are each readily observed. This is less likely in the NMR analysis where changes in chemical shifts are monitored. When a 1:1 complex is formed, various protons display a shift. Complexation of the second equivalent, however, often produces little change, as the chemical shifts in the singly bound and doubly bound complexes are similar. This similarity of the NMR chemical shifts of sequential complexes makes distinguishing multiple binding equilibria difficult in the NMR titration of dicarboxylates. The calorimetric analysis also points out that a curve fit analysis, involving receptors that contain multiple binding sites, may fail to include all binding events and is likely to be responsible for differences between ITC and NMR data.

These thermodynamic results are best viewed in concert with other thermodynamic data involving hydrogen-bonding receptors, where endothermic association has been observed for complexes with oxyanions. A bis-amidinium receptor formed endothermic complexes with glutarate and isophthalate in methanol.[7] Similar endothermic binding was observed between a zwitterionic bis-guanidinium and sulfate in both DMSO and in methanol.[8] As in the current study, association was more endothermic in methanol than DMSO. Peptide recognition by a tetra-guanidinium receptor in 10% aqueous methanol showed thermodynamic dependency on side chain identity.[6] Recognition via four aspartates was exothermic, while that through the binding of four glutamates was endothermic, and in the intermediate case of two aspartates and two glutarates, association was enthalpically neutral. Since the nature of the guanidinium−carboxylate interaction should be similar in each case, this enthalpic difference must be from the differing degree of solvation around the longer amino acid side chains.

All of these studies illustrate that solvent participation is an integral facet of association, as the thermodynamic results are a consequence of not only the receptor but also the chosen solvent. These effects appear to represent a delicate balance between enthalpic and entropic forces present in association in polar solvents. In the current study, there is a trend for association to become more endothermic, and therefore more entropically driven, as the solvent conditions change from DMSO to methanol. A similar situation was observed for the complexation of sulfate, though conditions where association becomes exothermic were not explored.[8] The generality of these thermodynamic effects with other hydrogen-bonding receptors and the ability to design receptors to take advantage of these effects remain to be seen.

Conclusions

Dicarboxylate recognition has been demonstrated by a variety of receptors that rely on the presentation of two bidentate binding groups around a central spacer. While previous results have shown that this complexation can be accomplished in polar organic solvents such as DMSO, this work also demonstrates the effectiveness of these receptors in polar protic solvents such as methanol and water. As the polarity and hydrogen-bonding ability of the solvent increases, binding diminishes.

Complementary analysis using NMR titration and ITC illustrates that the thermodynamics of association are also dependent on solvent. In less polar solvents such as DMSO, complex formation is enthalpically driven. In methanol and methanol/water mixtures, association becomes endothermic, with favorable entropy providing the associative force. This is thought to indicate a change from association that is promoted primarily by hydrogen bond formation to association that is driven by solvent liberation. Qualitative ITC indicates the presence of multiple binding equilibria in complexes of bis-guanidinums and dicarboxylates. The enthalpic nature of these secondary complexes changes with dicarboxylate. These results and others demonstrate the subtle balance between enthalpic and entropic forces necessary for host−guest complexation.

Experimental Section

General Methods. THF was obtained from Fisher and distilled from sodium benzophenone ketyl. All other reagents, unless otherwise noted, were obtained from the Aldrich Chemical Co. and used without further purification.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300 MHz). NMR chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were determined at the Department of Chemistry, University of Pittsburgh. EI and FAB mass spectra (MS) were obtained using a Varian MAT CH-5 or VG 7070 mass spectrometer under the direction of Dr. Kasi V. Somayajula. Melting points were determined using an electrothermal capillary melting point apparatus and are uncorrected. Elemental analysis was carried out by Atlantic Microlab, Inc., Norcross, GA.

Synthesis. 1,4-Bis[*N*,*N*′-(butylaminocarbonyl)aminomethyl]benzene (2). *p*-Xylyenediamine (1.00 g, 7.34 mmol) was dissolved in 50 mL of absolute THF. Butyl isocyanate (1.80 g, 18.16 mmol) was added, and the solution was stirred for 8 h. The reaction mixture was added to 250 mL of 10% HCl. The resulting precipitate was collected by filtration and washed with several volumes of methanol. The white solid (2.00 g, 79%) was dried under high vacuum: mp 210 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15 (s, 4H, Ar), 6.18 (br, 2H, NH), 5.84 (br, 2H), 4.14 (d, J = 3.8 Hz, 4H), 3.30 (br, 4H), 1.29 (m, 8H), 0.86 (t, J = 7.1 Hz, 6H); MS (EI) *m*/*e* calcd for $C_{14}H_{19}N_3O_2$ 261.1477, found 261.1472, 334 (1), 261 (34), 235 (8), 218 (25), 206 (5), 161 (23), 146 (21), 135 (7), 119 (100), 106 (44). Anal. Calcd for $C_{18}H_{30}N_4O_2$: C, 64.64; H, 9.04; N, 16.75. Found: C, 64.76; H, 8.97; N, 16.64.

1,4-Bis[*N*,*N*′-(butylaminothiocarbonyl)aminomethyl]benzene (3). *p*-Xylyenediamine (1.00 g, 7.34 mmol) was dissolved in 50 mL of absolute THF. Butyl isothiocyanate (2.10 g, 18.26 mmol) was added, and the solution was stirred for 8 h. The reaction mixture was added to 250 mL of 10% HCl. The resulting precipitate was collected by filtration and washed with several volumes of methanol. The white solid (2.10 g, 78%) was dried under high vacuum: mp 185.5−186 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (br, 2H), 7.46 (br, 2H), 7.22 (s, 4H), 4.62 (br, 4H), 3.38 (br, 4H), 1.44 (m, 4H), 1.27 (m, 4H), 0.87 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.4 (s), 138.0 (s), 127.2 (d), 46.7 (t), 43.4 (t), 30.9 (t), 19.6 (t), 13.8 (t); MS (EI) *m*/*e* calcd for $C_{14}H_{19}N_3S_2$ ($M^+ - NH_2Bu$): 293.1020, found 293.1013, 366 (1), 293 (59), 251 (33), 235 (22), 193 (11), 177 (18), 162 (48), 145 (8), 136 (23), 120 (74), 104 (100), 91 (53), 72 (53), 57 (31). Anal. Calcd for $C_{18}H_{30}N_4S_2$: C, 58.98; H, 8.25; N, 15.28. Found: C, 59.07; H, 8.22; N, 15.18.

***N*,*N*′-Bis(3,4-dihydro-1*H*-imidazol-2-yl)-1,4-bis(aminomethyl)benzene Diiodide (4).** *p*-Xylylenediamine (2.72 g, 20.0 mmol) and 2-methylmercapto-4,5-dihydroimidazole hydroiodide (9.76 g, 40.0 mmol) were dissolved in 25 mL of methanol. A condenser, equipped with a NaOH trap at the top (to catch evolving MeSH), was attached. The solution was

refluxed for 4 h and reduced to minimal volume under reduced pressure. Ethyl ether (50 mL) was added, producing a white precipitate. This was recrystallized in hot methanol yielding 5.84 g (55%) of pale yellow crystals: mp >260 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.7−7.8 (br, 6H), 7.31 (s, 4H), 4.38 (s, 4H), 3.60 (s, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.2, 136.4, 127.6, 45.2, 42.6. Anal. Calcd for: C, 31.84; H, 4.20; N, 15.91. Found: C, 31.87; H, 4.22; N, 15.

***N,N′*-Bis(3,4-dihydro-1*H*-imidazol-2-yl)-1,3-bis(aminomethyl)benzene Diiodide (5).** A solution of 2-methylmercapto-4,5-dihydroimidazole hydroiodide (7.17 g, 29.4 mmol) was prepared in methanol (100 mL), with a condenser leading to an aqueous NaOH trap. To this was added *m*-xylylene diamine (2.0 g, 14.7 mmol), and the solution was refluxed for 4 h. The volume of the solution was reduced to 10 mL, and the solution was cooled, at which point a white solid precipitated. Addition of diethyl ether to the remaining solution produced more precipitation. Both precipitates were filtered, combined, and recrystallized from hot methanol. White crystals (5.45 g, 70%) remained: mp 235−236 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (br, 2H, NH), 8.8−7.6 (br, 4H, NH), 7.40 (t, J = 7.3 Hz, 1H, Ar), 7.26 (s, 1H, Ar), 7.22 (d, J = 4.8 Hz, 2H, Ar), 4.38 (s, 4H, xylylenyl-CH_2), 3.61 (s, 8H, imidazoline-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.2 (s), 137.4 (s), 128.9 (d), 126.5 (d), 126.0 (d), 45.3 (t), 42.6 (t). Anal. Calcd for $C_{14}H_{22}I_2N_6$: C, 31.84; H, 4.20; N, 15.91. Found: C, 31.86; H, 4.25; N, 15.84.

Bis(tetrabutylammonium) Glutarate. Glutaric acid (1.0 g, 7.57 mmol) was dissolved in 10 mL of water. Tetrabutylammonium hydroxide (40% in water) was added until the pH = 8. Removal of solvent by lyophilization left a clear paste which solidified into a white solid (4.85 g, 96%) in several days under high vacuum: mp 78−79 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.17 (m, 16H), 1.67 (m, 4H), 1.55 (m, 18H), 1.29 (m, 16H), 0.91 (t, J = 7.2 Hz, 24H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.7, 64.6, 57.5, 24.6, 23.1, 19.2, 13.5. Anal. Calcd for $C_{37}H_{78}N_2O_4\cdot 3H_2O$: C, 66.42; H, 12.65; N, 4.19. Found: C, 66.18; H, 12.52; N, 4.14.

Bis(tetrabutylammonium) 1,3-Adamantanedicarboxylate. 1,3-Adamantanedicarboxylic acid (157.4 mg, 0.70 mmol) was dissolved in a minimum volume of methanol and diluted with 4 mL of water. Tetrabutylammonium hydroxide (40% in water) was added until the pH = 8. Some sonication was necessary to completely solubilize the solution. After neutralization was complete, the clear solution was concentrated to dryness by lyophilization leaving a clear paste. A white powder formed after several days under high vacuum (1.05 g, 99%): mp 91−92 °C; ^{1}H NMR (300 MHz, methanol-d_4) δ 3.21 (m, 16H, CH_2N), 2.03 (s, 2H, CH), 1.89 (s, 2H, O_2C−C−CH_2−C−CO_2), 1.78 (s, 8H, 4-CH_2), 1.63 (br, 18H), 1.40 (m, 16H, CH_2-CH_3), 1.00 (t, J = 7.2 Hz, 24H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.0 (s, CO_2), 57.5 (t), 43.8 (t), 41.4 (s), 40.5 (t), 37.2 (t), 29.4 (d), 23.1 (t), 19.2 (t), 13.5 (q). Anal. Calcd for $C_{44}H_{86}N_2O_4\cdot 4H_2O$: C, 67.82; H, 12.16; N, 3.59. Found: C, 67.84; H, 12.12; N, 3.58.

1,3,4,6,7,8-Hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine Tetraphenylborate (8). 1,3,4,6,7,8-Hexahydro-2*H*-pyrimido-[1,2-*a*]pyrimidine (0.10 g, 0.542 mmol) was dissolved in 10 mL of dilute aqueous hydrochloric acid. A solution of tetraphenylboron sodium (0.20 g, 0.584 mmol) in 10 mL of water was added resulting in the immediate formation of a white precipitate. This solid (0.14 g, 55%) was collected via filtration and washed with water: mp 180 °C (dec); ^{1}H NMR (300 MHz, CD_3CN) δ 7.26 (br, 8H, Ar), 7.00 (t, J = 7.2 Hz, 8H, Ar), 6.43 (t, J = 7.1 Hz, 4H, Ar), 5.80 (br, 2H, NH), 3.20 (m, 8H), 1.90 (m, 4H). Anal. Calcd for: C, 81.04; H, 7.46; N, 9.15. Found: C, 80.96; H, 7.48; N, 9.23.

Isothermal Titration Calorimetry. All binding experiments were performed on an isothermal titration calorimeter from Microcal Inc. (Northhampton, MA). In a typical ITC experiment, a 5 mM receptor solution is added to the calorimetry cell. A 100 mM solution of tetrabutylammonium acetate is introduced in 50 5-μL injections, for a total of 250 μL of added guest. Such high concentrations are necessary to generate the sharp curves required for acceptable curve fitting. The solution is continuously stirred to ensure rapid mixing and kept at an operating temperature of 25 °C through the combination of an external cooling bath (at 18 °C) and an internal heater. Dilution effects are determined by a second experiment by adding the same acetate solution into pure DMSO and subtracting this from the raw titration to produce the final binding curve.

Association parameters are found by applying either one-site or two-site models using the Origin software.[15] These methods rely on standard nonlinear least-squares regression[11] to fit the titration curves, taking into account the change in observable volume that occurs during the calorimetric titration. Since many of the derivatives that were studied form weak complexes with acetate, the stoichiometry of binding is fixed at 1 equiv in all binding analyses. Failure to fix this value can in some cases result in minimizations progressing to excessive values for binding stoichiometry.

NMR Binding Titration. All NMR experiments were performed at constant analyte concentration, typically 1 mM. A solution containing 1 mM of analyte and 20 mM of substrate was added in numerous aliquots, and the observed chemical shift was recorded at each concentration. Association constants were determined by nonlinear regression analysis of the resulting isotherm.

Acknowledgment. The authors wish to thank Professor Craig Wilcox of the University of Pittsburgh for providing the Hostest-II program and the National Institutes of Health (GM 35208) for financial support.

JO010413Y