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Calorimetric Investigation of Guanidinium-Carboxylate Interactions

Brian Linton and Andrew D. Hamilton*†

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260

†Current address: Sterling Chemistry Laboratory, Box 208107, Yale University, New Haven, CT 06511

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Abstract: Isothermal titration calorimetry was utilized to study the association between a series of guanidinium derivatives and tetrabutylammonium acetate. This technique provides a measure of association strength, stoichiometry of binding, as well as thermodynamic parameters of association from a single experiment. Guanidinium derivatives which can form bidentate linear hydrogen bonds with acetate show significant, exothermic binding in DMSO (Ka = 5600 M^{-1}; ΔH = -3.6 kcal/mol), while derivatives which lack this bidentate linear hydrogen bonding interaction result in complexes where association is weaker (Ka ≈ 100 M^{-1}) and enthalpically neutral or endothermic. Additionally calorimetry permits the complete assessment of the multiple binding equilibria when derivatives complex two equivalents of guest.

Keywords: Molecular recognition, calorimetry, guanidinium, carboxylate.

Introduction

Central to much of host-guest chemistry is the determination of the binding affinity between receptor and substrate. Typically this association strength is measured by spectrophotometric techniques such as UV absorption, fluorescence or nuclear magnetic resonance. An attractive alternative is titration calorimetry which can determine binding affinities as well as association thermodynamics of small molecule recognition by directly measuring the heat produced from host-guest complexation.

Isothermal Titration Calorimetry[1,2] measures the heat evolved or absorbed during the addition of aliquots of guest into host solution. This technique has been employed to investigate strongly associating species such as protein-DNA complexes,[3-7] enzyme substrates,[8] cyclodextrins[9,10] and antibodies[11] but the increased amounts of material required for accurate analysis of weakly bound species render this technique impractical for biological systems with lower association strength. This limitation is not a factor for smaller synthetic molecules, making calorimetry ideal for this type of host-guest complex.[12-15] This method is superior to other techniques not only in the combination of association and thermodynamic information provided from just one experiment, but also in the ability to successfully evaluate convoluted multiple binding equilibria.

Email: Andrew.Hamilton@yale.edu

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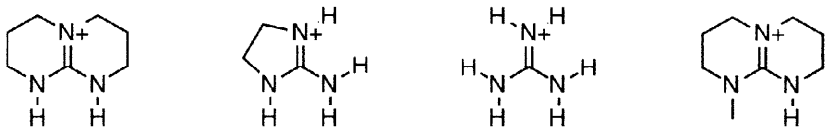
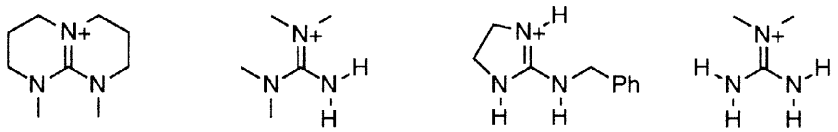
In the present study, an isothermal titration calorimeter (ITC) from Microcal Inc. (Northhampton, MA)[16] was used to investigate the association behavior of tetrabutylammonium acetate binding to a series of simple guanidinium derivatives (**1-8**) which differ in the number and orientation of available hydrogen bond donors. Each guanidinium has the potential to form a different host-guest complex with acetate by varying stoichiometry and mode of binding. All host-guest complexes were studied in DMSO where guanidinium-carboxylate complexes exhibit moderate association strength.[17,18] In addition to determining association strength, calorimetry is able to delineate both number of bound guests and thermodynamics of association for each guanidinium derivative.



1 **2** **3** **4**



5 **6** **7** **8**

Results and Discussion.

The calorimetric analysis of bicyclic guanidinium **1** is the most straightforward of the series due to the existence of one, strong binding site. Figure 1 shows the results of a calorimetric titration of the tetraphenylborate salt of **1** and tetrabutylammonium (TBA) acetate in DMSO. The top panel displays raw data; heat evolving with each injection over time. Initial injections produce a large signal from complete complexation of added guest, but this response decreases over time as binding sites become saturated. A binding isotherm (■) is generated by integrating each peak and plotting the resulting data versus mole ratio. Elevated concentrations of injectant result in a sizable heat of dilution which is measured in a separate titration (◆) and subtracted from the raw data (■) to produce the final binding curve (○), shown in the bottom panel. Also shown is the non-linear least squares fit of the subtracted curve using a one site binding model.[19] In all titrations the first several injections deviate from ideal binding behavior reflecting the effect of multiple guanidinium molecules associating with the limited acetate present and are not considered in the curve fitting analysis.

The final binding isotherm (○) displayed in Figure 1 is characteristic of a moderately strong, exothermic 1:1 complex. The equimolar stoichiometry is evident from the transition (inflection point) in the curve at one equivalent. Values for association constant (K_a = 5600 M^{-1}) and association enthalpy (ΔH = -3.6 kcal/mol) were determined by non-linear least squares fit using a one site binding model.[19] The strength and exothermic nature of this association suggests a complex held together by strong hydrogen bonding such as the bidentate interaction shown in Figure 2.

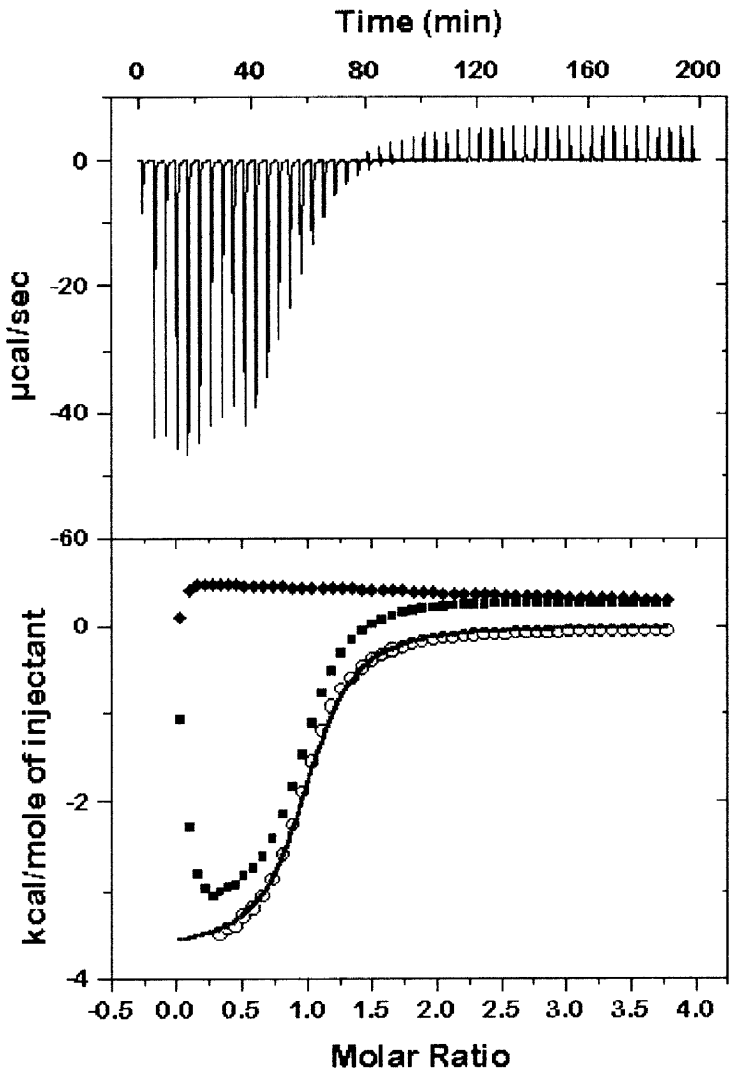
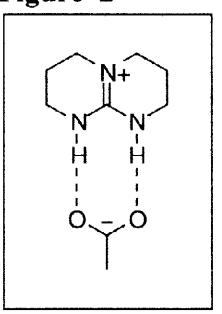


Figure 1. Calorimetric titration of tetrabutylammonium acetate added to **1** tetraphenylborate in DMSO. Top panel: raw data showing heat evolved with each injection of guest. Bottom panel: Integrated curves of raw titration(■), dilution(◆) and subtracted final binding curve(○). The solid line represents a non-linear least squares fit using a 1:1 binding model.

Figure 2



Association characteristics are also dependent on counterion and solvent. When the guanidinium counterion is changed to chloride, association strength is reduced in comparison to iodide or tetraphenylborate (TPB), as can be seen from binding data shown in Table 1. This effect is consistent with other anion-binding receptors which demonstrate weak binding to chloride, no binding to other halides, and strong association to carboxylates.[20] Since the chloride-guanidinium attraction reduces the net energy gain from acetate complexation, other derivatives were prepared as the iodide or TPB salts, dependent on synthetic availability .

Table 1. Binding constants (M^{-1}), association enthalpy (kcal/mol), and association entropy (cal/molK) for guanidinium derivatives and TBA Acetate in DMSO, at 25°C.

Guanidinium	Anion	$Ka_{(1:1)}$	$\Delta H_{(1:1)}$	$\Delta S_{(1:1)}$	$Ka_{(1:2)}$	$\Delta H_{(1:2)}$	$\Delta S_{(1:2)}$
1	Cl	2900±100	-3.3±0.1	+4.7±0.4			
1	I	5200±350	-3.7±0.1	+4.5±0.5			
1	BPh_4	5600±520	-3.6±0.1	+5.0±0.5			
2	BPh_4	8700±700	-3.1±0.1	+7.6±0.5	220±10	-3.7±0.1	-1.7±0.5
3	BPh_4	7900±900	-3.5±0.1	+6.0±0.6	170±10	-4.5±0.1	-4.9±0.5
4	BPh_4	*	*				
5	BPh_4	*	*				
6	BPh_4	110±10	+5.1±0.2	+26.4±0.9			
7	I	7200±370	-2.8±0.1	+9.4±0.5			
8	BPh_4	3400±100	-3.8±0.1	+3.3±0.4			

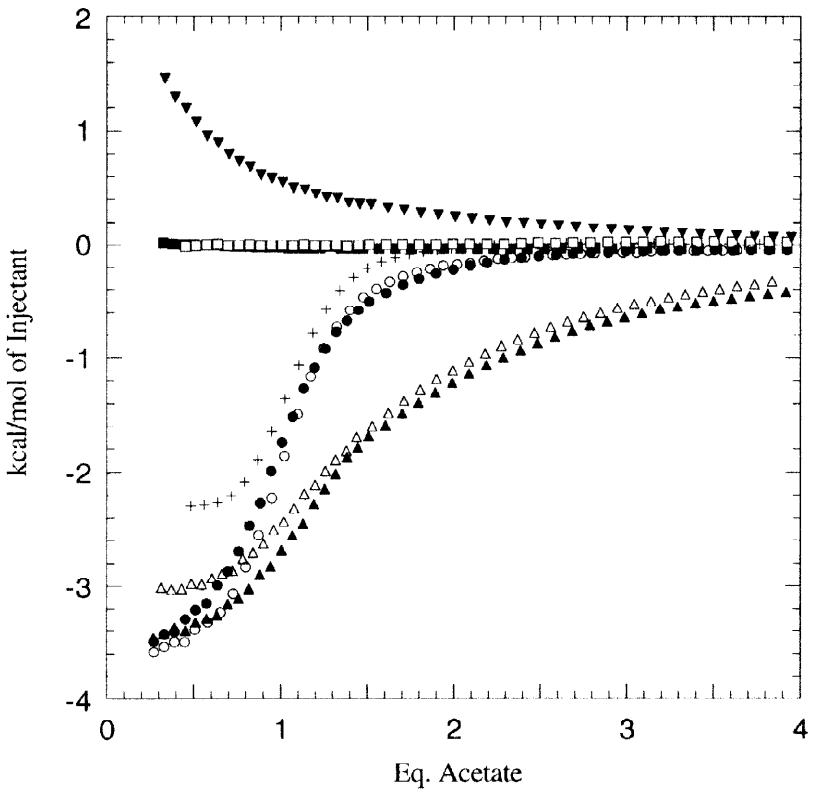
* Horizontal binding isotherm indicating no binding or enthalpically neutral binding.

Ka and ΔH are determined from non-linear least squares fit of the binding isotherm while ΔS is derived from the other two values. Data in this table represent the average value from duplicate experiments performed with fresh samples on different days. In each case 250uL of a 100mM solution of TBA acetate was added over 50 injections to a calorimetry cell containing 5mM guanidinium salt. A one site model was employed with stoichiometry fixed at one equivalent, with the exception of derivatives **2** and **3** which required a two site model.

In methanol, both the association strength and the thermodynamic nature of the complex are changed, due to increased solvation of both host and guest binding sites relative to DMSO. Complex formation must be preceded by energetically unfavorable desolvation of these sites, resulting in lower binding affinity (Ka = 100 M^{-1} for **1**•BPh4 and TBA•AcO). Additionally, complex formation becomes endothermic (ΔH = +1.0 kcal/mol, ΔS = +12.5 cal/molK), indicating bimolecular association promoted by the release of solvent molecules from binding sites to bulk solvent. Similar endothermic, entropy-driven association in methanol has been observed in complexes between bis-guanidinium receptors and dianions[15,21] suggesting a general mode of binding in methanol facilitated not by the formation of strong hydrogen bonds but rather by the liberation of bound solvent.

Alteration of the arrangement of available guanidinium hydrogen bonds produces modified complexes with acetate, directly observable through changes in the calorimetric binding curves. A comparison of the ITC binding isotherms produced by all eight guanidinium derivatives shown in Figure 3 displays four types of results. Guanidiniums **2** (Δ) and **3** (▲) produce curves which are more shallow than guanidinium **1** (○). Derivatives **4** (■) and **5** (□) produce no signal in the calorimetric titration resulting in a horizontal isotherm. The positive direction of the binding curve produced by **6** (▼) indicates endothermic association. Isotherms for guanidiniums **7** (+) and **8** (●) resemble the curve from derivative **1**. Variation in these titration curves reflects differences in binding affinity, association enthalpy and in some cases stoichiometry. In most cases binding isotherms are curve fit using a one site model assuming only 1:1 complexation, but several receptors (**2, 3**) complex an additional equivalent of acetate, and require the application of a two site binding model. Detailed association data for all guanidinium derivatives binding acetate in DMSO are collected in Table 1.

Figure 3. ITC binding curves from addition of TBA acetate to guanidiniums (1-8) in DMSO. Legend: ○ = **1**, Δ = **2**, ▲ = **3**, ■ = **4**, ❑ = **5**, ▼ = **6**, + = **7**, ● = **8**.



While guanidinium **1** is presumed to have one bidentate binding site, the availability of additional hydrogen bond donors in receptors **2** and **3** permits the complexation of additional equivalents of guest. This effect is illustrated by the differences in the calorimetric binding curves of receptor **1** and **2** seen in Figure 3. Although maintaining exothermic association, the more gradual curve of **2** (Δ) is indicative of either weaker complexation or the presence of a second binding event.

In an effort to determine the stoichiometry of association, both one site and two site binding models[12] were applied to the titration curves from guanidinium **2**. A one site model assumes formation of only a 1:1 complex while the two site model also incorporates the binding of a second equivalent of guest. Figure 4 displays the experimental data for the association of **2** and acetate (Δ) as well as the non-linear least squares analysis from each model. The two site model (Figure 4A) is in good statistical ($\chi^2 = 140$) and visual agreement with experimental data, indicating strong ($Ka_{1:1} = 8700\ M^{-1}$) exothermic 1:1 association and a weaker ($Ka_{1:2} = 220\ M^{-1}$) exothermic 1:2 association. Application of the one site model (Figure 4B) suggesting much weaker binding ($Ka = 200\ M^{-1}$) was rejected due to greater deviation from experimental data ($\chi^2 = 22000$) as well as binding data which is inconsistent with other bidentate guanidinium receptors. The success of the two site model corresponds to complexation of two equivalents of acetate as shown in Figure 5. Association of a second acetate to form a ternary complex is weaker than initial binding to the positively charged free guanidinium, and entropically unfavorable.

Figure 4. ITC curve fitting for guanidinium **2** and TBA acetate using one or two site models.
A: Fit using two-site binding model. **B**: Fit using one-site binding model

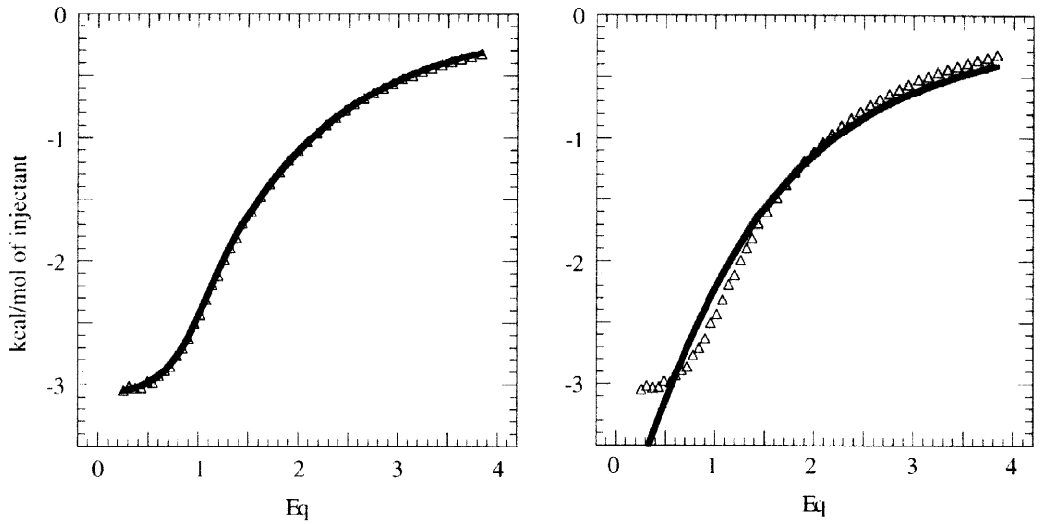
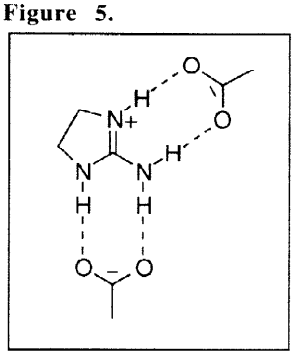


Figure 5.



Calorimetric titration of derivative **3** and acetate produces an experimental curve similar to **2** and comparable binding data using the two site model. In this case the availability of two additional hydrogen bonds permits the possible complexation of a third equivalent of guest. However, no indications of such association is observed in the calorimetric titration, presumably due to the extremely unfavorable binding of an anionic guest to the anionic guanidinium-bis(acetate) complex.

A similar analysis of the complex between receptor **2** and TBA acetate using NMR titration protocols results in binding curves where the stoichiometry of association is ambiguous. Figure 6 displays the changes in the chemical shift of the two imidazoline-NH protons of **2** at various equivalents of acetate. While both of these curves can be modeled satisfactorily using a 1:1 model (shown as best fit line in Figure 6) the resulting association constants are not in agreement with one another (Ka = 2600 and 750 M^{-1}), and both indicate weaker association than that observed for guanidiniums with only one binding site. The similarity in the chemical shift of singly and doubly bound complexes as well as uncertainty in the chemical shift of the 1:1 complex makes complete deconvolution of the multiple equilibrium with a two site model impossible, rather a large variety of binding constants model the experimental data with similar errors. A reverse titration where the acetate methyl signal is followed with added receptor is equally unsuccessful due to the similar chemical shifts of the methyl group when bound once or twice to guanidinium **2**. Thus, NMR titrations produce curves which are not successfully modeled by a two site model, while one site models produce inaccurate association data.

Figure 6. NMR titration of **2** with TBA acetate in DMSO. Lines represent a 1:1 binding model.

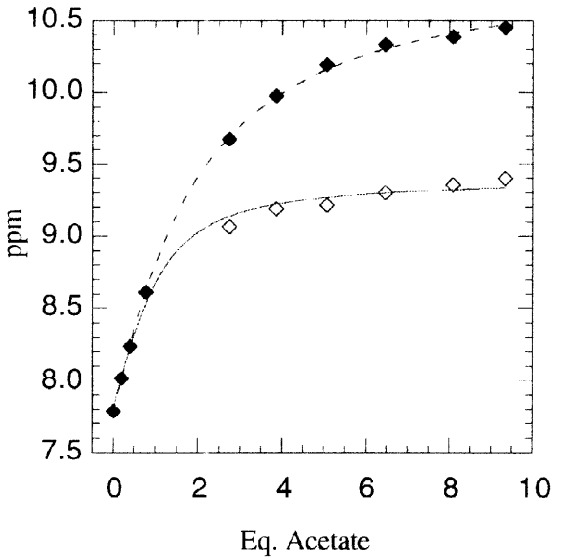
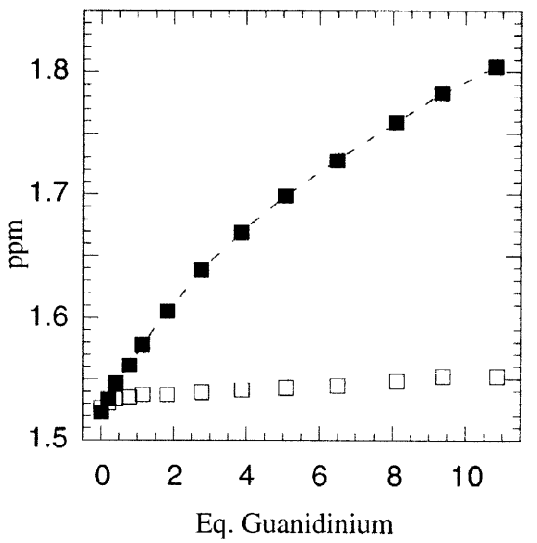


Figure 7. NMR titration of TBA acetate with **4** and **5** in DMSO. Legend: ■ = 4, ❑ = 5, line represents a 1:1 binding model.



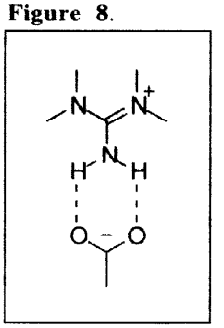
The importance of the bidentate hydrogen bonding interaction was investigated by removing available hydrogen bond donors and measuring association with acetate. Molecules **4** and **5** resemble bicyclic guanidinium **1**, but contain one and two additional methyl groups respectively in place of hydrogen bond donors. Receptor **4** is thus able to form only one hydrogen bond with guests while receptor **5** must interact entirely through electrostatic attraction. In both cases there is no response in the calorimetric titration, evident from the horizontal binding isotherms shown in Figure 3 (■ and ❑). Two explanations exist for such a phenomenon; either no association, or enthalpically neutral association. The calorimetric technique does not distinguish between these two possibilities, but does indicate that the elimination of even one hydrogen bond leads to a radically different interaction.

Distinguishing no association from enthalpically neutral association is accomplished by the corresponding NMR titration. When guanidiniums **4** and **5** are added to a constant concentration of acetate the methyl signals shift as shown in Figure 7. Mono-methylated bicyclic guanidinium **4** (■) does show modest association to acetate, Ka = 90 M^{-1}, indicating that the presence of only one hydrogen bond results in the formation of a weak host-guest complex. Dimethylated receptor **5** (❑) induces no change in the chemical shift of the acetate methyl group, indicating no association. These results suggest that the observed association between guanidiniums and carboxylates in DMSO is highly dependent on bidentate hydrogen bonding, with little electrostatic contribution.

Directionality of hydrogen bonds is also important in carboxylate recognition, with changes in the orientation of the hydrogen bonds resulting in a drastically different calorimetric titration (see Figure 3). Derivative **1** (○) contains two hydrogen bond donors and shows strong exothermic association with acetate, while tetramethyl-guanidinium **6** (▼) contains two hydrogen bond donors but demonstrates weak, endothermic binding (Ka = 110 M^{-1}, ΔH = +5.1 kcal/mol). The formation of a bidentate interaction between guanidinium **6** and acetate can only occur through the six-membered ring shown in Figure 8, rather than the eight-membered ring seen previously. While it is possible that this derivative forms only a monodentate hydrogen bonding interaction

with acetate, the endothermic nature of the association indicates a complex which differs from guanidinium **4**. Presumably this receptor binds acetate through the bidentate interaction shown in Figure 8, but in this orientation the bent hydrogen bonds are not as strong as the linear hydrogen bonds of the complex shown in Figure 2. The significantly positive association entropy of this interaction (ΔS = +26.4 cal/molK) suggests that solvent reorganization makes a sizable contribution to formation of this complex.

Figure 8.



Receptors **7** and **8** contain three and four possible hydrogen bond donors respectively with more than one potential binding site, however the ITC binding isotherms resemble the curve from receptor **1**, and are successfully modeled using a one site model indicating only 1:1 association. The lack of a second binding event agrees with the previous determination that a single hydrogen bond is insufficient for strong complexation, an event that would be reduced further in these receptors due to the second equivalent binding to a neutral 1:1 complex. Despite the presence of additional available hydrogen bonds, only a 1:1 complex is formed.

Conclusions.

Calorimetric determination of the association characteristics of a series of guanidinium derivatives binding to acetate illustrates that in DMSO, discrete hydrogen bonding is the driving force in complexation. Derivatives **1**, **2**, **3**, **7** and **8** which can form linear, bidentate hydrogen bonds produce moderately strong complexes with acetate in DMSO. Both association enthalpy and entropy are favorable indicating complex formation is accomplished through hydrogen bond formation and a contribution from the liberation of bound solvent molecules. In methanol binding is endothermic, suggesting a primary role of solvent reorganization in bimolecular association

In the case of guanidiniums **2** and **3** calorimetric titration shows the complexation of a second equivalent of acetate. Unlike other techniques, the fact that the second association produces a signal which is independent from the first allows both events to be successfully fit using a two site binding model. Complexation of a second equivalent of acetate is understandably weaker, predominantly a result of the unfavorable entropy of association.

Guanidinium **6** which is unable to form bidentate linear hydrogen bonds still associates with acetate, although weakly. This decrease in binding strength is reflected by the endothermic nature of this association, while the larger association entropy indicates solvent reorganization makes a sizable contribution to the strength of complexation. Indeed the positive enthalpy value suggests that the sum of all hydrogen bonds formed in the complex and by liberated solvent molecules is less than the total that existed between independent species and solvent before guanidinium-acetate association. In this case hydrogen bonding between guanidinium and acetate alone is not enough to direct complex formation, but rather association is facilitated by solvent reorganization.

This series of simple guanidinium derivatives was chosen to explore the detailed thermodynamic information alluded to by previous work in carboxylate recognition,[17] as well as to evaluate the ability of calorimetry to investigate this interaction. This study has shown that in DMSO strong complexation is mediated primarily by hydrogen bond formation with an additional favorable contribution from solvent reorganization. The success of isothermal titration calorimetry in delineating various aspects of this interaction encourages future application of this technique to other synthetic host-guest systems.

Experimental.

Isothermal Titration Calorimetry

All binding experiments were performed on an Isothermal Titration Calorimeter from Microcal Inc. (Northhampton, MA). In a typical ITC experiment a 5mM receptor solution is added to the calorimetry cell. A 100mM solution of tetrabutylammonium acetate is introduced in fifty 5μL injections, for a total of 250μL 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 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.[16] These methods rely on standard non-linear least-squares regression[19] to fit the titration curves, taking into account the change in observable volume that occurs during the calorimetric titration. Since many of the derivatives studied form weak complexes with acetate, the stoichiometry of binding is fixed at one equivalent in all binding analyses. Failure to fix this value can in some cases result in minimizations progressing to unbelievable values for binding stoichiometry.

NMR Binding Titration.

All NMR experiments were performed at constant analyte concentration, typically 1mM. A solution containing 1mM analyte and 20 mM substrate was added in numerous aliquots and the observed chemical shift was recorded at each concentration. Association constants were determined by non-linear regression analysis of the resulting isotherm.[22]

Synthesis of guanidiniums **1-8.**

General Methods. Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AF300 spectrometer , and chemical shifts are reported relative to internal Me_4Si. Elemental analysis was carried out by Atlantic Microlab Inc., Norcross, GA

1,3,4,6,7,8-Hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine hydrochloride (1•Cl).

1,3,4,6,7,8-Hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine (0.80g, 5.75 mMol) was dissolved in 50 mL absolute diethyl ether. Addition of ethyl ether saturated with gaseous HCl resulted in the formation of a white precipitate. This solid (1.00g, 100%) was collected via filtration: mp 165 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (br, 2H, NH), 3.25 (m, 4H), 3.17 (m, 4H), 1.85 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 150.9 (s), 46.1 (t), 37.3 (t), 20.3 (t); Analysis calc'd for $C_7H_{14}ClN_3 \cdot 1/2H_2O$: C, 45.53; H, 8.19; N, 22.75; found: C, 45.78; H, 7.94; N, 22.81.

1,3,4,6,7,8-Hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine hydroiodide (1•I). 1,3,4,6,7,8-Hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine (0.29g, 2.08 mMol) was dissolved in 20 mL water. Hydroiodic acid (4 mL) was added, and the solution was stirred for one hour. Concentration to dryness under reduced pressure resulted in a tan paste, which was dissolved in a minimal amount of dichloromethane. Addition of several volumes of THF caused the formation of a white precipitate (0.47g, 84%) which was collected via filtration and washed with THF: mp 117-118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (br, 2H, NH), 3.34 (m, 8H), 2.04 (m, 4H); Analysis calc'd for : C, 31.48; H, 5.28; N, 15.73; found: C, 31.58; H, 5.20; N, 15.60.

1,3,4,6,7,8-Hexahydro-2H-pyrimido[1,2-a]pyrimidine tetraphenylborate (1•BPh_4). 1,3,4,6,7,8-Hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine (0.10g, 0.542 mMol) was dissolved in 10 mL 10% HCl (aq.). A solution of tetraphenylboron sodium (0.20g, 0.584 mMol) in 10 mL water was added resulting in the immediate formation of a white precipitate. This solid (0.14g, 55%) was collected via filtration and washed with water: mp dec 180 °C; ^{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); Analysis calc'd for : C, 81.04; H, 7.46; N, 9.15; found: C, 80.96; H, 7.48; N, 9.23.

2-Aminoimidazolinium tetraphenylborate (2). 2-Aminoimidazolinium *p*-toluenesulfonate (0.20g, 7.71 mMol) was dissolved in 1 mL water. This solution was added to 10mL of water containing 0.29g (8.47 mMol) tetraphenylboron sodium. After stirring for one hour, the resulting precipitate was collected by filtration and washed with several volumes of water, yielding 0.28g (90%) white solid: mp 188-190 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 7.30 (br, 8H, Ar), 7.02 (t, J=7.2 Hz, 8H, Ar), 6.82 (t, J=7.0 Hz, 8H, Ar), 6.11 (br, 4H, NH), 3.54 (s, 4H, CH_2); ^{13}C NMR (75 MHz, CD_3CN) δ 166.0 (m), 137.3 (d), 127.3 (d), 123.4 (d), 44.3 (t); Analysis calc'd for $C_{27}H_{28}BN_3$: C, 80.00; H, 6.96; N, 10.337; found: C, 79.92; H, 7.04; N, 10.36.

Guanidinium tetraphenylborate (3). Guanidine hydrochloride (0.20g, 2.09 mMol) was dissolved in 1 mL water. This solution was added to 10mL of water containing 0.80g (2.34 mMol) tetraphenylboron sodium. After stirring for one hour, the resulting precipitate was collected by filtration and washed with several volumes of water yielding 0.69g (87%) white solid: mp 218-220 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 7.28 (br, 8H, Ar), 7.00 (t, J=7.2 Hz, 8H, Ar), 6.85 (t, J=7.2 Hz, 4H, Ar), 5.88 (br, 6H, NH); ^{13}C NMR (CD_3CN) δ 165 (m), 158.3 (s), 136.6 (d), 126.7 (d), 122.9 (d); Analysis calc'd for $C_{25}H_{26}BN_3 \cdot {}^1/_3H_2O$: C, 77.79; H, 7.14; N, 10.89; found: C, 77.75; H, 7.01; N, 10.96.

1,3,4,6,7,8-Hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine tetraphenylborate (4). 1,3,4,6,7,8-Hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (0.10g, 0.65 mMol) was dissolved in 2 mL water and treated with 2 mL 10% aqueous HCl. After stirring five minutes this solution was poured into 10mL water containing sodium tetraphenylborate (0.25g, 0.73 mMol). A white precipitate formed immediately. After

swirling intermittently for ten minutes, the solid was collected by filtration and washed with several volumes of water. A white solid (0.29g, 93%) remained: mp dec 165 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 7.26 (br, 8H, Ar), 6.98 (t, J=7.4 Hz, 8H, Ar), 6.84 (t, J=7.2 Hz, 4H, Ar), 5.92 (br, 1H, NH), 3.22 (m, 8H, CH_2), 1.93 (m, 4H, CH_2); Analysis calc'd for $C_{32}H_{36}BN_3$: C, 81.18; H, 7.66; N, 8.87; found: C, 80.99; H, 7.67; N, 8.76.

1,3,4,6,7,8-Hexahydro-1,8-dimethyl-2*H*-pyrimido[1,2-*a*]pyrimidine tetraphenylborate (5). 1,3,4,6,7,8-Hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (100mg, 0.65mMol) was dissolved in 10 mL anhydrous THF. Iodomethane (130mg, 0.92 mMol) was added and the solution stirred for four hours. An off-white precipitate formed several minutes after addition was complete, with more solidifying over time. The solid was collected by filtration and washed several times with THF, whereas a pale yellow solid (0.16g, 83%) remained: mp 220-222 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 3.34 (m, 4H, CH_2), 3.22 (m, 4H, CH_2), 2.93 (s, 6H, CH_3), 1.95 (m, 4H, CH_2).

1,1,3,3-Tetramethylguanidinium tetraphenylborate (6). 1,1,3,3-tetramethylguanidine (116mg, 1.01 mMol) was dissolved in 1 mL water. This solution was added to 1 mL 10% HCl (aq) followed by 10mL of water containing 0.38g (1.11 mMol) tetraphenylboron sodium. After swirling for one hour, the resulting precipitate was collected by filtration and washed with several volumes of water, yielding 0.41g (94%) white solid: mp dec. 208 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 7.29 (br, 8H, Ar), 7.01 (t, 7.2H, Ar), 6.84 (m, 4H, Ar), 6.04 (br, 2H, NH), 2.85 (s, 12H, CH_3); Analysis calc'd for $C_{28}H_{34}BN_3$: C, 80.00; H, 7.87; N, 9.65; found: C, 79.87; H, 7.84; N, 9.58.

N-(3,4-Dihydro-1*H*-imidazol-2-yl)-aminomethyl benzene hydroiodide (7). A solution of 2-methylmercapto-4,5-dihydroimidazole hydroiodide (1.00g, 4.10 mMol) was prepared in t-butanol (10 mL) with a condenser leading to an aqueous NaOH trap. To this was added benzylamine (0.44g, 6.58 mMol) and the solution was refluxed for four hours. Ethyl ether (50mL) was added causing the formation of a white solid. This precipitate was collected by filtration yielding 1.01g (81%) white solid: mp 138-139 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (br, 1H, NH), 7.40 (m, 2H), 7.30 (m, 3H), 4.32 (d, J=5.6 Hz, 2H), 3.59 (s, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.2 (s), 137.0 (s), 128.6 (d), 127.6 (d), 127.3 (d), 45.5 (t), 42.6 (t); Analysis calc'd for $C_{10}H_{14}IN_3$: C, 39.62; H, 4.65; N, 13.86; found: C, 39.58; H, 4.63; N, 13.84.

1,1-Dimethylguanidine tetraphenylborate (8). 1,1-Dimethylguanidine sulfate (103mg, 0.38 mMol) was dissolved in 1 mL water. This solution was added to 10mL of water containing 150mg (0.44 mMol) tetraphenylboron sodium. After swirling for one hour, the resulting precipitate was collected by filtration and washed with several volumes of water, yielding 0.14g (91%) white solid: mp 212-213 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 7.31 (br, 8H, Ar), 7.03 (t, J=7.3 Hz, 8H, Ar), 6.87 (t, J=7.2 Hz, 4H, Ar), 5.72 (br, 4H, NH), 2.88 (s, 6H, CH_3); ^{13}C NMR (75 MHz, CD_3CN) δ 164.1 (m, Ar-B), 136.7 (d), 126.6 (d), 122.8 (d), 38.7 (q); Analysis calc'd for $C_{27}H_{30}BN_3$: C, 79.61; H, 7.42; N, 10.32; found: C, 79.79; H, 7.40; N, 10.40.

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