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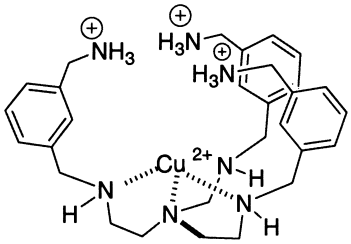

$$\Delta G^\circ_S = \Delta G^\circ_A + \Delta G^\circ_B - \Delta G^\circ_{AB}$$

$$\Delta H^\circ_S = \Delta H^\circ_A + \Delta H^\circ_B - \Delta H^\circ_{AB}$$

$$T\Delta S^\circ_S = T\Delta S^\circ_A + T\Delta S^\circ_B - T\Delta S^\circ_{AB}$$

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Studies into the Thermodynamic Origin of Negative Cooperativity in Ion-Pairing Molecular Recognition

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Abstract: The association of synthetic receptors to target guests often proceeds through the cooperative action of multiple binding forces. An investigation into the thermodynamic origin of cooperativity in ion-pairing host−guest binding in water is described. The binding affinities of 1,2,3,4-butanetetracarboxylate, tricarballate, glutarate, and acetate to a C_{3v} symmetric metallo-host (**1**) are characterized in terms of the binding constants (K_a) and the thermodynamic parameters $\Delta G°$, $\Delta H°$, and $\Delta S°$, as determined by isothermal titration calorimetry (ITC). These values are used to determine the individual contributions of the binding interaction to the overall binding. Several ways to view the combination of the individual binding events that make up the whole are analyzed, all of which lead to the conclusion of negative cooperativity. Combined, the data were used to evaluate the thermodynamic origin of negative cooperativity for this series of guests, revealing that entropy is the largest contributing factor. An interpretation of this result focuses upon differences in the number of water molecules displaced upon binding.

Introduction

The association of small molecules to form complexes through weak noncovalent interactions lies at the heart of molecular recognition. Several methods have been developed to quantify the strength of host−guest interactions.[1] The strength of a host−guest association is commonly reported as the binding constant (K_a) or as the Gibbs' free energy of binding ($\Delta G°$), but the contributions of the enthalpy change ($\Delta H°$) and the entropy change ($\Delta S°$) to the binding are not routinely measured. The quantification of the $\Delta H°$ and $\Delta S°$ components of the Gibbs' free energy of binding has become an area of increasing interest in light of the application of isothermal titration calorimetry (ITC) techniques[2−5] to molecular recognition.[6−15] This permits direct measurement of the heat of binding, from which K_a, $\Delta G°$, $\Delta H°$, and $\Delta S°$ values can be accurately determined. Herein we describe the use of ITC to analyze cooperative binding.

A common design principle in molecular recognition entails the pairwise matching of molecular recognition contacts between the host and the guest. This is generally thought to increase the Gibbs free energy of binding by increasing the number of weak binding interactions that hold the complex together.[16−19] One expects electrostatic interactions to manifest themselves in terms of enthalpy changes, yet in protic media, ion pairing is sometimes thermal neutral or even endothermic.[7,13,20]

Understanding any molecular recognition event requires one to consider the differences between the energies of solvation of the free host and free guest relative to solvation of the host−guest complex, the interactions between the host and the guest, and the cohesive interactions between the released solvent. Ion-pairing is often thermoneutral, and the solvation/desolvation processes influence binding mostly via entropic changes. It follows that in a system where multiple attractive electrostatic interactions are operative, the binding event should be more exothermic and there would also be more solvent release.[21−25] Quantitatively, a higher-affinity complex would be observed

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relative to a system with fewer such binding interactions. We test this concept herein by studying the electrostatic interactions between a polycationic host and various anionic guests.

The above analysis does not take into account the fact that the individual binding interactions involved in a host−guest association are not independent of one another, a phenomenon known as cooperativity. Positive cooperativity is observed when the overall binding is greater than the mere summation of the Gibbs' free energies of binding for the individual interactions. Alternatively, negative cooperativity arises when the overall binding is weaker than the combined binding energies of the parts. It is important to realize for the purposes of this work that as the individual binding interactions are tethered together the Gibbs free energies of binding increases, whether positive or negative cooperativity is present. Negative cooperativity indicates that the increase in Gibbs free energy of binding is smaller than what could have been achieved. An extensive review on the thermodynamic aspects of cooperativity is provided by Whitesides[26] and co-workers. From a survey of the literature, it is clear that negative cooperativity is the norm. There appear to be few studies that address the enthalpic and entropic origin of cooperativity, albeit negative or positive, for binding events using designed synthetic receptors.[27−30]

The thermodynamic aspects of cooperativity have been discussed by both Jencks and Williams. An analysis by Jencks in 1981 gave concrete definitions and mathematical relationships that define negative and positive cooperativity.[31] Jencks proposed that the Gibbs' free energy of binding A−B ($\Delta G_{AB}°$) is a summation of the free energies of binding for the individual parts A ($\Delta G_A°$) and B ($\Delta G_B°$) plus an additional term, the Gibbs' free energy of connection ($\Delta G_S°$) that arises from the presence of the tether (eq 1). By convention, a positive $\Delta G_S°$ represents positive cooperativity and a negative $\Delta G_S°$ represents negative cooperativity.

$$\Delta G_S° = \Delta G_A° + \Delta G_B° - \Delta G_{AB}° \qquad (1)$$

His approach predicted that entropy is the largest contributing factor for positive cooperativity. The basis of Jencks' proposal relies on an analysis of a protein containing two binding pockets that are complementary to A and B moieties. In his analysis, both A and B bind to their respective pockets. However, when A is tethered to B (A−B), the binding of A will assist the binding of B by increasing the effective molarity of B, thereby imposing an entropic gain on the binding pair (Figure 1). The price for unfavorable entropy changes derived from the association of the binding partners is paid once in the case of the tethered moieties. In Jencks' analysis, negative cooperativity can arise from decreased enthalpy. For example, if the tether between A and B is of insufficient length to allow the binding moieties to realize their full enthalpic potential (Figure 1), negative cooperativity will result.

Recent work by Williams and Westwell[16,17,32] provides another approach to understanding the enthalpy and entropy

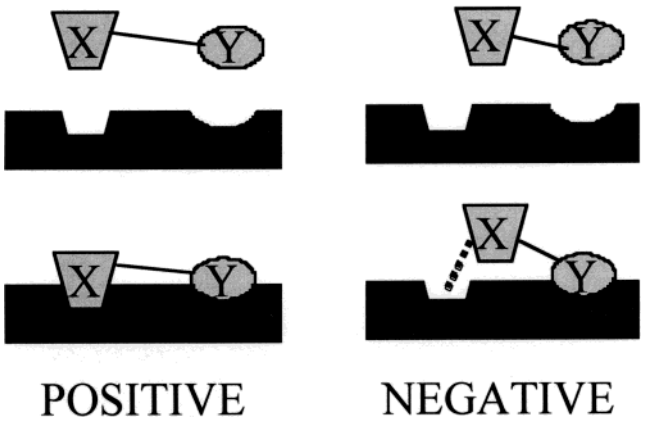

Figure 1. Depiction of the binding of A−B to their respective binding sites on the host. The drawing on the left represents positive cooperativity, and that on the right represents negative cooperativity.

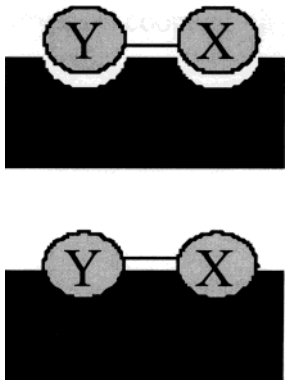

Figure 2. Model used by Williams to describe cooperativity. The tethering of A and B leads to a more tightly held complex due to shorter contacts, albeit at the cost of less residual motion.

parameters that characterize cooperativity. Their analysis relies on enthalpy−entropy compensation effects[33−37] in which high-affinity complexes display large exothermic values but less residual motion. Williams proposes that a complex held together by noncovalent interactions has less residual motion than that of a complex held together by a less extensive network of similar interactions. The enhanced binding derives from the increased enthalpy of the interactions that are enforced by the presence of the tether. The increased enthalpy is a consequence of shorter contact distances between the binding groups in A−B with the host versus A and B with the host individually (Figure 2). Williams proposes that positive cooperativity will have a significant enthalpic component and that negative cooperativity can arise from either enthalpy or entropy, depending on the extent to which they compensate each other. One goal of the study presented here was to determine the extent to which the Jencks and Williams viewpoints explain cooperativity in ion-pairing interactions. To do this, we needed to extend the Jencks approach of defining a Gibbs free energy of connection to enthalpy and entropy changes.

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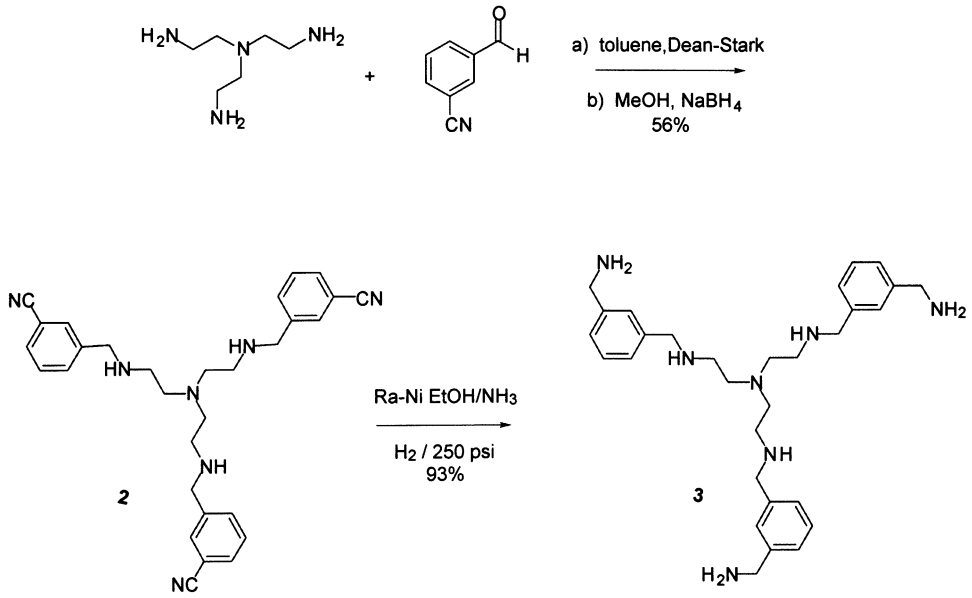
Scheme 1. Synthesis of Tripodal Ligand Precursor to Receptor **1**



The approach to breaking down the origin of cooperativity introduced herein relies on the quantification of the $\Delta H°$ and $\Delta S°$ values for A−B, A, and B as guests. A comparison of such parameters will offer insight into the origin of the observed cooperativity. A dissection of the $\Delta H°$ and $T\Delta S°$, analogous to Jencks' analysis, yields $\Delta H_S°$ and $T\Delta S_S°$ terms. As with the Jencks equations, the signs of the resulting $\Delta H_S°$ and $T\Delta S_S°$ values are important to the analysis. A positive $\Delta H_S°$ value indicates that the binding enthalpy change for A−B is more favorable than the sum of the enthalpy changes for A and B. Conversely, negative $\Delta H_S°$ values tell us that the binding enthalpy change for A−B is less favorable than the combined enthalpy changes of A and B. A positive sign with regard to the $T\Delta S_S°$ value indicates that the binding entropy change of A−B is not as favorable as the combined entropy changes for A and B. It follows that a negative $T\Delta S_S°$ means that the binding entropy change of A−B is more favorable than a sum of the entropy changes for A and B.

$$\Delta H_S° = \Delta H_A° + \Delta H_B° - \Delta H_{AB}° \quad (2)$$

$$T\Delta S_S° = T\Delta S_A° + T\Delta S_B° - T\Delta S_{AB}° \quad (3)$$

Having established a mathematical basis for our studies, we sought to explore whether increasing the number of ion-pairing interactions would lead to increased binding and whether that occurred as a result in increased favorable enthalpy or entropy. From prior studies (discussed above), we expected entropy to dominate the Gibbs free energy of binding, but it was not clear if entropy or enthalpy would increase as the number of ion-pair contacts was increased. Second, we sought to discover if the increased binding affinities were indicative of positive or negative cooperativity as defined by Jencks (eq 1) and expected to find negative cooperativity. Last, whatever form of cooperativity resulted, we sought to discover whether it primarily resulted from favorable or unfavorable enthalpies and entropies of connection (eqs 2 and 3).

Results and Discussion

Design Criteria. The host was chosen to provide a total of four binding sites (three ammonium groups and a metal) to complement guests having negatively charged functional groups (carboxylates). The energetics of the binding of guests having one to four functional carboxylates to the host were quantified and used to dissect the contributions of the thermodynamic parameters of the "parts" to those of the "whole".

The host (**1**) features a C_{3v} symmetric cavity derived from the preorganization of a tripodal ligand around a Cu(II) center, reminiscent of receptors from Fabbrizzi and others.[38−43] The Cu(II) center and the three ammonium groups on the periphery of the cavity were intended to provide a total of four binding sites for anionic guests. We recently reported that this host has a high selectivity and affinity for phosphate in water at neutral pH,[44] but it is also suitable for binding oligocarboxylate guests for the purposes of this study. Host−guest binding was expected to occur through the action of multiple complementary electrostatic interactions between functional groups of each of the binding partners. The ammonium groups of **1** are not specifically preorganized to complement any particular one of the carboxylate guests, and hence, we fully expected entropy consequences from the restriction of host conformations upon guest complexation.

Synthesis. The tripodal ligand (**3**) used to generate **1** was obtained through the reductive amination of tris(2-aminoethyl)-amine with 3-cyanobenzaldehyde. The resulting nitrile **2** was reduced using H_2 at 250 psi over a Raney-nickel catalyst in an ammonia-saturated ethanol solution to yield **3**. Simply stirring with 1 equiv of $CuCl_2$ in water resulted in complete metalation, as determined by UV/vis spectroscopy. Counterions to the ammonium groups used in the binding studies were chlorides.

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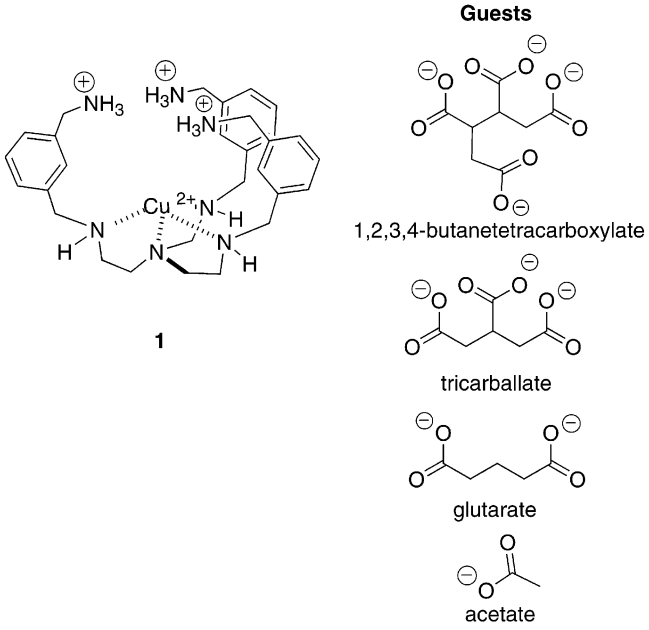

Gibbs Free Energies of Binding. A series of carboxylates were used as guests for **1**. The binding of 1,2,3,4-butanetetracarboxylate, tricarballate, glutarate, and acetate to **1** were quantified by observing the change in the absorbance of **1** as aliquots of a guest solution were added to a HEPES-buffered (5 mM) solution of **1**. The binding curves generated for each guest were fit with a 1:1 binding algorithm[1] to yield binding constants (Table 1). These data reveal that the tetracarboxylate and tricarballate complexes with **1** have binding affinities on the same order of magnitude (both near $K_a = 10^5$ M^{-1}, near -7 kcal/mol), but the binding of the tetracarboxylate is stronger by around 0.5 kcal/mol. Conversely, the binding constant for glutarate to **1** is nearly 2 orders of magnitude smaller ($K_a = 3.0 \times 10^3$ M^{-1}, -4.5 kcal/mol) than for the tetra- or tricarboxylate guests. Acetate had a smaller binding constant ($K_a = 9.0 \times 10^2$ M^{-1}, -4.1 kcal/mol) than glutarate, as it can only interact with one of the four binding sites on **1**, likely the Cu(II) center. This affinity is reasonable because it is comparable to a binding affinity of 50 M^{-1} determined for the binding of acetate to a phenanthroline-bound Cu(II) center in water.[45] Yet, the increase observed for glutarate over acetate is not large, only being around 0.5 kcal/mol.

Inspection of just these binding constants indicates that there is indeed cooperativity between the binding groups on the host for complexation of the various guests when proceeding from acetate to glutarate to tricarballate and tetracarboxylate because the affinities increase. However, it is definitely clear that the addition of another carboxylate to tricarballate, making 1,2,3,4-butanetetracarboxylate, does not result in a large increase in affinity; therefore, cooperativity is expected to be strongly negative here. Similarly, the addition of a carboxylate to acetate to give glutarate does not significantly increase binding. Instead, a relatively large increase in affinity arises from adding one or two more carboxylates to glutarate (giving either the tri- or tetracarboxylate). What is not clear is whether the cooperativity in comparing glutarate to the tri- or tetracarboxylate is negative or positive and if the associated large increase in binding has primarily an enthalpic or entropic origin.

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As described below, ITC was used to uncover the enthalpic/entropic origin of the cooperativity. In these studies, 5 μL aliquots of a solution of **1** were added to a HEPES-buffered (10 mM) solution of guest (Table 1). By ITC, the binding constants were found to range from 1.8×10^4 to 1.9×10^4 M^{-1} (-5.8 kcal/mol) at the high for the tetracarboxylate/tricarballate, down to 3.3×10^2 M^{-1} (-3.4 kcal/mol) for acetate. Figure 3 shows some representative ITC curves. These values are slightly smaller compared to those from the UV/vis titrations, possibly due to competition from the buffer that is present in a higher concentration in the ITC studies. Importantly, the affinity constants, and therefore the Gibbs free energies of binding, are comparable from both the UV/vis and ITC data, and the trend is identical: acetate and glutarate binding affinities are comparable and the tricarballate and 1,2,3,4-butanetetracarboxylate binding affinities are on the same order of magnitude. Additionally, there is a large increase in affinity when moving from acetate or glutarate to tricarballate or 1,2,3,4-butanetetracarboxylate.

We are confident that a carboxylate to Cu(II) ligation is always the primary interaction for the binding of any oligocarboxylate to **1** based on the Gibbs free energy of binding of acetate, which is between 3.5 and 4 kcal/mol. The ammonium groups play an important role as they add 2.5 to 3.2 kcal/mol in binding energy for potentially three ammonium−carboxylate interactions, but this total is less than the single interaction with Cu(II).

Actually, it is likely that only two ammonium−carboxylate interactions are gained when comparing acetate to 1,2,3,4-butanetetracarboxylate because the binding of this tetracarboxylate is nearly identical to that of tricarballate. Tricarballate can only form two ammonium−carboxylate interactions once one of the three carboxylates is bound to the Cu(II). Our determination of 2.5 to 3.2 kcal/mol for likely two, but possibly three, ammonium−carboxylate interactions is consistent with a variety of literature values. Schneider has determined that, on average, an ammonium to carboxylate interaction is worth 1.2 kcal/mol in water.[46] Fersht has determined 3−9 kcal/mol for charged interactions in water within hydrophobic enzyme active sites.[47,48] Fersht also estimates that neutral hydrogen bonds are worth about 0.5 to 1.8 kcal/mol in water in natural systems.[47,48] We are not aware of many examples for the enthalpy and entropy for binding of a carboxylate and ammonium in water. However, Rebek has estimated that a hydrogen bond in water that is worth -0.2 kcal/mol in Gibbs energy is formed from -0.8 and -1.5 kcal/mol $\Delta H°$ and $T\Delta S°$ values, respectively.[49]

Analysis of Cooperativity. The series of carboxylate guests provide an opportunity to quantitatively analyze the presence of cooperativity in the binding of the guests using Jencks' and Williams' A−B (whole) versus A and B (parts) approach. The tetracarboxylate (the whole) can be thought of as a combination of the tricarballate and the acetate (the parts) or as a combination of two glutarates (the parts). Similarly, tricarballate can be derived from glutarate and acetate. This "whole" versus the

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Table 1. Binding Constants and Thermodynamic Parameters of Anions Binding to **1**

guest	K_a (M^{-1}) UV/vis[a]	K_a (M^{-1}) ITC[b]	$\Delta G°$ (kcal/mol) UV/vis	$\Delta G°$ (kcal/mol) ITC	$\Delta H°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
1,2,3,4-butanetetracarboxylate	2.2×10^5 ($\pm 2 \times 10^4$)	1.8×10^4 ($\pm 1 \times 10^3$)	−7.3	−5.8	−0.29 (±0.01)	+5.4
tricarballate	9.0×10^4 ($\pm 4 \times 10^3$)	1.9×10^4 ($\pm 3 \times 10^3$)	−6.8	−5.8	−0.47 (±0.01)	+5.4
glutarate	2.0×10^3 ($\pm 2 \times 10^2$)	4×10^2 ($\pm 1 \times 10^2$)	−4.5	−3.6	+3.3 (±0.5)	+6.8
acetate	9×10^2 ($\pm 2 \times 10^2$)	3×10^2 ($\pm 1 \times 10^2$)	−4.1	−3.4	+0.7 (±0.5)	+4.1

[a] The UV/vis data obtained from the addition of 5 μL aliquots of a 15.0 mM solution of guest to a solution buffered with HEPES (5 mM) of **1** (0.69 mM) at pH 7.4. The errors as reported are the calculated standard deviation of data for three titrations. [b] The ITC data were obtained for a binding isotherm generated from 40 injections of a 20.0 mM solution of **1** to a 1.18 mM solution of guest solution buffered with HEPES (10 mM) at pH 7.4. The errors reported are those generated from the curve fit, thus reflecting the fit of the data. NOTE: The values obtained from the ITC data were corrected for the heat generated from dilution of the host. As can be noted, both the standard deviation and error from the curve fits for $\Delta G°$ are similar, around 10% on the higher values and around 25% for the lower values. For the tetracarboxylate and the tricarballate, the points at the end of the titration curve were slightly less exothermic than the parallel dilution titration; therefore, the values were adjusted accordingly to provide a more realistic curve fit.

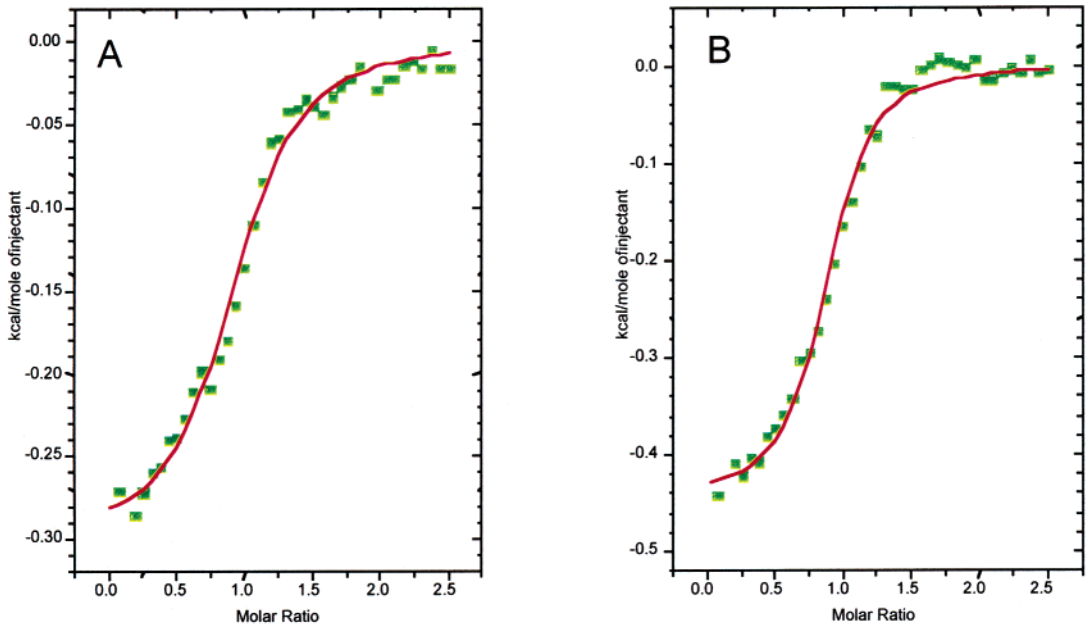

Figure 3. (A) Binding isotherm derived from titration of 1,2,3,4-butanetetracarboxylic acid (1.18 mM) with **1** (20.0 mM) at pH 7.4. The 1:1 curve fit parameters are $n = 0.97$, $K = 1.3 \times 10^4$ M^{-1}, $\Delta\Delta H = -300$ cal/mol, $\Delta S = 17.7$ cal/mol. (B) Binding isotherm derived from titration of tricarballic acid (1.19 mM) with **1** (20.0 mM) at pH 7.4. The 1:1 curve fit parameters are $n = 0.90$, $K = 2.5 \times 10^4$ M^{-1}, $\Delta H° = -444$ cal/mol, $\Delta S° = 18.6$ cal/mol.

"parts" provides an opportunity to explore the additivity of the $\Delta G°$ values of the parts ($\Delta G_A°$, $\Delta G_B°$) compared to the overall $\Delta G_{AB}°$, giving the Gibbs free energy of connection $\Delta G_S°$ (eq 1).

The analysis can be done using either the UV/vis or ITC data. The trends are the same, and to simplify the discussion the UV/vis data are discussed and the ITC data are given here in parentheses. The tabulated values (Table 2) indicate that $\Delta G_S°$ for the tetracarboxylate are −3.6 (−3.4 from ITC) and −1.8 (−1.4 from ITC) kcal/mol when this guest is considered to be a combination of tricarballate and acetate or two glutarates, respectively. Negative cooperativity is indicated for either analysis. The largest negative cooperativity arises when the acetate−tricarballate pair is compared to the tetracarboxylate binding. We indicated above that this is expected, as the affinities of tricarballate and 1,2,3,4-tetracarboxylate are the same within our experimental error for both the UV/vis and ITC analysis. Yet, even the cooperativity obtained when analyzing the increase in affinity found when going from glutarate to the tetracarboxylate or tricarboxylate is negative. When the tricarballate is considered as a combination of glutarate and acetate, negative cooperativity is again found, with a $\Delta G_S°$ of −1.8 kcal/mol (−1.2 kcal/mol by ITC).

To see how valid the analysis is relative to our errors in determining the values, we present one example of the propagation of errors. The K_a value for the tetracarboxylate ranges between 2.0×10^5 and 2.4×10^5 M^{-1} with our given error ($K_a = 2.2 \times 10^5 \pm 2 \times 10^4$ M^{-1}). Therefore, the $\Delta G_{AB}°$ for tetracarboxylate is -7.3 ± 0.05 kcal/mol. Using tricarballate and acetate as the parts leads in a manner similar to $\Delta G_A° + \Delta G_B° = (-6.8 \pm 0.03) + (-4.1 \pm 0.14) = -10.9 \pm 0.17$ kcal/mol. This leads to a $\Delta G_S°$ value of -3.6 ± 0.22 kcal/mol. Hence, the propagation of errors is below our ability to measure the values, and confident trends can be created. Table 1 lists values for the propagation of error on the $\Delta G_S°$ values determined in this manner.

How Valid Is the A−B versus A and B Analysis? The authors acknowledge that the manner in which we approach the "whole" versus the "parts" analysis is not exactly the same as described by Jencks (Figures 1 and 2). For example, let us

Table 2. Tabulated Data for the "Whole" versus the "Parts" Analysis of the Series of Carboxylate Guests[a]

A–B	A + B	ΔG_{AB}° (kcal/mol)	$\Delta G_A^{\circ} + \Delta G_B^{\circ}$ (kcal/mol)	$\Delta G_S^{\circ a}$ (kcal/mol)
		UV/vis		
1,2,3,4-butanetetracarboxylate	tricarballate + acetate	−7.3	−10.9	−3.6 ± 0.2
tricarballate	glutarate + acetate	−6.8	−8.6	−1.8 ± 0.3
1,2,3,4-butanetetracarboxylate	glutarate + glutarate	−7.3	−9.1	−1.8 ± 0.3
		ITC		
1,2,3,4-butanetetracarboxylate	tricarballate + acetate	−5.8	−9.3	−3.4 ± 0.3
tricarballate	glutarate + acetate	−5.8	−7.1	−1.2 ± 0.4
1,2,3,4-butanetetracarboxylate	glutarate + glutarate	−5.8	−7.2	−1.4 ± 0.3
A–B	A + B	ΔH_{AB}° (kcal/mol)	$\Delta H_A^{\circ} + \Delta H_B^{\circ}$ (kcal/mol)	$\Delta H_S^{\circ *}$ (kcal/mol)
1,2,3,4-butanetetracarboxylate	tricarballate + acetate	−0.3	+0.2	+0.5 ± 0.7
tricarballate	glutarate + acetate	−0.5	+4.0	+4.5 ± 1.1
1,2,3,4-butanetetracarboxylate	glutarate + glutarate	−0.3	+6.6	+6.7 ± 1.1
		$T\Delta S_{AB}^{\circ}$ (kcal/mol)	$T\Delta S_A + T\Delta S_B$ (kcal/mol)	$T\Delta S_S^{\circ *}$ (kcal/mol)
1,2,3,4-butanetetracarboxylate	tricarballate + acetate	+5.4	+9.5	+4.1 ± 1.0
tricarballate	glutarate + acetate	+5.4	+11.0	+5.6 ± 1.5
1,2,3,4-butanetetracarboxylate	glutarate + glutarate	+5.4	+13.7	+8.3 ± 1.4

[a] Values listed in this column show propagated error in the analysis.

consider our dissection of the tetracarboxylate into acetate and tricarballate. The ΔG° value for the acetate component reflects the binding of a carboxylate to the Cu(II) center, whereas the tricarballate ΔG° value reflects the binding of one carboxylate group to the Cu(II) center and two carboxylate groups potentially to two ammonium groups. Therefore, the analysis includes interaction to the Cu(II) for both the A and B fragments. A comparison more appropriate to Jencks' A−B versus A and B dissection would be the combination of only one Cu(II)−carboxylate interaction with three ammonium−carboxylate interactions.

Therefore, as stated, the manner in which the "whole" is cut into "parts" in this study is not exactly as postulated by Jencks. Our justification of the dissection procedure given resides in analyzing all the components simply as ion-pairing interactions. In this sense, we are correlating the cooperativity to the number of ion pairs formed, and we are not concerning ourselves with the specific nature of the ion pairs. However, this is an important caveat to our study that should be recognized.

Obviously some ion-pair strengths are larger than others, and here we find that a carboxylate−Cu(II) pair has a more favorable free energy of binding than a carboxylate−ammonium pair. Yet, as we describe below, the thermodynamic driving force for ligating a carboxylate to Cu(II) derives almost entirely from an increase in entropy. The binding is not due to a stronger electrostatic attraction formed between a Cu(II) and a negative carboxylate relative to water solvation of the Cu(II) and the carboxylate (it is actually slightly endothermic). Further, our study is consistent with literature precedent, which indicates that ligation of Cu(II) with carboxylates is driven by entropy, not enthalpy.[50,51] This is analogous to an emerging body of literature that indicates ion-pairing interactions between organic functional groups in water are primarily entropy driven, and one expects the association of an ammonium with a carboxylate to be driven by an increase in entropy.[7,11,13,20] Therefore, all our "parts" have the same primary driving force: entropy.

Two questions addressed herein are whether tethering all these entropy-driven interactions together will give rise to favorable enthalpy and will the entropy of connection be positive or negative. As now described, the analysis gives an interesting lesson for cooperativity in ion pairing that is primarily focused upon differences in entropies of connection while chelation of ion pairs results in small positive cooperativity in enthalpy.

Interpretation of the Enthalpy and Entropy Changes of Binding. Before examining the "whole" versus the "parts" analysis, we describe the most obvious interpretation of the enthalpy and entropy of binding data (Table 1). The ΔH° values are exothermic for the tetra- and tricarboxylate guests (−0.29 and −0.47 kcal/mol, respectively). However, the ΔH° values are endothermic for both the glutarate (+3.2 kcal/mol) and acetate guests (+0.71 kcal/mol). Additionally, the binding of all four guests is characterized by a positive $T\Delta S^{\circ}$ term.

The endothermic binding of the glutarate and the acetate to **1** indicates that the primary mode of binding to the metal center has an unfavorable enthalpy change as the guest exchanges for the counterions and/or solvent on the Cu(II) center, but their release still drives binding. The endothermic binding of a carboxylate to a Cu(II) center has been previously described in the literature and attributed to the reorganization of solvent molecules.[50,51] While the driving force of the binding of the tetra- and tricarboxylate guests is primarily entropic, the additional carboxylates relative to glutarate and acetate offer favorable ΔH° to enhance the association due to increased electrostatic interactions. With increasing carboxylate−ammonium interactions, the endothermic binding of acetate and glutarate converts to exothermic. Williams postulates that as the interactions between a host and guest increase, they become increasingly exothermic because there is less residual motion and their contacts are tighter. Our data support this view.

The favorable entropy change seen in ion-pairing interactions is postulated to arise from the displacement of waters of solvation and/or counterions from both the host and the guest into solution, thereby increasing the entropy of the system as a

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whole.[10,52−54] One would expect the solvent displacement by the tetra- and tricarboxylate to be greater relative to glutarate and acetate due to increased ion-pairing interactions. However, the $\Delta S°$ values are similar for the tetracarboxylate, tricarballate, and glutarate, but are indeed lower for acetate. It is likely that the increase in favorable entropy derived from solvent release with the larger anions is in part opposed by decreased residual motion in the complex as more binding contacts are formed. This makes the tetra- and tricarboxylate show a decreased $\Delta S°$ value relative to glutarate, with a concomitant increase in favorable $\Delta H°$.

On the Origin of Negative Cooperativity. The enthalpy and entropy values can be used to characterize the enthalpy and entropy source of the negative cooperativity by using our "whole" versus the "parts" dissection that use eqs 2 and 3 (Table 2). Treatment of the tetracarboxylate as the combination of tricarballate and acetate gives a positive $\Delta H_S°$ value (+0.53 kcal/mol) and a positive $T\Delta S_S°$ value (+4.12 kcal/mol). The data show that there is a gain in enthalpy of binding when connecting A and B (tetracarboxylate) relative to A and B separately. The enthalpy is more exothermic (−0.29 kcal/mol) than the combined enthalpy changes of A (tricarballate) and B (acetate) individually (+0.24 kcal/mol). As mentioned above, we postulate that this arises from having more ion-pairing interactions and/or the presence of shorter contact distances between binding functionalities. However, the $T\Delta S°$ values show that the binding of A−B is favorable (+5.4 kcal/mol) but to a lesser extent than simple summation of the $T\Delta S°$ values of binding of A and B separately (+9.5 kcal/mol).

The difference in $T\Delta S°$ values for binding A−B relative to A and B can have three contributing factors: (1) the presence of the tether, (2) the residual motion of the guest, and (3) the role of the solvent and/or counterions. As Jencks discussed,[31] the loss in translational entropy paid once in the case of A−B versus twice in the case of A and B offers an entropic gain to the binding of A−B. Conversely, the binding of A−B places more restrictions on the residual motions (vibrational and rotational) compared to those of A and B separately, thereby introducing an entropic loss to the binding of A−B versus A and B. Further, the associations of A−B, A, and B to the host all displace solvent and/or counterion molecules from the binding pocket, leading to an increase in the entropy of the system. It appears that the enthalpy gain of binding A−B is outweighed by the contribution of the loss of residual motion and/or decreased solvent release, thereby identifying entropy as the thermodynamic origin for the observed negative cooperativity.

The tetracarboxylate (the whole) can also be treated as the combination of two glutarate molecules (the parts). The thermodynamic data for this analysis indicate that the enthalpy change for the association of A−B (tetracarboxylate) is more exothermic (−0.3 kcal/mol) than summation of the $\Delta H°$ values (+6.6 kcal/mol) for A (glutarate) and B (glutarate) separately. This gain in enthalpy can be interpreted in an analogous manner to the tricarballate−acetate dissection. The values for the glutarate−glutarate pair reveal a positive $T\Delta S°$ (+5.4 kcal/mol) for the binding of the A−B system, but it is again less positive that the summation of the $T\Delta S°$ values (+13.7 kcal/mol) for A and B. The glutarate−glutarate combination demonstrates a greater loss in entropy for A−B versus A and B than the tricarballate−acetate pair. As discussed above, the apparent gain in enthalpy for the A−B system is outweighed by the loss in residual motion and/or decreased solvent release, which highlights entropy as being the origin of the negative cooperativity.

Last, we examine the tricarballate guest as a combination of glutarate and acetate. As in the case of the other two A−B pairs, tricarballate binding is more exothermic (−0.5 kcal/mol) than the summation of the enthalpy changes (+4.0 kcal/mol) for the individual parts. Further, as found for the tetracarboxylate, the $T\Delta S°$ value (+5.4 kcal/mol) for binding tricarballate is positive, albeit smaller than the summation (+11.0 kcal/mol) of the $T\Delta S°$ terms for the glutarate and the acetate. This again indicates that the gain in enthalpy for binding A−B is outweighed by a loss in residual motion and/or lower solvent release, pointing to entropy as the source for the negative cooperativity.

Of the three contributions to the entropy of binding, two factors must contribute to the reduced entropy of binding A−B versus A and B: loss of residual motion and decreased solvent and/or counterion release in binding A−B versus A and B. In all three analyses above, we postulate that a lower release of solvent/counterions is the major contributor. The experimental data show that the $T\Delta S_S°$ term is highly unfavorable, and it seems unlikely that this is primarily a result of decreased residual motions in binding A−B versus A or B individually. Thus, decreased solvent and/or counterion release upon binding A−B versus A and B must contribute significantly.

The introduction of a tether or covalent bond in A−B inherently leads to the occupation of a smaller volume within the host cavity than A and B separately. Additionally, individual A and B molecules have a larger solvation sphere than an A−B molecule. Therefore, upon binding A−B fewer solvents and/or counterion molecules would be released to bulk solution compared to A and B alone. Indeed, the data agree with this as the entropy changes for A−B binding are smaller than the entropy changes for A and B combined. Our study focuses upon a single system, and it remains to be seen if this is a general phenomenon. However, if this "volume analysis" is general, it may be difficult to achieve positive cooperativity in ion-pairing molecular recognition in water.

Conclusions

In summary, the experimental approach reported herein demonstrates the efficacy of using a synthetic receptor to explore the thermodynamic origin of cooperativity through binding a series of carboxylate-containing guests. The data suggest that entropy leads to the negative cooperativity of the host−guest complexes in water. This entropic contribution may arise from loss in residual motions and/or attenuated solvent or counterion release. We propose that reduced solvent/counterion release in binding A−B versus A and B individually is dominant. This is reasonable when considering the occupied volumes of the guests and the relative amounts of solvent release.

Although isolated to a specific host−guest system, these results provide a first look into the enthalpy/entropy origin of cooperativity in ion-pairing molecular recognition using a synthetic receptor in aqueous media. The study highlights the strength and value of the experimental approach, as it can be

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used to explore the cooperativity of different binding interactions within a single host design. Insights into the thermodynamic profile of binding and cooperativity can advance the field of molecular recognition to yet another level as we attempt to understand the energetics of the binding forces that promote host−guest complexation. We are extending the analysis given herein to several other host−guest systems.

Experimental Section

General Considerations. The chemicals used were obtained from Aldrich and were used without further purification, except where noted. Methanol was refluxed over magnesium and distilled. Flash chromatography was performed on Whatman 60 Å 230−400 mesh silica gel. ^{1}H (300 MHz) and ^{13}C (75 MHz) spectra were measured by a Varian Unity Plus spectrometer. Mass spectra were recorded on a Finnigan VG analytical ZAB2-E spectrometer. UV/vis spectra were collected on a Beckman DU-640 at 25 °C unless noted otherwise.

UV/Vis Titrations. The titrations were performed on a Beckman DU-640 UV/vis instrument. A typical titration is described below, although concentrations varied from experiment to experiment. A solution of the receptor (4.87 mM) was prepared and buffered with HEPES (5 mM) at pH 7.4. A similar solution of the guest (19.94 mM) was prepared. A cuvette was then filled with 860 μL of a HEPES (5 mM) solution and scanned as the blank reading. The host solution (140 μL) was introduced to the cuvette (total host concentration of 0.68 mM), and the absorbance was recorded. Aliquots of a stock solution were then added to the cuvette, and the absorbance was recorded after each addition. The stock solution contained the host (0.68 mM) and guest (12.61 mM) in HEPES buffer (5 mM). The absorbances for each addition, at a chosen wavelength, were used to calculate the δ absorbances relative to the first absorbance reading. These values were then plotted versus the concentration of the added guest for each aliquot. The binding isotherm from this raw data were curve fitted using the 1:1 binding equation (either done manually in Excel or done iteratively in Origin).

Microcalorimetric Measurements. An isothermal titration calorimeter (ITC), purchased from Microcal, Inc., MA, was used in all microcalorimetric experiments. Titration microcalorimetry[14] allows one to determine simultaneously the enthalpy and equilibrium constant from a single titration curve. ORIGIN 5.0 software (Microcal, Inc.) was used to calculate the equilibrium constant and standard molar enthalpy of reaction from the titration curves for 1:1 complexation. A typical titration is described, although concentrations and parameters varied from experiment to experiment. The reference cell was filled with a buffer solution (HEPES, 10 mM) identical to that in the titration cell. The titration cell was filled with a HEPES-buffered (10 mM, pH 7.4) solution of the guest (1.18 mM). The syringe was filled with approximately 250 μL of a solution of the host (20.0 mM) buffered with HEPES (10 mM, pH 7.4). The concentration of the syringe contents is typically 20 times that of the concentration of the cell contents. The syringe was fitted above the cell and the following parameters set: Injection size: 5 μL, number of injections: 35 at a minimum, temperature: 25 °C, injection interval: 300 s, cell feedback: 20 μcal. Following data collection, the Origin software was used to apply a 1:1 binding algorithm to the data, the fit of which yields a binding affinity, enthalpy change, entropy change, and binding stoichiometry for the titration.

***N*-(4-benzyl)*N*′-[2-(3-aminomethyl-benzylamino)-ethyl]*N*′-[(4-cyano-benzylamino)-methyl]-ethane-1,2-diamine (2).** To a flask fitted with a Dean−Stark apparatus containing toluene (150 mL) was added 3-cyanobenzaldehyde (1.9 g, 15.2 mmol). Tris-(2-aminoethyl)amine (0.76 g, 5.1 mmol) was added via syringe. The reaction mixture was heated to reflux (solution was yellow in color) for 2 h to ensure removal of water. The contents of the reaction flask were cooled, and the toluene was removed by rotary evaporation. The crude oil was dissolved in dry MeOH (150 mL) and stirred under an inert atmosphere. To the solution was added sodium borohydride (0.57 g, 15.2 mmol) as a solid portion. The reaction mixture was stirred for 1 h. Water was added dropwise to quench any remaining $NaBH_4$ and then concentrated in vacuo without further workup. The crude mixture was purified by silica gel chromatography, using 2% NH_3 saturated and MeOH in CH_2Cl_2 as the eluent. The desired product was isolated as a yellow oil in a 56% yield (1.4 g, 2.9 mmol).

^{1}H NMR (CD_3CN): δ 7.63 (s, 3H), 7.55 (d, 6H, J = 7.8 Hz), 7.39 (t, 3H, J = 7.8 Hz), 3.71 (s, 6H), 2.54 (m, 12H). ^{13}C NMR (CD_3CN): δ 143.9, 133.5, 132.3, 131.3, 130.1, 119.9, 112.7, 55.0, 53.4, 47.8. HRMS (CI+) *m/z*: 492.2871; calcd 492.2875. IR: (2224 cm^{-1})

***N*-(4-aminomethyl-benzyl)*N*′-[2-(3-aminomethyl-benzylamino)-ethyl]*N*′-[(4-aminomethyl-benzylamino)-methyl]-ethane-1,2-diamine (3).** To an ethanolic solution (20 mL) of **2** (375 mg, 0.76 mmol) saturated with NH_3(g) was added a Raney-nickel catalyst (pipet tip). The reaction mixture was sealed in a high-pressure apparatus. H_2(g) was introduced to the reaction flask at 250 psi for 24 h. The crude mixture was filtered over Celite, dried over Na_2SO_4, and concentrated by rotary evaporation to yield a yellow oil in 93% yield (353 mg, 0.70 mmol).

^{1}H NMR (CD_3CN): δ 7.17 (m, 12H), 3.69 (s, 6H), 3.66 (s, 6H), 2.54 (m, 12H). ^{13}C NMR δ 144.0, 141.1, 128.5, 127.1, 126.5, 125.7, 54.3, 53.5, 46.9, 45.9. HRMS (CI+) *m/z:* 504.3817; calcd 504.3814.

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Supporting Information Available: Isothermal calorimetry plots of binding between **1** and acetate and glutarate. UV/vis titrations of **1** with tetracarboxylate, tricarballate, glutarate, and acetate (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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