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Energetics of a Low Barrier Hydrogen Bond in Nonpolar Solvents

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Abstract: A measure of the strength of a low barrier hydrogen bond (LBHB) in apolar organic media was obtained using synthetic molecules derived from Kemp's triacid. The structures feature unusually rigid conformations that enforce intramolecular hydrogen bonds in a dicarboxylic acid, its corresponding acid-amide and their respective conjugate bases. Analysis of proton and deuterium NMR spectra established the formation of a LBHB in the conjugate base of the diacid and a conventional hydrogen bond in the conjugate base of the acid-amide. Through deprotonation equilibria with organic bases, it was determined that the conjugate base of the diacid was more stable than the conjugate base of the acid-amide by 2.4 kcal/mol in benzene and 1.4 kcal/mol in dichloromethane. These figures set the upper limits for the free energy of the **additional** stabilization arising from the LBHB at 25 °C. This value is far lower than many estimates but is closer to the recent determinations of Schwartz and Drueckhammer [*J. Am. Chem. Soc.* **1995**, *117*, 11902−11905].

Introduction

Unusually strong hydrogen bonds, known as low barrier hydrogen bonds (LBHB), have emerged as a new rationale for the exceptional catalytic abilities of some enzymes.[1−5] The existence of LBHB's in solution and especially in the solid state and gas phase has been established for some time[6,7] and the criteria for their detection are well established, but the energetics involved are harder to assess. Recently, there has been some debate on the magnitude of the energetics of the LBHB.[8−12] For condensed phases, some estimates for the **additional** stabilization from a LBHB (vs a conventional hydrogen bond) exceed 10 kcal/mol. However, a recent study of mesaconic and citraconic acid derivatives in DMSO solution indicated a considerably lower value of 4.4 kcal/mol for the extra stabilization.[13] Another study that compares enzyme-inhibitor complexes comes to an even smaller figure of ∼2 kcal/mol.[14] This value is more in line with those determined for conventional hydrogen bonds as obtained through site directed mutagenesis[15] or in unnatural amino acid replacement experiments.[16] To complement these recent findings and estimates, we examined LBHB's in low dielectric media using synthetic structures. During the review of this manuscript, a study of LBHB's in organic solvents was published.[17] This gave a value of 4−5 kcal/mol for phthalate monoanion derivatives in DMSO and 2.7−6 kcal/mol for range of phenol-phenolate derivatives in THF; no evidence for special stabilization due to p*K*a-matched LBHB's was found. During the revision of this manuscript, an analysis of hydrogen bonding in solution appeared in which the relevance of spectroscopic features of LBHB's to their strength was seriously questioned.[18] The studies described below are consistent with these recent developments.

The system involves *m*-xylidenediamine-bis(Kemp's triacid)-imide (XDK) **1a** (Figure 1a), a structure of some utility as a model in bioorganic and bioinorganic chemistry.[19] XDK features two unconjugated acids, rigidly locked into a conformation that enforces the formation of two intramolecular hydrogen bonds.[20] Of the four types of hydrogen bonds that could be formed between two carboxylic acids (Figure 1b), the arrangement in XDK allows only the syn-syn pattern. Upon deprotonation of XDK, a monoanion with an intramolecular hydrogen bond is obtained; the symmetry of this species requires that the acid and base components have precisely the same strength. This balance of p*K*a's is believed to be an essential feature of the LBHB.[4,5]

Diacid **1a** shows peculiar p*K*a's in aqueous ethanol.[21,22] The first p*K*a (4.8) is only slightly lower than that of other analogous

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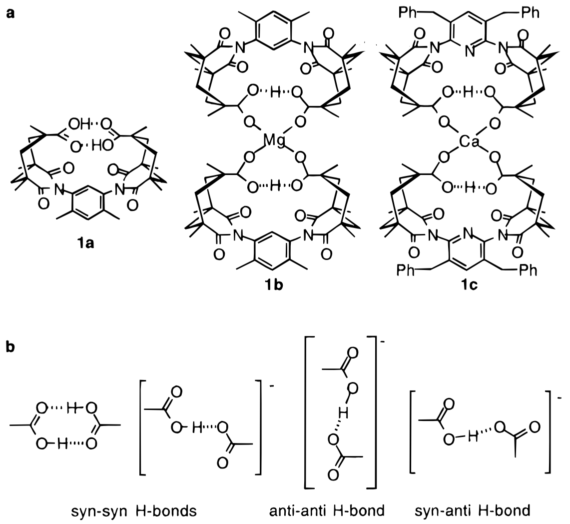

Figure 1. (a) Previously studied XDK derivatives. (b) Four types of hydrogen bonds which can be formed between two carboxylic acids.

diacids under comparable conditions, but the second p*K*a of 11.1 is remarkably high. The large second p*K*a has been attributed to the repulsion of two negative charges at a small distance, especially since the more basic syn lone pairs of the carboxylates are directed at one another in the dianion. Large Δp*K*a's in aqueous solution are also characteristic properties of hydrogen maleate and hydrogen phthalate, which form short and (presumably strong) hydrogen bonds. A strong LBHB in the XDK monoanion might also explain the large Δp*K*a's in **1a**, although it is generally believed that such hydrogen bonds do not persist in polar protic media.[23]

The magnesium complex **1b** in DMSO does indeed show a downfield NMR signal at 19 ppm, a clear indication of a strongly hydrogen bonded proton.[24] We had also observed a short hydrogen bond in the crystal structure of calcium complex **1c**. The relevant O−O distance was 2.43 Å in **1c**, while in **1a** the O−O distance of the two conventional hydrogen bonds were 2.68 and 2.69 Å.[21]

A highly lipophilic hexabenzyloxymethyl-XDK diacid **2a** was synthesized for study in apolar media. The corresponding monoamide-monoacid **3**, which offers a control was also prepared, as was the diamide **4**. The crystalline−calcium complex **2b** enabled analysis of the geometry of the LBHB-ed complex. The syntheses were carried out as outlined in Figure 2. Conversion of benzyloxymethyl-Kemp's anhydride-acid[25] to acid chloride **5** proceeded smoothly with oxalyl chloride. This material was used to acylate diaminoxylene in refluxing pyridine to yield XDK diacid **2a** in 70% yield. The corresponding diacid chloride **6** was obtained by treatment of **2a** with oxalyl chloride. When 3 equiv of ammonia was placed with diacid chloride **6** in the presence of triethylamine (TEA) in a warm sealed tube, diamide **4** was obtained in 78% yield. When compound **6** was reacted with only 1 equiv of ammonia under the same conditions, both XDK acid-amide **3** (6% yield) and diamide **4** (16% yield) could be isolated. The 2:1 diacid/calcium complex (**2b**) was prepared by treating **2a** with 0.5 equiv of CaH_2.

Characterization of the Hydrogen Bonding in Solution

The titration of diacid **2a** with triethylamine in anhydrous CD_2Cl_2 was monitored by ^1H NMR at 25 °C. The diacid signal at 12.60 ppm, characteristic of a neutral, normal hydrogen bond, was replaced by a signal at 18.0 ppm when 1 equiv of triethylamine was added. This downfield signal is consistent with that expected for a LBHB. When the system was cooled to −70 °C, this resonance moved even further downfield to 19.2 ppm. Excess triethylamine (4 equiv) was added, but the peak positions did not change beyond those observed at 1 equiv. Accordingly, the second deprotonation of the diacid does not occur under these conditions.

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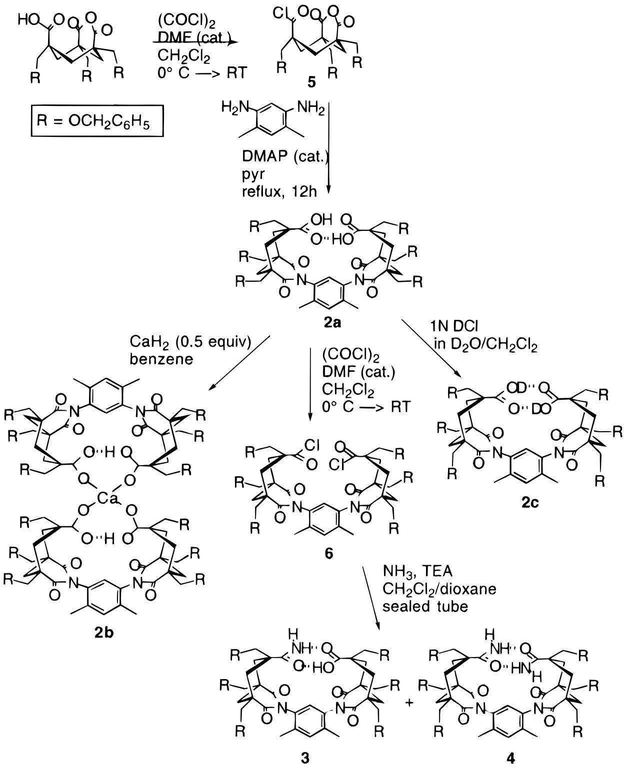

Figure 2. Synthesis of XDK derivatives.

For acid-amide **3** in $CDCl_3$ the chemical shift was at 13.80 ppm, and amide protons appeared at 8.50 and 6.03 ppm. When approximately 1 equiv of triethylamine was added, the acid signal disappeared, and the downfield (H_Z) amide signal moved to 11.6 ppm. This signal, while unusually downfield for an amide, is far upfield from the region (16−20 ppm) associated with strong hydrogen bonds. The amide H_E signal at 6.0 ppm moved only very slightly. Accordingly, the hydrogen bonds formed appear to be of a conventional type in both the neutral and monodeprotonated forms of **3**. A recent study of an amide-acid pair in peptide helices reports a much more modest shift (from 7.6 to 7.8 ppm) when a neutral glutamine−aspartate pair was deprotonated. The hydrogen bond in that case was with the H_E proton of the amide; the H_Z proton shift (~7.0 ppm) remained unchanged.[26] In diamide **4**, the chemical shifts of the amide protons were at 8.90 and 5.86 ppm; these signals also remained unchanged in the presence of added organic bases.

A deuterium isotope shift experiment[27] gave evidence that the hydrogen bond in the monoanion of **2a** could be further characterized as a double-well LBHB. The acid protons in **2a** were exchanged with deuterium by treatment with DCl in D_2O. The signal for the diacid in the ^2H NMR of **2c** (Figure 2) at

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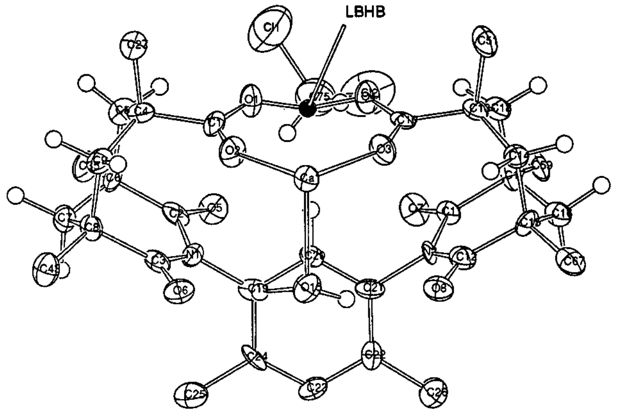


Figure 3. Front view of the **2b** structure showing the water molecule coordinated to the calcium and hydrogen-bonded to the carbonyl oxygens of the imides.

room temperature in dry CH_2Cl_2 moved to 11.86 ppm, a $\Delta\delta$(H, D) of 0.74 ppm. When 1 equiv of triethylamine was added, and the monoanion form was cooled to −70 °C, a broad peak at 17.8 ppm for the acid deuteron was observed. An energy barrier for the deuterium transfer between the two oxygens was confirmed from the sizable positive $\Delta\delta$(H, D) value of 1.4 ppm. From the chemical shifts and the isotope shifts, the diacid appears to exhibit conventional hydrogen bonds, while the monoanion forms a LBHB rather than a single-well hydrogen bond.

There exists, nonetheless, some uncertainty about the solution structure of the hydrogen bonds featured in the monoanions. There appears to be enough flexibility in the skeleton of XDK to permit independent motions of the two ends of the system.[28] The syn−syn arrangement is forced on the molecules and adjustment of the structure to give the best hydrogen bonds with guest species has been observed.[29] It is likely that the monoanion of **2a** can also attain the best geometry for intramolecular hydrogen bonds. In the absence of a metal ion, the arrangement with the spectator oxygens well-separated (as shown in Figure 1b) should be the best arrangement. However, a bifurcated hydrogen bond with the acid placed between the oxygens cannot be excluded by the evidence outlined above. Similar considerations apply to the structure of the monoanion of **3**. As we shall see, the presence of a metal ion stabilizes the spectator oxygens to give rise to another array, that observed in the solid state.

Crystallography

Recrystallization of **2b** from CH_2Cl_2 and isooctane yielded single crystals suitable for X-ray structure determination. A single crystal X-ray diffraction analysis unambiguously defined the structure of **2b** (Figures 3 and 4). The overall geometry of **2b** was analogous to that obtained earlier for **1c**.[21] The O−O distance of the short hydrogen bond was 2.42 Å, within the range for a LBHB. This short bond was in the same plane as the xylene atoms of **2b**, causing the cyclohexyl rings of the Kemp's imide-acid to be tilted out of the plane of xylene . The calcium had octahedral coordination with two molecules of **2b** positioned in the equatorial plane and two water molecules coordinated in the axial positions. The oxygen of the water molecule was coplanar with the carbonyl oxygens of the two imides, and each hydrogen of the water formed a hydrogen bond to the carbonyl oxygens of the two imides. The CH_2Cl_2 molecule filling the void cavity on the face of the xylene opposite the calcium had the two chlorine atoms in close proximity (~3.5 Å) to the acid and imide oxygens.

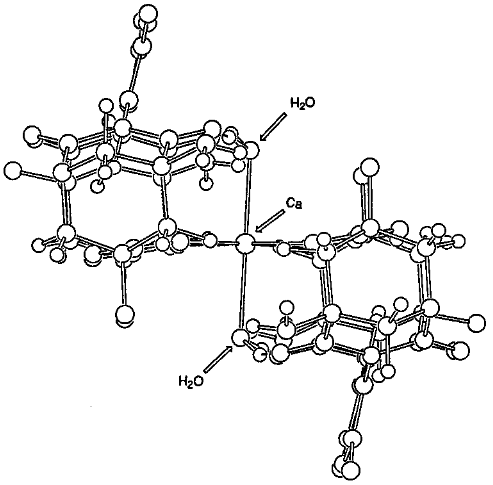


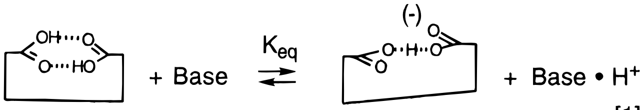
Figure 4. Side view of the **2b** complex showing the geometry of the calcium ion (benzyloxy groups and CH_2Cl_2 molecules were omitted for clarity).

Table 1. Summary of Deprotonation Studies at 25 °C

solvent	base	substrate	K_{eq} (sd)	ΔG (kcal/mol)
benzene	DPG	**2a**	2.2 (± 0.2) × 10^6	−8.3 ± 0.1
		3	2.1 (± 0.7) × 10^4	−5.9 ± 0.2
CH_2Cl_2	TEA	**2a**	6.6 (± 3.1) × 10^4	−6.2 ± 0.4
		3	3.5 (± 1.5) × 10^3	−4.8 ± 0.3

Equilibria and Energetics

Deprotonations of **2a** and **3** with diphenyl guanidine (DPG) were examined in benzene, using procedures developed to measure acid−base equilibria and p*K*a's in nonpolar organic solvents.[30] The indicator dye bromophthalein magenta E was applied with triethylamine as the base in CH_2Cl_2 at 25 °C. The equilibrium constant (K_{eq}) determined for the acid−base reactions 1 and 2 are summarized in Table 1.



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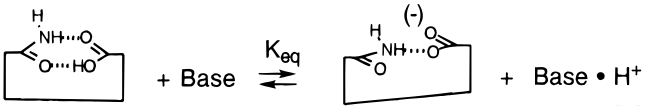

[2]

The deprotonation of the diacid was easier than the deprotonation of acid-amide **3**, a result that anticipates the generation of a stronger hydrogen bond in reaction 1 than in reaction 2. Control experiments using various amounts of diamide **4** and the indicator-base solution, showed that the nonacidic portions of the molecule neither affect the equilibrium nor alter the absorption of the indicator-base salt.

From the K_{eq} for eqs 1 and 2, the free energy of a single deprotonation to form a charged hydrogen bond was calculated, using a statistical correction for the two acid sites for the

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deprotonation of **2a** (Table 1). The difference in the free energies for the diacid and acid-amide can be partially attributed to the extra stabilization energy arising from a charged LBHB. The values in benzene and CH_2Cl_2 were determined to be -2.4 and -1.4 kcal/mol, respectively.

Discussion

This values obtained above are in general agreement with the results of Schwartz and Drueckhammer, who recently showed that the anti−anti LBHB of a carboxylate-acid pair was 4.4 kcal/mol more stable in DMSO than the hydrogen bond of the corresponding carboxylate-amide pair.[14] Our results are somewhat lower, and even then are likely to be an overestimate of the stabilization afforded by a LBHB vs its conventional counterpart. The reason is that, as in the Schwartz and Drueckhammer study, the hydrogen bonds being compared are to two acids of different acidity—the carboxylic acid and the primary amide. To be sure, the acid is unusually weak ($pKa = 11.1$ in aqueous ethanol) but still several orders of magnitude stronger than the value estimated for a primary amide ($pKa \sim 15$). In addition, the primary amide features different electrostatics from the carboxylic acid as presented to the carboxylate. According to Gilli et al.,[7] this situation leads to inherently weaker strength of a heteronuclear (N−H···O) hydrogen bond vs a homonuclear (N−H···N or O−H···O) one. A primary alcohol opposite the carboxylate would be a better reference compound from these perspectives and we are hopeful that it can be prepared for parallel studies.

The recent correlation study[5] of ΔpKa to hydrogen bond strength also showed no special energetics for the hydrogen bond forming at $\Delta pKa = 0$. Even though these studies involved different geometries, solvents, and methods, it appears that the contributions of p*K*a matched LBHB's vs p*K*a mismatched hydrogen bonds are quite modest in solution. The implications of these findings for biological catalysis will be left for others to define.[17,18]

Experimental Section

General Methods. Air-/water-sensitive reactions were performed in flame-dried glassware under argon. Tetrahydrofuran and dioxane were distilled from Na/benzophenone ketyl. Methylene chloride was distilled from P_2O_5. Unless otherwise stated, all other commercially available reagents were used without further purification.

NMR spectra were obtained on Bruker AC-250, Varian XL-300, Varian UN-300, and Varian VXR-500 spectrometers. All chemical shift values are reported in parts per million. Spectra taken in $CDCl_3$ were referenced to residual $CHCl_3$ (7.26). Spectra taken in DMSO-d_6 were referenced to residual solvent (2.49). Fourier transform infrared spectra were taken on a Perkin-Elmer infrared spectrometer. UV measurements were taken on a Perkin-Elmer Lambda2 spectrophotometer. High-resolution mass spectra were obtained with a Finnegan Mat 8200 instrument. Melting points were taken on a Elctrothermal 9100 melting-point apparatus. Flash chromatography[31] was performed using Silica Gel 60 (ICN, 230−400 mesh).

Synthesis. Anhydride Acid Chloride 5. Oxalyl chloride (120 μL, 8 equiv) was added to a cooled (ice bath) solution of Kemp's anhydride acid (100 mg, 0.174 mmol) in CH_2Cl_2 (5 mL) and DMF (5 μL) and was stirred for 5 min. The solution was warmed to room temperature and stirred overnight. The solvent was then removed *in vacuo*, and the resulting white solid was used immediately without further purification.

2,4-Bis{((*cis,cis*-2,4-dioxo-1,5,7-tris((benzyloxy)methyl)-3-azabicyclo-[3.3.1]non-7-carboxylic Acid *m*-Xylene (2a). 2,4-Dinitro-*m*-xylene (176 mg, 0.897 mmol) and 10% Pd−C (18 mg) in tetrahydrofuran (4 mL) was stirred under a H_2 atmosphere at room temperature for 24 h. The reaction mixture was filtered through Celite and then concentrated in vacuo to give a faintly purple crystalline solid. This was immediately treated with acid chloride **5** (1.00 g, 1.74 mmol) and DMAP (2 mg, 0.016 mmol) dissolved in pyridine (30 mL). The mixture was heated at reflux for 18 h under Ar. The reaction mixture was concentrated, and the brownish yellow residue was taken up in EtOAc. The organic phase was washed with 1 N $HCl_{(aq)}$ (2 × 50 mL) and brine (50 mL), then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified using flash chromatography through a silica gel column (20, 30, 40% EtOAc/CH_2Cl_2) to give crude diacid **2a** (778 mg, 70%). This was recrystallized from CH_2Cl_2/Et_2O to give 608 mg (56%) of pure **2a** as white solid: mp 187.6−188.4 °C; IR (KBr) 3488, 2862, 1735, 1686, 1648, 1453, 1364, 1192, 1097, 737, 697 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 18.70 (br s, 1 H), 7.32−7.23 (m, 31 H), 6.982 (s, 1 H), 4.486 (s, 8 H), 4.437 (s, 4 H), 3.802 (d, 4 H, $J = 8.8$ Hz), 3.348 (d, 4 H, $J = 8.8$ Hz), 3.197 (s, 4 H), 2.588 (d, 2 H, $J = 12.7$ Hz), 2.343 (d, 4 H, $J = 13.2$ Hz), 1.792 (s, 6 H), 1.591 (d, 4 H, $J = 12.2$ Hz), 1.536 (d, 2 H, $J = 12.7$ Hz); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{74}H_{77}N_2O_{14}$ (M + H), 1217.5375; found, 1217.5382.

Deuterated Diacid 2b. Diacid **2a** was dissolved in CH_2Cl_2 and washed with 1 N DCl in D_2O (3 × 8 mL). The organic phase was separated, concentrated, and dried in a vacuum desiccator.

Diacid Calcium Complex 2c. Diacid **2a** (30 mg, 0.025 mmol) and CaH_2 (0.5 mg, 0.5 equiv) were placed in dry benzene (1 mL) and stirred at room temperature for 1 h. The solution was filtered through glass wool and concentrated in vacuo: mp 200.9−203.2 °C; IR (KBr) 3488, 2862, 1735, 1686, 1648, 1453, 1364, 1192, 1097, 737, 697 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 19.4 (br s, 2 H), 7.36−7.12 (m, 62 H), 7.028 (s, 2 H), 4.58−4.47 (m, 16 H), 4.394 (d, 4 H, $J = 12.2$ Hz), 4.281 (d, 4 H, $J = 11.7$ Hz), 3.964 (d, 4 H, $J = 8.8$ Hz), 3.912 (d, 4 H, $J = 9.3$ Hz), 3.51−3.44 (m, 12 H), 3.349 (d, 4 H, $J = 8.3$ Hz), 2.769 (d, 4 H, $J = 13.2$ Hz), 2.676 (d, 4 H, $J = 13.2$ Hz), 2.590 (d, 4 H, $J = 14.2$ Hz), 1.91−1.84 (m, 16 H), 1.702 (d, 4 H, $J = 12.7$ Hz), 1.629 (d, 4 H, $J = 13.2$ Hz).

Single Crystal X-ray Diffraction Analysis of Diacid Calcium Complex 2b. Compound **2b** crystallized as clear rods from slow diffusion of isooctane into CH_2Cl_2 containing **2b**. A specimen with approximate dimensions 0.4 × 0.3 × 0.3 × mm was selected for analysis and was mounted on a fiber embedded in a matrix of Paraton N. Data were collected at −66 °C on a Siemens CCD diffractometer (equipped with an automated three circle goniometer and a solid state generator) using graphite-monochromatized Mo, K_α (0.710690 Å) by the ω scan method operating under the program SMART.[32] A total of 15 frames at 20 s measured at 0.3° increments of ω at three different values of 2θ and ϕ were collected, and, after least squares, a preliminary unit cell was obtained. For data collection three sets of frames of 20 s exposure were collected. Data were collected in three distinct shells. For the first shell, 606 frames were collected with values of $\phi = 0°$ and $\omega = -26°$. For the second shell, 435 frames were collected with $\phi = 88°$ and $\omega = -21°$, and, for the third shell, values of $\phi = 180°$ and $\omega = -23°$ were used to collect 230 frames. At the end of the data collection, the first 50 frames of the first shell were recollected to correct for any crystal decay, but no anomalies were observed. The data were integrated using the program SAINT.[33] The integrated intensities of the three shells were merged into one reflection file. The data were filtered to reject outliers based on the agreement of the intensity of the reflection and the average of the symmetry equivalents to which the reflection belongs. A total of 26 381 reflections were measured, ($2\theta_{max} = 46.6°$) of which 9955 (R_{int} for averaged reflections $= 0.06$).

The systematic extinctions and crystal density were consistent with the monoclinic space group $P2_1/n$ with half the molecule, one CH_2Cl_2 and one water of composition $C_{75}H_{80}N_2O_{15}Cl_2Ca_{0.5}$ forming the asymmetric unit. The unit cell dimensions were $a = 16.631(1)$, $b = 23.990(2)$, and $c = 17.267(1)$ Å, $\beta = 95.9645(1)°$, $V = 6852.0(6)$ Å^3 with $Z = 4$. The structure was solved using the direct methods program

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Sir92 of the TeXsan[34] crystallographic package of the Molecular Structure Corporation. The final full-matrix least-squares refinements with 4477 reflections ($2.7° < 2\theta < 40.9°$, $I > 3\sigma$) with anisotropic thermal parameters for all non-hydrogen atoms and located and riding isotropic hydrogens converged smoothly to a final $R = 0.072$, $R_w = 0.085$. A computer-generated perspective model of the final model is given in Figure 1.

Diacid Chloride 6. Oxalyl chloride (100 μL, 14 equiv) was added to diacid **2a** (100 mg, 0.082 mmol) dissolved in CH_2Cl_2 (15 mL) and DMF (5 μL) and cooled in an ice bath. After stirring for 10 min, the bath was removed and stirred for 3 h at room temperature. The clear solution gradually became cloudy. Concentration *in vacuo* yielded the diacid chloride as a white solid, and this material was used without further purification: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.4−7.2 (m, 31 H), 7.047 (d, 1 H, $J = 6.1$ Hz), 4.572 (d, 4 H, $J = 12.0$ Hz), 4.494 (s, 4 H), 4.483 (d, 4 H, $J = 12.0$ Hz), 3.889 (d, 4 H, $J = 9.0$ Hz), 3.518 (s, 4 H), 3.502 (d, 4 H, $J = 7.7$ Hz), 2.724 (d, 6 H, $J = 14.2$ Hz), 1.907 (s, 6 H), 1.717 (d, 2 H, $J = 12.7$ Hz), 1.662 (d, 4 H, $J = 14.4$ Hz).

Acid-Amide 3. Triethylamine (250 μL) was added to diacid chloride **6** (0.103 mmol) dissolved in dioxane (20 mL), and the flask was sealed with a septum. Ammonia (0.5 M in dioxane, 205 μL, 1 equiv) was added through the septum, and the slightly cloudy mixture was stirred at room temperature for 20 h. An aqueous solution of 1 N HCl (1 mL) was added to quench the reaction. The solvent was removed under reduced pressure, and the residue was taken up in CH_2Cl_2 (20 mL) and washed with 1 N $HCl_{(aq)}$ (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography through a silica gel column (using a solvent system gradient of 5, 7, 20% acetone/CH_2Cl_2) to give a mixture of acid-amide **3** and diamide **4** (30 mg). Diacid was recovered (26.6 mg, 22%). Rechromatography of the mixture (4, 8% acetone/CH_2Cl_2) yielded pure acid-amide **3** (7 mg, 6%) and diamide **4** (20 mg, 16%) as white solids: for **3** mp 86.1−87.5 °C; IR (KBr) 3421, 3179, 2860, 1700, 1453, 1400, 1365, 1193, 1101, 737, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.80 (br s, 1 H), 8.495 (br s, 1 H), 7.35−7.19 (m, 30 H), 7.095 (s, 1 H), 6.828 (2, 1 H), 6.030 (br s, 1 H), 4.59−4.43 (m, 12 H), 3.892 (t, 4 H, $J = 9.9$ Hz), 3.467 (dd, 4 H, $J = 2.7$, 8.8 Hz), 3.397 (s, 2 H), 3.311 (s, 2 H), 2.747 (t, 2 H, $J = 12.2$ Hz), 2.570 (d, 2 H, $J = 13.7$ Hz), 2.434 (d, 2 H, $J = 14.4$ Hz), 1.935 (s, 3 H), 1.927 (s, 3 H), 1.73−1.59 (m, 6 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{74}H_{78}N_3O_{13}$ (M + H), 1216.5535; found, 1216.55215.

Diamide 4. Acid chloride **6** (0.0821 mmol) dioxane (3 mL), and TEA (200 μL, 17 equiv) were combined in a round-bottomed flask and then sealed with a septum. Ammonia solution (0.5 M in dioxane, 500 μL, 3 equiv) was added through the septum, and the suspension was stirred for 24 h. The reaction mixture was concentrated, taken up in CH_2Cl_2 (20 mL), and washed with 1 N HCl (20 mL). The layers were separated, and the organic layer was washed with 1 N HCl (2 × 10 mL) and with brine (10 mL), then dried over Na_2SO_4, filtered, and concentrated. The crude white solid was purified by flash chromatography through a silica gel column (5, 10% acetone/$CHCl_3$) to give diamide **4** (78.2 mg) in 78% yield: mp 88.4−89.7 °C; IR (KBr) 3477, 3168, 2859, 1735, 1694, 1496, 1453, 1400, 1365, 1194, 1100, 736, 697 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.903 (br s, 2 H), 7.35−7.24 (m, 30 H), 7.079 (s, 1 H), 7.015 (s, 1 H), 5.856 (br s, 2 H), 4.572 (d, 4 H, $J = 12.32$ Hz), 4.490 (d, 4 H, $J = 11.2$ Hz), 4.471 (s, 4 H), 3.889 (d, 4 H, $J = 9.4$ Hz), 3.475 (d, 4H, $J = 8.5$ Hz), 3.323 (s, 4 H), 2.767 (d, 2 H, $J = 13.0$ Hz), 2.404 (d, 4 H, $J = 14.3$ Hz), 1.917 (s, 6 H), 1.689 (d, 2 H, $J = 13.0$ Hz), 1.618 (d, 4 H, $J = 14.9$ Hz); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{74}H_{79}N_4O_{12}$ (M + H), 1215.5694; found, 1215.5704.

Determination of Equilibrium Constants by UV. Reagent grade benzene, dried over 4Å molecular sieves and subsequently stored over fresh 4Å molecular sieves, and anhydrous methylene chloride stored over 4A molecular sieves, were used as solvent. Teflon stoppered quartz cuvettes (10 mm cell length) were dried in a vacuum desiccator prior to use. The 3′,3″,5′,5″-tetrabromophenolphthalein ethyl ester (Bromophthalein magenta E), diphenyl guanidine, and substrates **2a**, **3**, and **4** were stored in a desiccator prior to use. TEA was dried over 4Å molecular sieves.

Stock solutions of bromophthalein magenta E (BME) indicator (2.5×10^{-4} M, 5×10^{-4} M) and substrates **2a** (2.5×10^{-4} M, 5×10^{-4} M) and **3** (2.5×10^{-4} M, 1.25×10^{-3} M, 1.0×10^{-3} M) were prepared in both benzene and CH_2Cl_2. Solutions of diphenyl guanidine in benzene (5×10^{-4} M), triethylamine in CH_2Cl_2 (5×10^{-4} M), and diamide **4** (5×10^{-4} M) in benzene were prepared. Aliquots of indicator, base, and substrate (**2a**, **3**, or **4**) were added to the cuvette and diluted with either benzene or CH_2Cl_2 to 1.00 mL such that the indicator was 2.5×10^{-5} M, the base concentrations were 0.14−4.0 equiv, and the substrate concentration was in the range of 1−15 times that of the indicator. The solutions were capped immediately and were mixed well using a vortex mixer. The cuvettes were placed in a temperature-controlled cell holder at 25 °C and were equilibrated for 15-20 min before making absorbance measurements. A cuvette containing 1 mL of solvent was used as reference. The changes in absorbance that accompanied the conversion of BME to its diphenylguanidinium salt (540 nm in benzene) or triethylammonium salt (577 nm in CH_2Cl_2) were recorded.

A literature value for the equilibrium constant for the reaction of BME and diphenyl guanine forming the salt complex in benzene at 25 °C was used.[30] The equilibrium constant of BME and TEA in CH_2Cl_2 at 25 °C was determined from a constant indicator UV titration: aliquots of a solution of 2.5×10^{-4}, 1.0×10^{-3}, 2.5×10^{-3} M TEA in 2.5×10^{-5} M BME were added to a 2.5×10^{-5} M BME solution. The resulting curve (18 point) obtained by following the absorbance of the indicator-TEA salt was fitted to the 1:1 binding isotherm.[35] Nonlinear least-squares regression was used to curve-fit the experimental data with the Simplex algorithm as implemented in Systat 5.2.[36] Equilibrium constants for the deprotonation of the substrate were calculated as described by Davis et al.[30] At least 10 reliable measurements were taken and averaged to obtain the value for each equilibrium constant.

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[36] *Systat 5.2*, 5.2 ed.; Systat Inc.: Evanston, IL, 1992.