Molecular Recognition of Enolates of Active Methylene Compounds in Acetonitrile. The Interplay between Complementarity and Basicity, and the Use of Hydrogen Bonding to Lower Guest pK_as

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Abstract: A model system for enolase and racemase enzymes was used to explore the extent to which traditional hydrogen bonds can increase carbon acidity. Polyazaclefts **1** and **2** were investigated as receptors for active methylene enolates **10** to **19** in acetonitrile. Receptor **1** was designed to form four hydrogen bonds to the heteroatoms of the enolate guests. The synthesis of the receptors began with the central pyridine ring followed by formation of the peripheral pyrrole rings. Binding constants for **1** with the enolates **10** to **17** in acetonitrile vary from 1.75×10^2 to 2.72×10^4 M^{-1}. The nature of both the enolate functionality and the counterion were found to affect the strength of complexation. Molecular mechanics and X-ray analysis of host **1** were used to predict the geometries of the guest-host complexes. Nonaqueous titrations with picric acid were performed on enolates **10** to **17** in order to assess the degree to which complexation was changed by the basicity of the enolate. Complementarity of the guest to host **1** was found to be the dominant factor in enolate binding and not guest basicity. However, a correlation was found to exist between basicity and binding for enolates of the same shape and functionality. Binding by host **1** was found to increase the acidity of 1,3-cyclohexanedione by 1.0 pK_a unit in acetonitrile. Therefore, traditional hydrogen bonds exert only enough anion stabilization to account for a small fraction of the large pK_a shifts found for enolase and racemase enzymes. Nevertheless, this acidity increase can be exploited as a means to induce deprotonation of 1,3-cyclohexanedione in the presence of **1**.

Introduction

Both kinetic and thermodynamic barriers must be overcome by enolase and racemase enzymes in the deprotonation of the α-carbon of carbonyl substrates.[1] It has been proposed that the resulting enolate anions may be too high in energy to be viable intermediates in an enzyme-catalyzed process.[2] However, stabilization of anionic tetrahedral intermediates by amide NH groups in the oxyanion hole of chymotrypsin is well accepted.[3] Similarly, enolases and racemases may use hydrogen bonding from amino acid side chains and/or amide NHs to stabilize enolate anions, thereby increasing carbon acidity.

Recently, a different concept has been invoked to describe hydrogen bonding in enzymes: "low-barrier" hydrogen bonds.[4] When the pK_as of the anionic intermediate's conjugate acid and the hydrogen bond donor are nearly identical, exceptionally strong hydrogen bonds termed "low barrier" are postulated to form. If, however, the pK_as are significantly different, the hydrogen bonding is viewed as primarily electrostatic and is considered a traditional hydrogen bond (THB). In the case of mandelate racemase a low-barrier hydrogen bond (LBHB) has been postulated to be partially responsible for a total 25 pK_a unit decrease, thus increasing the substrate acidity by stabilization of the resulting enolate.[5] Therefore, LBHBs may yield enough stabilization to overcome the intrinsic thermodynamic problem of carbon acidity.[6] Other researchers, however, have postulated that electrostatic catalysis involving traditional hydrogen bonds and metals is sufficient to explain the increase in carbon acidity induced by mandelate racemase.[7] Independent of whether the acidity is increased by THBs or LBHBs, it is clear that hydrogen bonding is an effective means of stabilizing negative charges and increasing the acidity of carbonyl substrates.

Stabilization of an anion and the consequential increase in α-carbon acidity of a carbonyl substrate can be probed with a synthetic receptor that mimics the active site of a racemase or enolase enzyme. Such studies can lend insight into the stabilization imparted by substrate-enzyme interactions such as THBs. These studies can also probe if further interactions such as LBHBs or metal ligations need be invoked to explain the large enzyme-induced increases in carbon acidity. With this as our premise, we have examined increases in carbon acidity induced by a synthetic receptor which forms THBs in host-guest complexes.[8a] The guests examined are active methylene compounds, and the host (**1**) possesses amide-like NHs. These guests were chosen due to their increased functionality compared to simple carbonyl compounds such as cyclohexane. The increased functionality yields more sites for potential hydrogen

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(1) Kresge, A. J. *Acc. Chem. Res.* **1975**, *8*, 354. Kresge, A. J. *Pure Appl. Chem.* **1991**, *63*, 213. Gerlt, J. A.; Kozarich, J. W.; Kenyon, G. L.; Gassman, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9667. Chiang, Y.; Kresge, A. J.; Pruszynski, P.; Schepp, N. P.; Wirz, *J. Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 792.

(2) Kluger, R. *Chem. Rev.* **1990**, *90*, 1151. Thibblin, A.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 4963.

(3) Robertus, J. D.; Kraut, J.; Alden, R. A.; Birktoft, J. J. *Biochemistry* **1972**, *11*, 4302.

(4) (a) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887. (b) Cleland, W. W. *Biochemistry* **1992**, *31*, 317.

(5) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 5928.

(6) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943.

(7) Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc.* **1993**, *115*, 11569.

(8) (a) Kelly-Rowley, A. M.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1991**, *113*, 9687. (b) The *K* values reported in this preliminary communication are lower than reported herein. This is due to slight amounts of water in the earlier studies.

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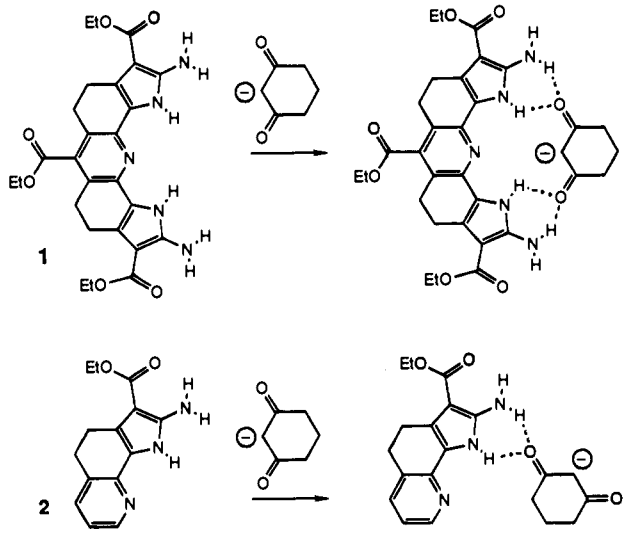
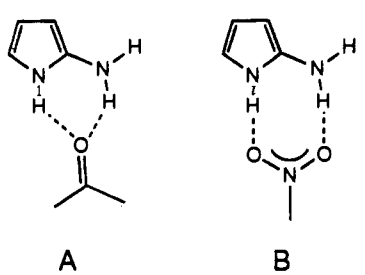


Figure 1. Idealized binding modes for **1** and **2**.

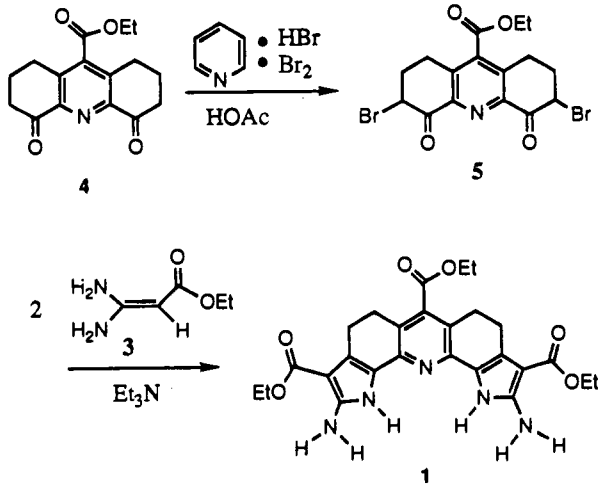
bonding compared to a monocarbonyl compound. Before the potential of **1** to shift guest pK_as was explored, experiments aimed at deciphering those factors that affect the strength of enolate binding to **1** were performed. Herein, we describe studies exploring the interplay between the complementarity and basicity of the guest. In addition, the ability of THBs to increase substrate acidity, thereby inducing substrate deprotonation only in the presence of the host, is examined. We conclude that the electrostatic interactions involved in THBs are not enough to produce large pK_a changes in dielectric media similar to enzyme active sites and that either metals or LBHBs are necessary to bring about large pK_a shifts.

Results and Discussion

A. Design Criteria. Figure 1 shows the host (**1**) studied as a model of an enolase or racemase enzyme, as well as an idealized binding mode for 1,3-cyclohexanedionate. This enolate was the guest for which the receptor was designed. It was chosen as the primary target because it possesses preorganized 1,3-diketone moieties, thereby allowing a confident prediction as to the optimum spatial positions of host hydrogen bond donors. The host possesses four hydrogen bond donors on the concave face of a cresent moon shaped cavity.[9] The two hydrogen bonds formed to each guest oxygen (A) are similar to those of the oxyanion hole of chymotrypsin, formed from the amides of Ser-195 and Gly-193.[3] In addition, such geminal positioning of the NH and NH_2 groups allows for the formation of two linear hydrogen bonds to an acceptor such as a nitro group (B). In order to enhance the hydrogen bond donating ability of these amines, an electron-withdrawing group (ethylcarboxyl) was placed on the β-carbon. Furthermore, the pyridine and pyrrole rings of **1** are fused with ethanediyl linkers to impart rigidity and preorganize the hydrogen bonding groups.[10] Host **2** was also studied as a means to evaluate the cooperativity of the C_2 symmetric sides of **1**.



Scheme 1. Synthesis of Host **1**



The electrostatic attraction that has been postulated to increase carbon acidity at active sites of enolases and racemases is predicted to be dependent upon the local solvation.[7] In addition, the ability to form LBHBs has been linked to a requirement of substrate and enolate desolvation at enzyme active sites.[4a] If reliable conclusions are to be drawn from synthetic systems as to the extent to which THBs can lower substrate pK_as, one must reproduce the local microenvironment around an enzyme hydrogen bond as closely as possible. As a mimic of the interior of an enzyme, we chose acetonitrile, which has a moderate dielectric constant (37.5), between that of chloroform (4.8) and water (78.3),[11] but does not form strong hydrogen bonds. For comparison, the dielectric constant used for calculating the electrostatic stabilization imparted by mandelate racemase was 15.[7]

B. Synthesis. The synthesis of 2-amino-3-(ethoxycarbonyl)-pyrroles can be accomplished by condensation of ethyl 3,3-diamino-2-propenoate (**3**)[12] with α-bromo ketones.[13] Hosts **1** and **2** were synthesized using this strategy. Compound **4** was formed according to a literature procedure[14] and then treated with pyridinium bromide perbromide in glacial acetic acid to give **5** in 60% yield (Scheme 1). Treatment of compound **5** with 2 equiv of **3** gave host **1** in 35% yield after purification. Host **2** was prepared in a similar manner starting from 5,6,7,8-tetrahydro-8-quinolone (**6**),[15] as shown in Scheme 2. However, direct treatment of **6** with pyridinium bromide perbromide resulted in a mixture of mostly di-α-brominated product and starting material, with very little formation of **8**. Control of the ketone reactivity was accomplished by converting it to the silyl enol ether **7**. Treatment of **6** with tBDMS-CF_3SO_3 and Et_3N in dry CH_2Cl_2 gave **7** in 93% yield after chromatography. Treatment of **7** with pyridinium bromide perbromide gave **8** as the primary product. Condensation of **3** and **8** resulted in an 82% yield of **2**.

The reaction of **3** with **5** or **8** could potentially yield different isomers, although condensation resulted in only one product

(9) For the advantages of such "crescent moon" shaped receptors see: Perreault, D.; Chen, X.; Anslyn, E. V. *Tetrahedron* **1995**, *51*, 353.

(10) Preorganization has been found to be crucial in similar systems. Zimmerman, S. C.; Mrksich, M.; Baloga, M. *J. Am. Chem. Soc.* **1989**, *111*, 8528.

(11) Dean, J. A. *Lange's Handbook of Chemistry*, 13th ed.; McGraw Hill Book Co.: New York, 1985.

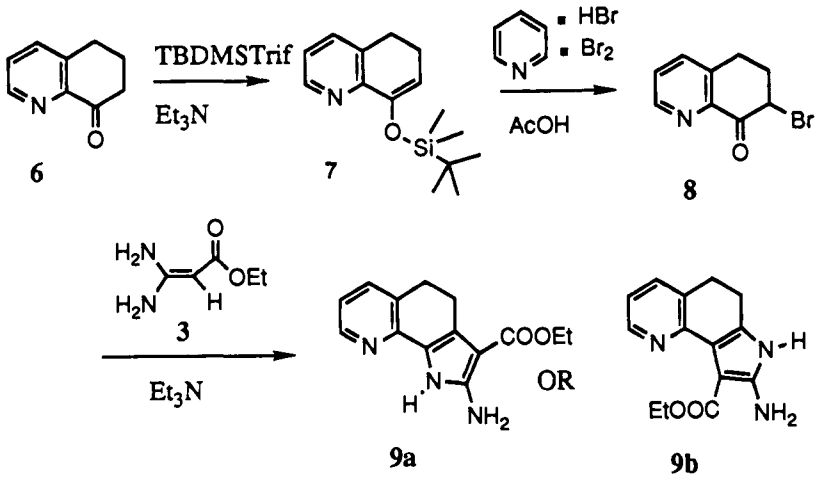
(12) Meyer, H.; Bossert, F.; Horstmann, H. *Liebigs Ann. Chem.* **1977**, 1895.

(13) Toja, E.; DePaoli, A.; Tuan, G.; Kettenring, J. *Synthesis* **1987**, 272.

(14) Kneeland, D. M.; Ariga, K.; Lynch, V. M.; Huang, C.-Y.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 10042.

(15) Thummel, R. P.; Lefoulon, F.; Cantu, D.; Mahadevan, R. *J. Org. Chem.* **1984**, *49*, 2208.

Scheme 2. Synthesis of Host **2**

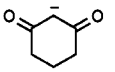
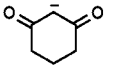
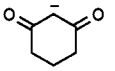
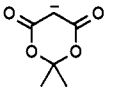
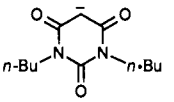
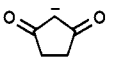
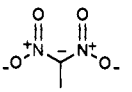
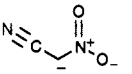
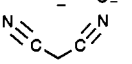
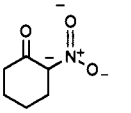
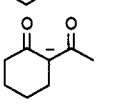
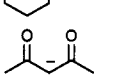


(see Scheme 2, **9a** and **9b**). The regiochemistry of addition of **3** with α-bromo ketones has been previously examined by analysis of mass spectra ion fragments.[13] This precedent predicts the regiochemistry shown in **9a**. To support or refute this prediction, a $^{13}C-^{13}C$-correlated 2-D NMR study was performed (see the supplementary material). The C−C connectivity was consistent with **9a** (henceforth called compound **2**).

C. Complexation Studies. a. Preliminary Considerations. In order to determine which enolates of active methylene compounds are the best candidates for probing pK_a shifts in the presence of **1**, a variety of guests possessing different functional groups were studied (Table 1). Addition of these enolates to **1** or **2** resulted in 1 to 2 ppm downfield shifts of the NH and NH_2 ^1H NMR resonances, with very little change in any other host chemical shifts (Figure 2). Since the chemical shifts of acidic groups such as OHs and NHs are quite sensitive to aggregation, a dilution study was performed to assure the absence of host aggregation. A saturated host solution in CD_3CN, prepared in the same manner as that used in the binding studies (see the Experimental Section), was incrementally titrated with CD_3CN. The resultant plot of chemical shift vs concentration showed that the pyrrole NH resonances were shifted downfield by a total of 0.024 ppm and the NH_2 resonances were shifted downfield by 0.001 ppm. These small shifts give no evidence for aggregation. Further, these changes are negligible compared to the 1 to 2 ppm changes observed in host−guest binding.

b. Binding. The interaction of host **1** in CD_3CN with enolates **10** to **19** (Table 1) was investigated by ^1H NMR. In each binding experiment, the host concentration was kept nearly constant while the concentration of the guest was incrementally increased. The guest to host ratio was initially 0 and was usually increased to 6, although some experiments were taken to 12 when binding was weak. The NH resonances occasionally became too broad to measure, in which case the NH_2 chemical shifts were used to generate the binding isotherm. Plots of downfield chemical shift movement versus guest to host ratio conformed to the typical 1:1 binding algorithm (Figure 2)[16] and were modeled with a computer program.[17] Table 1 summarizes the binding constants determined for the two hosts and various enolate guests.[8b] The counterion used for all anions was 15-crown-5 sodium. In addition, 1,3-cyclohexanedionate was also examined with two other counterions: sodium [2.2.1]cryptand and bis(triphenylphosphoranylidene)ammonium (PPN^+).

Table 1: List of Anions Studied as Guests with **1**, the Anion numbers, pK_as, and Binding Constants

anion[a]	number	pK_a	K (M^{-1}) to **1**	K (M^{-1}) to **2**
	**10a**	20.7	1.35×10^4	1.35×10^2
	**10b**[b]		2.72×10^4	
	**10c**[c]		1.16×10^4	
	**11**	19.2	7.90×10^2	
	**12**	19.5	1.48×10^3	
	**13**	19.2	5.88×10^3	
	**14**	16.9	3.93×10^3	
	**15**	15.9	6.70×10^2	
	**16**	21.2	1.75×10^2	
	**17**	20.4	8.12×10^3	1.60×10^2
	**18**		insignificant binding	
	**19**		insignificant binding	

[a] All counterions are 15-crown-5 Na^+, except for *b* [2.2.1]cryptand Na^+ and *c* bis(triphenylphosphoranylidene)ammonium. Temperature was 23 °C.

c. Analysis of Binding Data. Inspection of Table 1 reveals several key points. **Point 1.** Comparison of binding constants for the various enolates reveals that ketones and nitro groups

(16) (a) Wilcox, C. S.; Cowart, M. D. *Tetrahedron Lett.* **1986**, *27*, 5563. (b) Sheridan, R. E.; Whitlock, H. W. *J. Am. Chem. Soc.* **1986**, *108*, 7120.
(17) Neder, K. M.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 9412. Bell, D. A.; Anslyn, E. V. *J. Org. Chem.* **1994**, *59*, 512.

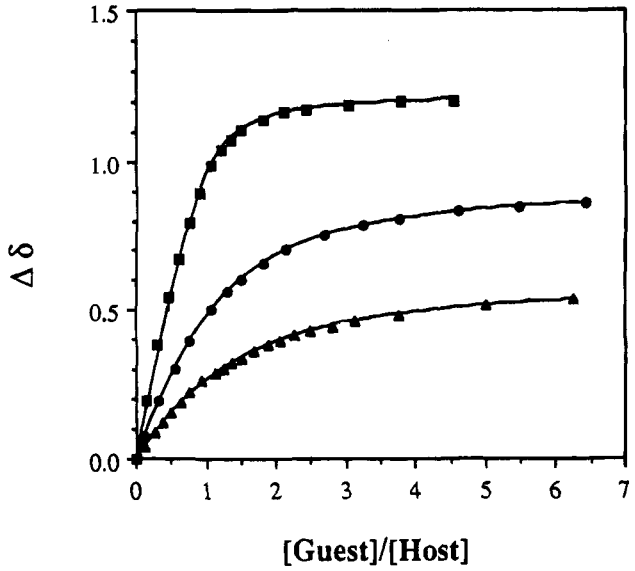


Figure 2. Change in the ^{1}H NMR chemical shift of the host pyrrole protons with increasing concentration of anionic guests. (■) is **10a**, (●) is **12**, and (▲) is **11**.

are bound more strongly than cyano groups. This is likely due to the fact that the heteroatoms on ketones and nitro groups can adopt the A and B binding mode discussed previously, whereas cyano groups may form only one hydrogen bond.

Point 2. Enolates which possess conformational freedom (**18** and **19**) do not show evidence of binding. Apparently the stability gained upon complexation with the host does not compensate for the energy required to assume a higher energy conformation[19b] that is complementary to the host. These problems do not arise in the conformationally restricted enolates.

Point 3. Of the 15-crown-5 sodium enolates studied, **10** possessed the strongest binding and is therefore presumed to be the most complementary to the host. However, there is a complicating factor in that there should be a correlation between the basicity of the anion and the strength of complexation.[17] This complication will be examined further in section E, Nonaqueous Titrations.

Point 4. The nature of the counterion affects the binding properties of an enolate, as revealed by a comparison of the binding constants of enolates **10a**, **10b**, and **10c**. The more free the anion is of its counterion, the stronger the binding observed with host **1**. Crystal structures of 15-crown-5 sodium reveal an open coordination site on the sodium which can be filled by an enolate anion.[18] In contrast, [2.2.1]cryptand leaves no open coordination sites; therefore, an enolate counterion is more "naked".[19a] The complete saturation of sodium coordination sites by [2.2.1]cryptand resulted in a 2-fold increase in binding constant compared to 15-crown-5. Apparently the PPN^+ counterion yields an ion pairing strength with the enolate that is even stronger than the [2.2.1]cryptand and 15-crown-5 sodium since **10c** has the lowest binding constant.

Point 5. The two symmetrical sides of **1** act cooperatively in the binding of enolates. Jencks has discussed the issues for determining cooperativity that are appropriate to our system.[20] He defines a term, ΔG_s, as a connection Gibbs energy that represents the change in the binding probability of two groups, A and B (eq 1). From eq 1, ΔG_s can be found to be eq 2,

$$\Delta G_{AB} = \Delta G_A + \Delta G_B + \Delta G_S \quad (1)$$

$$\Delta G_S = RT \ln(K_{AB}/K_A K_B) \quad (2)$$

where ΔG_A and ΔG_B represent the intrinsic binding energy of two different regions of a molecule A–B. We can consider **1** as the connection of two compounds like **2**. Therefore the binding of **10** with **1** is defined as K_{AB}, whereas the binding of **10** with **2** is K_A. The K_B term is more difficult to define. This binding constant represents the Gibbs free energy of binding the second ketone in **10** after the first ketone has been bound. This is clearly not as large as K_A since the enolate charge has already been stabilized and partially neutralized by hydrogen bonding. As an estimate of K_B, the binding constant of neutral 1,3-cyclohexanedione to **2** is used. This binding between 1,3-cyclohexanedione and **2** should be similar to K_B since it is between a neutral ketone and **2**, thereby mimicking the second ketone of **10** whose charge has already been significantly neutralized by hydrogen bonding. The binding of 1,3-cyclohexanedione is extremely weak, and we estimate an upper limit on the bonding constant of 5 M^{-1}. Using this as K_B, we calculated $\Delta G_s = 1.8$ kcal/mol. This is a significant chelation effect and demonstrates that the two halves of **1** are acting cooperatively. Although a K_B lower than 5 M^{-1} would increase the ΔG_s, the estimated 1.8 kcal/mol is best considered as an upper limit. The calculation is based upon the assumption that binding by the first host pyrrole localizes all of the negative charge on one oxygen and the other oxygen is consequently similar to a neutral ketone.

The binding studies show that enolates complex **1** with moderate to large binding constants and that the symmetrical halves of **1** act cooperatively. Thus, receptor **1** can be confidently used as a model of enolate anion stabilization by enolase or racemase enzymes.

D. Structural Studies. To further assess the predictive power of using **1** as a model for enzymatic enolate stabilization, it was important to determine the number of hydrogen bonds that form between **1** and the enolates. Unfortunately, attempts to obtain X-ray quality crystals of enolate–host complexes were unsuccessful. Cocrystals do form, but they are very thin needles which are disordered. However, the crystal structure of host **1** with picric acid was obtained, and an Ortep diagram is shown in Figure 3A. The central pyridine ring of the host has been protonated, and the picrate anion is bound in the cavity. The host is nearly planar: only the ethanediyl linkages and one ester are out of plane. The phenol oxygen of the anionic guest is bound to both a pyrrole and an amino group hydrogen on one-half of the host. The nitro group of the guest is hydrogen bound to the second NH and NH_2 group, but the anion is slipped toward the protonated central pyridine ring as it is the most acidic hydrogen on the host.

The picrate counterion mimics enolate **17**. The picrate phenol oxygen and a nitro group are in essentially identical positions to the ketone oxygen and nitro group of **17**, and thus the binding mode of picrate should be similar to that of **17**. Thus, a binding mode as shown in complex **20** is predicted for the complex between 1 and **17**. Furthermore, the geometrical placement of the phenol oxygen and the aromatic ring of picrate also mimicks one ketone and the cyclohexane ring of **10**. Using an arrangement of **10** similar to that of picrate as a starting point for molecular mechanics,[21] the structure shown in Figure 3B is obtained after minimization. As shown by the idealized structure in Figure 1, the enolate forms four hydrogen bonds to

(18) Bush, M. A.; Truter, M. R. *J. Chem. Soc., Perkin Trans. II* **1972**, 341.

(19) (a) Mathieu, F.; Metz, B.; Moras, D.; Weiss, R. *J. Am. Chem. Soc.* **1978**, *100*, 4412. (b) Bernasconi, C. F.; Kanavarioti, A. *J. Am. Chem. Soc.* **1986**, *108*, 7744.

(20) Jencks, W. P. *Proc. Natl. Acad. Sci., U.S.A.* **1981**, *78*, 4046.

(21) Still, C. MACROMODEL, version 2.5. Columbia University, Amber Force Field.

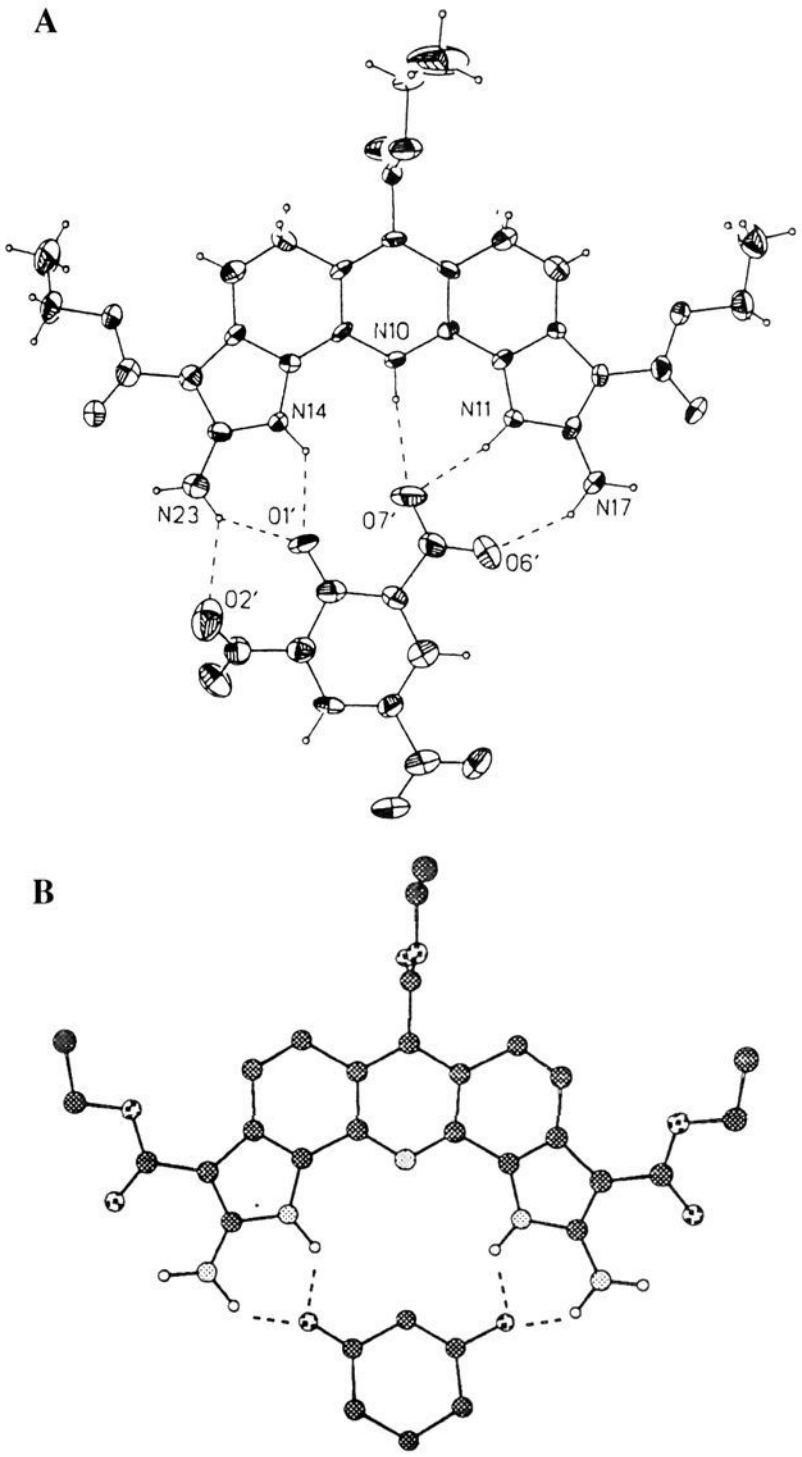
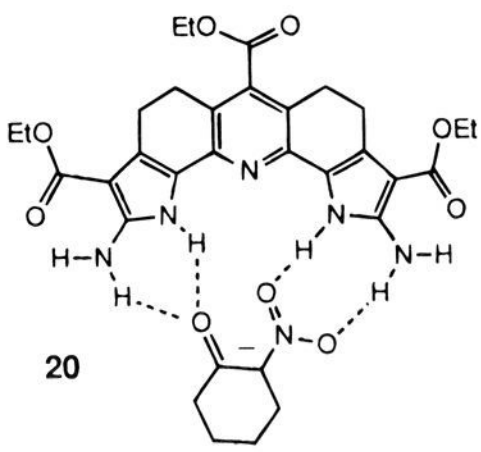


Figure 3. (A) X-ray structure of host **1** with picric acid. (B) Molecular mechanics derived structure for host **1** and anion **10**.

the receptor. Each oxygen of **10** is placed between an NH and NH_2 of the host. All the heteroatom distances between the host Ns and guest Os are less than 3.0 Å, indicating optimum hydrogen bonding. The evidence from the crystal structures and the molecular modeling supports four hydrogen bonds between the enolates and host **1**. Four hydrogen bonds should be a sufficient number for modeling the extent to which neutral hydrogen bonds will shift the pK_a of bound guests.



20

E. Nonaqueous Titrations. The guest pK_as must be known in acetonitrile in order to evaluate the potential for shifting these pK_as with **1**. In addition, in section C it was suggested that the basicity of the anions as well as the complementarity should play a role in determining the strength of complexation to **1**. To test these concepts, the determination of the pK_a values of the anions was necessary. Nonaqueous titration methods are well established in the literature.[22] Solvent differences such as dielectric constant and hydrogen bonding ability cause the measured pK_a values in acetonitrile to be higher than those found in aqueous titrations. The following sections describe the determination of the pK_a values for the enolates studied and the effect of binding on the measured pK_a values of 1,3-cyclohexanedione.

a. Enolate Titrations. Titrations were carried out on the 15-crown-5 sodium salts of the guests. This was done so that direct comparison to the association constants given in Table 1 could be made. The titrant chosen was picric acid (pK_a =11.0 in acetonitrile)[23] since it is a strong acid in acetonitrile in comparison to all the enolates titrated.[24] pHs in acetonitrile were measured by converting millivolt readings generated by a pH single-junction electrode and a $Ag/AgNO_3$ reference electrode as described in detail in the Experimental Section.

In aqueous solutions it is possible to describe the pH titration curve of weak acids and bases around a single pK_a using the Henderson−Hasselbach equation. In nonaqueous titrations this relationship may or may not describe the pH around the pK_a. The slope may be unusually steep or flat. For example, titrations of phenols in several inert solvents have resulted in unusually steep slopes which are attributed to associations of the phenols with phenolate ions,[25] a phenomenon called homoconjugation.[26] The explanation is that, early in the titration, the phenol is more acidic since the resulting phenolate becomes stabilized by hydrogen bonding. As the titration proceeds, the phenol becomes less acidic since the acidic hydrogen is involved in intermolecular hydrogen bonding. Similarly, a flat curve is indicative of the base being less basic early in the titration and becoming increasingly basic as the titration progresses. Several of the experimentally determined titration curves for enolates **10**−**17** were found to be quite flat, whereas others could be well modeled with the Henderson−Hasselbach equation. Two examples of our experimental curves along with the best fit Henderson−Hasselbach curves are shown in Figure 4. Figure 4A is an example of a "flat" curve which deviates significantly from the theoretical curve, whereas Figure 4B shows a curve which more closely conforms to the Henderson−Hasselbach equation. In general, for the 15-crown-5 sodium salts, the more basic the anion, the flatter the curve. We propose that intermolecular association of these guests with their corresponding counterions is occurring and that this association causes the flat titration curves. As discussed in section C, the X-ray structure of benzo-15-crown-5 sodium shows an open site which would allow complexation of an enolate to the 15-crown-5 sodium counterion.[18] Strong interaction with the counterion would be expected to affect the shape of the titration curve by making the anion less basic early in the titration curve. The more basic the enolate, the stronger the complexation to the counterion and, therefore, the more pronounced the "flattening" effect. In support of this postulate, a titration of sodium [2.2.1]-cryptand **10** yielded a curve possessing very definite upward

(22) (a) Fritz, J. S. *Acid-Base Titrations in Nonaqueous Solvents*; Allyn and Bacon: Boston, 1973. (b) Reference 22a, p 13.

(23) Kolthoff, I. M.; Chantooni, M. K., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 4428.

(24) Kolthoff, I. M.; Bruckenstein, S.; Chantooni, M. K., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 3917.

(25) Van der Heijde, H. B. *Anal. Chim. Acta* **1957**, *16*, 392. Harlow, G. A.; Bruss, D. B. *Anal. Chem.* **1958**, *30*, 1836. Fritz, J. S.; Yamamura, S. S. *Anal. Chem.* **1957**, *29*, 1079.

(26) See 27 as well as Harlow, G. A.; Bruss, D. B. *Anal. Chem.* **1958**, *30*, 1833. Coetzee, J. F.; Padmanabhan, G. R. *J. Am. Chem. Soc.* **1965**, *87*, 5005. Kolthoff, I. M.; Chantooni, M. K., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 4428.

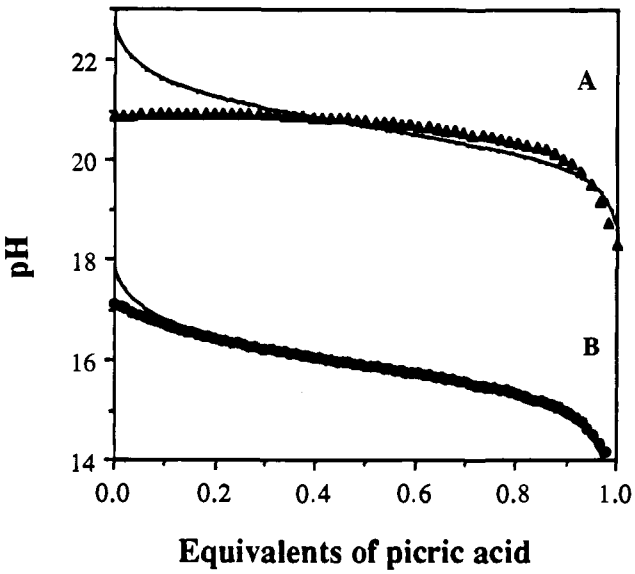


Figure 4. pH titration curve vs the theoretical curve for sodium 15-crown-5 1,3-cyclohexanedionate (▲), and the pH titration curve vs the theoretical curve for the sodium 15-crown-5 enolate of cyanonitromethane (●).

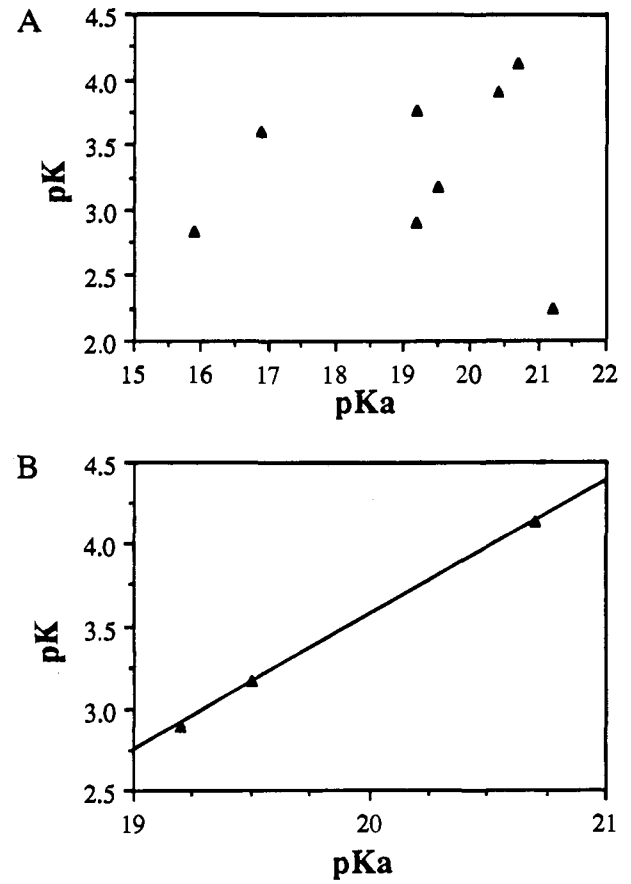


Figure 5. (A) Plot of pK vs pK_a for all of the anions. (B) Plot of pK vs pK_a for 1,3-diketone enolates **10a**, **11**, and **12**.

slope throughout the titration and conformed to the Henderson–Hallselbach equation (supplementary material). This enolate salt cannot form a direct coordination between the sodium and the enolate; therefore it does not show a "flat" titration curve.

Table 1 lists the pK_a values found for the enolates that were studied for binding to **1**. A plot of the pK_a values vs the pK values is shown in Figure 5A. This shows that there is no simple relationship between binding with host **1** and the pK_a of the anion. That is, an increase in basicity does not imply an increase in binding constant. To the contrary, the dominant factor is the geometric complementarity to the host, with the divergent 1,3-diketone structures being the most complementary. However, when a series of 1,3-diketone enolates with similar shape are compared, as in Figure 5B, there is a linear relationship between the pK and the enolate pK_a. Thus, within a series of similar guests, the basicity is of paramount importance in determining binding strength.

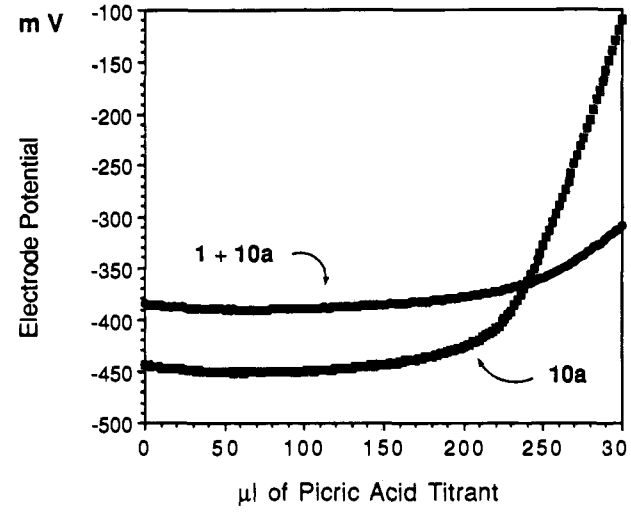


Figure 6. Titration of the **10a** in the presence and absence of host **1**.

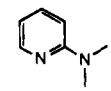
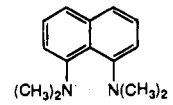
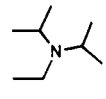
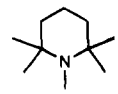
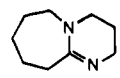
b. pK_a Shift Experiment. As discussed in the Introduction, stabilization of an enolate would be expected to cause the conjugate acid to become more acidic. The purpose of these experiments was to determine if this is indeed the case and to what extent traditional hydrogen bonds can shift enolate pK_as in a microenvironment similar to that of an enzyme active site. The strong binding of anions such as **10** indicated that 1,3-diketone enolates are the best targets for measuring pK_a shifts with **1**.

Figure 6 shows the resulting titration curves for **10a** alone and the complex between **1** and **10a**. A 1.0 ± 0.3 pK_a unit difference was found. The 1.0 pK_a unit difference for **10a** in the presence of **1** represents the minimum pK_a shift induced by **1**. At the start of the titration, **10a** was 93% bound to host **1**. Anions **11** and **12** do not bind as tightly and are only 41 and 57% bound to host **1** at the start of titration. pK_a shifts of 0.7 and 0.8 were found for these anions when bound to **1**. A decrease in pK_a of only 1 unit indicates that amide-like NH groups do not bring about large pK_a changes in moderate dielectric media even when four hydrogen bonds are formed between the carbonyl substrate oxygens and the receptor.

F. Selective Deprotonation. In the enolization catalyzed by mandelate racemase, a lysine (aqueous pK_a near 10.5) efficiently deprotonates the carbon acid substrate (pK_a near 29–32). Even though host **1** induced only a small pK_a change in 1,3-cyclohexanedione, we were intrigued at the possibility of using **1** to trigger deprotonation of the ketone guest in a similar manner to mandelate racemase. As with mandelate racemase, the strategy was to choose a base whose pK_a is lower than the pK_a of the guest but close enough to the pK_a of the host–guest complex to allow deprotonation. Thus, knowledge of the pK_as of a variety of bases in acetonitrile was required.

Table 2 shows the bases and pK_as which were determined. The bases were titrated in acetonitrile with picric acid as described in the Experimental Section. All the experimentally determined titration curves for these amines conformed to the

Table 2: List of Bases Studied for Inducing Binding of **1** and **10**, and Their pK_as in Acetonitrile

Compound	pKa
	13.4
	17.8
	18.1
	18.2
	23.9[32]

Henderson–Hasselbach equation. These pK_as allow predictions to be made as to whether deprotonation of 1,3-cyclohexanedione (pK_a 20.7) can be induced by the presence of **1**. For example, a base such as DBU, which has a much higher pK_a of 23.9, will completely deprotonate the guest *irrespective* of the presence of **1**, whereas an amine such as 2-(*N*,*N*-dimethylamino)pyridine (pK_a = 13.4) will barely deprotonate the guest independent of the presence of **1**. However, a base such as 1,2,2,6,6-pentamethylpiperidine (pK_a = 18.2)[27] should deprotonate a larger percentage of 1,3-cyclohexanedione when in the presence of **1** than without addition of **1**. Monitoring the NH_2 groups of **1** gives the extent to which the host–guest recognition is triggered by the addition of the various bases and thereby yields the extent to which **1** can be used to induce deprotonation of the guest.

Figure 7 shows the movement of the NH_2 ^1H NMR resonances of **1** when in the presence of 2 equiv of 1,3-cyclohexanedione and various concentrations of amines. The strong base DBU gives a curve reminiscent of the binding isotherm shown in Figure 2 since as predicted, DBU completely deprotonates the neutral guest. In complete contrast, addition of 2-(*N*,*N*-dimethylamino)pyridine leads to absolutely no complexation. An intermediate response to the basic trigger was found for pentamethylpiperidine (PMP). For this amine the calculated curve for the extent of binding based upon a 1,3-cyclohexanedione pK_a of 20.7 is shown in Figure 7. The calculated curve based upon the pK_a of unbound guest does not match the experimentally determined curve. Instead, significantly more 1,3-cyclohexanedione is bound than would be predicted on the basis of its pK_a. This experiment demonstrates that the addition of a hydrogen bonding receptor with amide-like NHs can reduce the pK_a of a carbon acid and thereby allow for deprotonation by a base which otherwise would not significantly deprotonate the acid. Therefore receptor **1** can reduce the thermodynamic barrier to deprotonation of a carbon acid in a similar manner to mandelate racemase, albeit to a much less dramatic extent.

(27) For comparison, the pK_a of triethylamine in acetonitrile has been reported to be 18.46. Coetzee, J. F.; Padmanabhan, G. R. *J. Am. Chem. Soc.* **1965**, *87*, 5005.

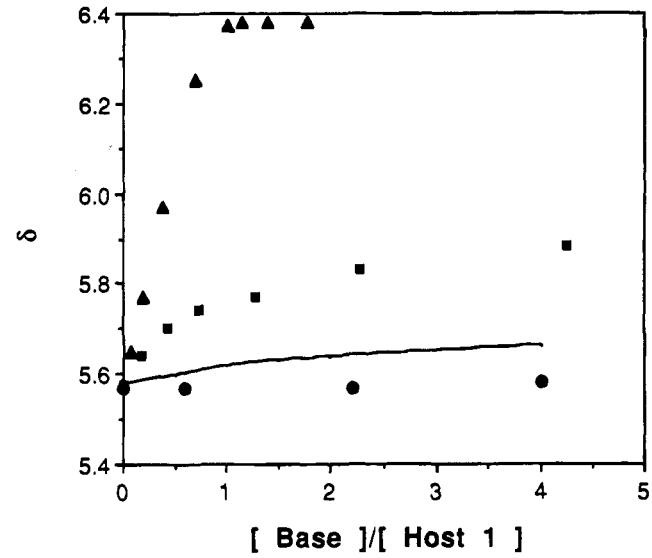


Figure 7. Binding isotherm for **10** and **1** as a function of concentration of various bases. ▲ = DBU, ■ = 1,2,2,6,6-pentamethylpiperidine, ● = (2-*N*,*N*-dimethylamino)pyridine. The line represents the isotherm that would be generated with PMP if there was no change in guest pK_a upon binding to **1**.

Conclusions

Traditional hydrogen bonding has been used to bind enolates in acetonitrile. The binding with **1** is selective for divergent 1,3-diketone-like enolates even though these enolates are less basic than others that were investigated. However, within a series of similar enolates, increased basicity leads to increased binding. In addition, the hydrogen bonding stabilizes the enolate, rendering the corresponding conjugate acid more acidic. While we were able to demonstrate that the pK_a shift can in principle induce deprotonation of the guest, the pK_a shift was quite small. Even with four hydrogen bonds, THBs from NH groups only gave a 1.4 kcal/mol increase in acidity. Therefore, THBs are only responsible for a small fraction of the pK_a shifts found for enolase and racemase enzymes.

Charged electrophilic groups in a synthetic host or an enzyme will likely increase the magnitude of carbon acid pK_a shifts. For example, charged hydrogen bonds donated from basic amino acid side chains would be expected to be stronger, thereby potentially increasing the pK_a shift. In addition, other electrophiles such as metals would likely increase guest acidity further via large electrostatic effects in the low-polarity microenvironment of an enzyme active site. Finally, LBHBs may account for part of the much larger enzyme-induced pK_a shifts. We are currently exploring synthetic hosts incorporating metals and/or hydrogen bond donors with pK_as more closely matched to the guests as a means to lower the thermodynamic barrier to carbon acidity.

Experimental Section

A. General Considerations. Instrumentation. ^1H and ^{13}C NMR spectra were obtained using a Bruker AC-250 or General Electric QE-300 spectrometer. 2-D spectra were recorded on a General Electric GN-500. Melting points were measured on a Thomas Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc. Low-resolution mass spectra were measured in CI mode with a Finnigan TSQ70 spectrometer. High-resolution mass spectra were recorded with a CEC 21-110B instrument in CI mode.

Materials. Preparative flash chromatography was performed on Scientific Adsorbents Incorporated silica gel 40 μM. Analytical TLC was performed on precoated silica gel 60 F-254 plates. Ether and THF

were distilled from sodium and benzophenone. Dichloromethane, acetonitrile, and triethylamine were refluxed and distilled from calcium hydride. The ozonolysis reactions were carried out on a Welsbach T-816 ozone generator at 90 V and 1−2 L/min. Compounds and solvents were dried and stored in a Vacuum Atmosphere drybox MO-20.

Compounds. Compound **4** was synthesized as described previously.[14] Compound **6** was synthesized according to the method of Thummel.[15] Compound **3** was synthesized according to the method of Meyer.[12] 1,3-Di-*n*-butylbarbituric acid was prepared according to the method of Goldner.[28] 1,1-Dinitroethane was prepared according to the method of Borgardt.[29] Cyanonitromethane was prepared by the method of Reid and Kohler from methazonic acid,[30] which was prepared by the method of Dunstan and Goulding.[31] The starting materials for all syntheses were purchased from Aldrich Chemical Co.

B. Analytical Studies. a. Binding Studies. All ^{1}H NMR binding studies were carried out on a Bruker AC-250 NMR spectrometer using CD_3CN which had been distilled from calcium hydride. All solutions were prepared in the drybox. Septa and nitrogen gas balloons were used to exclude water from the solutions when outside of the drybox. All glassware such as vials and NMR tubes was dried in an oven and stored in the drybox. Anions were prepared from neutral substrates under nitrogen and were either kept in the drybox or in a desiccator at all times.

The solubility of host **1** is low in acetonitrile. Accordingly, saturated solutions of **1** (1.4×10^{-3} M) were prepared in the drybox 12 to 24 h in advance of a study, and **2** was studied at approximately the same concentration. The concentrations of hosts **1** and **2** were determined by UV−vis spectroscopy on a Beckman DU-70 UV−vis spectrophotometer. The extinction coefficient of host **1** at 327 nm is 26 975 cm^{-1} m^{-1}. The extinction coefficient of host **2** at 290 nm is 8538 cm^{-1} m^{-1}. Just prior to the start of a study, 500 μL of a host stock solution was transferred via syringe into an NMR tube which was then capped with a septum. The concentration was determined by UV−vis spectroscopy. A guest stock solution needed to reach the desired guest to host ratio in a maximum of 100 μL was prepared. To accurately determine the concentration of the guest stock solution, integration of guest peaks against host peaks was performed.

The ^{1}H NMR spectrum of the initial pure host solution was recorded, and then some volume (typically 2 μL at the beginning of a study) of the guest stock was transferred into the NMR tube via a syringe. The spectrum was recorded and then the process was repeated until the desired guest to host ratio was obtained.

b. Nonaqueous Titrations. All pK_a measurements were carried out in Omnisolve "low water" acetonitrile (11 ppm water) which was used as received. Hamilton gas-tight syringes and glassware were dried, and solvent transfers were performed via syringe through a septum or in a glovebag or drybox under N_2. Picric acid was recrystallized from acetone and dried under vacuum over P_2O_5. The electrolyte, Et_4NClO_4, was purchased from Eastman Kodak and used as received. Tetraethylammonium picrate was prepared and dried according to the method of Coetzee and Padmanabhan.[26] The $DBUH^+Ph_4B^-$ was synthesized as described in the synthesis section of this paper. Standards were run before and after each titration to allow conversion of millivolts to pH. The two standards used were a 1:1 mixture (pK_a = 11)[23] of picric acid (PA) with tetraethylammonium picrate ($Et_4N^+PA^-$) and a 1:1 mixture (pK_a = 23.9)[32] of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with its conjugate acid tetraphenylborate salt ($DBUH^+TPB^-$). Identical electrolyte concentration to the anion solution which was being titrated was included. The electrode was allowed to equilibrate with the solution for 30 to 40 min until the readings stabilized.

Titration of enolates or bases was performed with picric acid in acetonitrile. Readings were taken on an Orion Model 720A pH meter in millivolt mode. Titrant was added using a programmable Harvard Apparatus syringe infusion pump 22. An IBM compatible computer was used to record millivolt and volume readings. An Orion glass pH electrode Model 91-01 was used in conjunction with a silver/silver nitrate reference electrode.

The titration cell was constructed of Pyrex with three glass joints at the top. One was an Ace-Thred (Ace Glass Co.), and the other two were female 14/20 standard taper. The Orion glass electrode was then placed through the Ace-Thred bushing. One of the 14/20 joints was sealed with a septum. This position was used to introduce solutions and titrant via syringe and a nitrogen balloon. The other 14/20 joint was used for the reference electrode, the outer section of which was constructed with a 14/20 male joint. The inner section of the reference electrode was constructed of a rod of silver extending 4.5 cm below where it is sealed with glue into a glass tube. The silver rod was soldered to a silver wire which was then connected to the wire leading to the reference jack of the pH meter via an alligator clip. The reference electrode is separated from the titration solution by a Vycor tip purchased from Bioanalytical Systems, Inc. The inner portion is filled with 0.01 M $AgNO_3$ and 0.1 M Et_4NClO_4 in dry acetonitrile.

For a direct comparison between the titration curve and a curve generated by the Henderson−Hasselbach equation, it was necessary to convert the curves from millivolt readings to pH. A FORTRAN program[33] was used to generate the pH values for each point based on the values of two standards described above. The same program included a subroutine for the generation of the theoretical titration curve for any given pK_a value.

c. Titration of a Series of Enolates. A typical experiment is described. Stock solutions of anion (1.34×10^{-3} M) and electrolyte (5.0×10^{-5} M) were prepared. Portions were added to the above described cell such that the desired concentrations would be present upon dilution to 5 mL. For example, addition of 1.5 mL of anion stock, 1.0 mL of electrolyte stock, and 2.5 mL of CH_3CN results in concentrations of 4.02×10^{-4} M anion and 1.0×10^{-5} M electrolyte in the titration cell. The concentration of picric acid titrant was typically ten times more concentrated than the anion, so that equivalence would be reached at approximately 500 μL. Titrant was added at a rate of 8.3 μL/min. Titrant was added in 5−10 μL portions.

d. Titration of Enolates with and without Host 1. Details unique to this experiment are described. The concentrations in the titration cell of anion, electrolyte, and host were 2.20×10^{-4}, 9.52×10^{-6}, and 1.26×10^{-3} M, respectively. The concentration of picric acid titrant was approximately 20 times the anion concentration (4.02×10^{-3} M) to minimize dilution during titration. The syringe pump was programmed to dispense 2 μL at a time. Total titration time was 1 h. Titrations of **11** and **12** were performed similarly.

e. NMR Titration of 1,3-Cyclohexanedione with Bases. A 0.303 M stock solution of 1,3-cyclohexanedione was prepared, and 20 μL was added to 2.0 mL of a saturated host solution. The initial concentration of the host was 1.49×10^{-3} M, and the concentration of substrate was 3.00×10^{-3} M (2 substrate/host). Three 500 μL portions of the host/substrate solution were placed in individual NMR tubes and sealed with a septum in the drybox. Three bases were examined: DBU, 1,2,2,6,6-pentamethylpiperidine, and (2-*N*,*N*-dimethylamino)-pyridine. Stock solutions were made of each base, and portions were added to their respective NMR tubes such that the base concentration was incrementally increased. The ^{1}H NMR chemical shift of the amine protons of the host were monitored as a function of the base/substrate ratio.

C. Structural Studies. X-ray Experimental for 1, $(C_{26}H_{30}N_5O_6)^+$-$(C_6H_2N_3O_7)^-$. Purplish crystals were grown by slow evaporation from an ethyl acetate solution. The data were collected at 183 K on a Nicolet P3 diffractometer, equipped with a Nicolet LT-2 low-temperature device and using a graphite monochromator with Mo Kα radiation (λ = 0.71073 Å). Crystals of **1** are triclinic with space group $P\bar{1}$, $Z = 2$, $F(000) = 768$, $a = 7.989(2)$, $b = 10.782(3)$, and $c = 19.161(5)$ Å, $\alpha = 89.18(2)$, $\beta = 78.17(2)$, and $\gamma = 85.18(2)°$, $V = 1609.7(7)$ Å^3 having a calculated density = 1.52 g cm^{-3} for $(C_{26}H_{30}N_5O_6)^+(C_6H_2N_3O_7)^-$. Four reflections were remeasured every 96 reflections to monitor instrument and crystal stability. A smoothed curve of the intensities of these check reflections was used to scale the data. The scaling factor

(28) Goldner, H.; Dietz, G.; Carstens, E. *Ann. Chem.* **1966**, *691*, 142−158.
(29) Borgardt, F. G.; Seeler, A. K.; Nobel, P., Jr. *J. Org. Chem.* **1966**, *31*, 2806.
(30) Reid, W.; Kohler, E. *Ann. Chem.* **1956**, *598*, 145.
(31) Dunstan, W. R.; Goulding, E. *J. Chem. Soc.* **1900**, *77*, 1264.
(32) Leffek, K. T.; Pruszynski, P.; Thanapaalasingham, K. *Can. J. Chem.* **1986**, *67*, 590.
(33) Program available upon request.

ranged from 0.978 to 1.02. The data were corrected for Lp effects but not absorption ($m = 1.124$ cm^{-1}). Data reduction and decay correction were performed using the SHELXTL-*Plus* software package.[34] The structure was solved by direct methods and refined on F by full-matrix least-squares with anisotropic thermal parameters for the non-H atoms. The hydrogen atoms were calculated in idealized positions (C−H 0.96 Å, N−H 0.90 Å) with U_{iso} set to 1.2 × U_{eq} of the relevant non-hydrogen atom. A total of 478 parameters were refined using 2043 reflections to an $R(F) = 0.0693$, $wR(F) = 0.0623$, with a goodness of fit = 1.576. The function, $\sum w(|F_o| - |F_c|)^2$, was minimized, where $w = 1/(s(F_o))^2$ and $s(F_o) = \{0.5kI^{-1/2}[(s(I))^2 + (0.02I)^2]^{1/2}\}$. The intensity, I, is given by $(I_{peak} - I_{background})$(scan rate). The value 0.02 is a factor to downweigh intense reflections and to account for instrument instability, and k is the correction due to Lp effects and decay. $\sigma(I)$ was estimated from counting statistics; $\sigma(I) = [(I_{peak} + I_{background})^{1/2}$(scan rate)]. Neutral atom scattering factors for the non-H atoms were taken from Cromer and Mann,[35] with the anomalous-dispersion corrections taken from the work of Cromer and Liberman.[36] The scattering factors for the H atoms were obtained from Stewart, Davidson, and Simpson.[37] Values used to calculate the linear absorption coefficient are from the *International Tables for X-ray Crystallography* (1974).[38] Other computer programs used in this work are listed elsewhere.[39] All figures were generated using SHELXTL-*Plus*.[34] Full details of the X-ray experiment, tables of positional and thermal parameters, geometric parameters, figures, and a list of observed and calculated structure factors are located in the supplementary material.

D. Synthesis. Tetraphenylborate Salt of Protonated 1,8-Diazabicyclo[5.4.0]undec-7-ene (TPB$^-$DBUH$^+$). Into a 50-mL round-bottomed flask were placed 1.0 mL (6.69 mmol) of DBU and 10 mL of absolute ethanol. An excess of HCl gas was bubbled through the solution. The ethanol was then removed at reduced pressure. To remove residual hydrochloric acid, the residue was dissolved in distilled water and then lyophilized to give a white solid. The chloride salt was dissolved in 10 mL of distilled water and mixed with an aqueous solution of $NaBPh_4$ (2.29 g, 1.0 equiv, in 10 mL of distilled water). The resultant white precipitate was collected and dried in a drying pistol under vacuum over P_2O_5 using refluxing ethyl acetate. Mp: 198.5−202.0 °C dec. ^{1}H NMR (CD_3CN, 250 MHz): δ 7.43 (s, $^+$N-*H*, 1H), 7.26 (m, Ph-*H*, 8H), 7.00 (t, Ph-*H*, 8H), 6.85 (t, Ph-*H*, 4H), 3.45 (m, NCH_2, 2H), 3.38 (t, NCH_2, 2H), 3.19 (m, NCH_2, 2H), 2.51 (m, CH_2C=N, 2H), 1.91 (m, CH_2, 2H), 1.68 (m, CH_2, 6H). ^{13}C {^{1}H} NMR (CD_3CN, 62.5 MHz): δ 167.0, 164.8 (q), 136.7, 126.6, 122.8, 55.2, 49.3, 39.1, 33.9, 29.4, 26.9, 24.3, 19.8. Anal. Calcd for $C_{33}H_{37}N_2B$: C, 83.89; H, 7.89; N, 5.93. Found: C, 82.30; H, 7.87; N, 6.03.

3,6-Dibromo-1,2,3,4,5,6,7,8-octahydro-4,5-dioxo-9-acridinecarboxylic Acid, Ethyl Ester (5). Into a 100-mL round-bottomed flask was placed 0.71 g (2.49 × 10^{-3} mol) of compound **4**. To this were added 20 mL acetic acid and 1.59 g (4.49 × 10^{-3} moles) pyridinium bromide perbromide. After the mixture was stirred for 12 h under a nitrogen atmosphere, the acetic acid was removed under vacuum to give a red oil. This was neutralized with a 7% aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic solution was dried over Na_2SO_4 and then condensed at reduced pressure to give an oil. The crude product was purified using flash chromatography on silica gel using 50% CH_2Cl_2 in EtOAc as eluent. The band with an R_f of 0.67 was collected. After condensation at reduced pressure, 0.67 g of a fluffy yellow solid (**5**) was obtained (62% yield). Mp: 83−85 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 4.85 (t, C*H*Br, 2H), 4.55 (q, OCH_2-CH_3, 2H), 3.4 (m, COCHBrC*H*H, 2H), 3.0 (m, COCHBrCH*H*, 2H), 2.55 (m, CH_2CH$_2$CHBr, 4H), 1.45 (t, OCH$_2$CH_3, 3H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz; both *meso* and *d,l* forms are present; the values in parentheses represent the same carbon on the different isomers): 186.2, 165.4, 145.9, 142.7, (138.4, 138.3), 62.7, (49.0, 48.9), (29.6, 29.4), (23.3, 23.1), 14.2. MS−CI$^+$: *m/z* 448 (MW + 4, 1.9), 446 (MW + 2, 2.7), 444 (MW, 2.6), 287 (100). HRMS−CI$^+$: *m/z* calcd for $C_{16}H_{16}NO_4Br_2$ 445.9439, found 445.9426. Anal. Calcd for $C_{16}H_{15}NO_4Br_2$: C, 43.18; H, 3.40; N, 3.15. Found: C, 41.87; H, 3.45; N, 3.00.

2,10-Diamino-1,4,5,7,8,11-hexahydro-dipyrrolo[3,2-*c*:2′,3′-*h*]acridine-3,6,9-tricarboxylic Acid, Triethyl Ester (1). Into an oven-dried 100-mL round-bottomed flask were placed 1.72 g (3.88 × 10^{-3} mol) of compound **5**, 1.21 g (9.30 × 10^{-3} mol, 2.4 equiv) of ethyl 3,3-diamino-2-propenoate (**3**), 2 mL of dry Et_3N, and 6 mL of dry THF, forming a black solution. The reaction was allowed to stir for 20 h under a dry N_2 atmosphere and was protected from light by foil. The solvent was removed under vacuum to give a thick paste. After an additional 24 h of standing, the paste was dissolved in CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2, and the resulting organic solution dried over anhydrous Na_2SO_4. The solution was condensed at reduced pressure to give a red oil which upon crystallization from EtOAc gave an orange-brown solid. Recrystallization of the crude solid from CH_3CN gave 0.68 g of compound **1** as bright yellow crystals (35% yield). Mp: 219−221 °C dec. ^{1}H NMR (CD_3CN, 250 MHz): δ 8.96 (s, N*H*, 2H), 5.57 (s, NH_2, 4H), 4.37 (q, OCH_2-CH_3, 2H), 4.19 (q, OCH_2CH$_3$, 4H), 2.81 (m, CH_2CH_2, 8H), 1.35 (t, OCH$_2$CH_3, 3H), 1.28 (t, OCH$_2$CH_3, 6H). ^{13}C{^{1}H} NMR (DMF-d_7, 62.5 MHz): δ 168.4, 166.5, 150.5, 145.9, 139.2, 123.0, 121.2, 118.9, 93.1, 61.9, 58.9, 26.2, 21.9, 14.9, 14.4. MS-CI$^+$: *m/z* 508 (MW + 1). HRMS-CI$^+$: *m/z* calcd for $C_{26}H_{29}N_5O_6$ 507.2118, found 507.2134. Anal. Calcd for $C_{26}H_{29}N_5O_6$: C, 61.53; H, 5.76; N, 13.8. Found: C, 60.85; H, 5.62; N, 13.59.

8-(*tert*-Butyldimethylsiloxy)-5,6-dihydroquinoline (7). Into a flame-dried 50-mL round-bottomed flask were added 104 mg (0.709 mmol) of **6**, 20 mL of dry CH_2Cl_2, and 200 μL (1.4 mmol, 2 equiv) of dry triethylamine. To this was added 310 μL (1.35 mmol, 1.9 equiv) of *tert*-butyldimethylsilyl trifluoromethanesulfonate. The reaction mixture was allowed to stir under a dry nitrogen atmosphere for 1.5 h and the reaction was quenched with 10 mL of saturated aqueous $NaHCO_3$. The aqueous solution was extracted with CH_2Cl_2, and the resultant organic solution was dried over anhydrous Na_2SO_4. Condensation of the solution under reduced pressure gave an oil which was purified by flash column chromatography on silica gel; elution was with 80:20 CH_2Cl_2:hexane. The product silyl enol ether **7** possessed an R_f of 0.42. Upon condensation of the solution a light yellow oil was obtained in 93% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.38 (d, Pyr-*H*, 1H), 7.33 (d, Pyr-*H*, 1H), 6.99 (t, Pyr-*H*, 1H), 5.53 (t, C=C*H*CH$_2$, 1H), 2.76 (t, CCH_2-CH$_2$CH=C, 2H), 2.31 (q, CCH$_2$CH_2CH=C, 2H), 0.98 (s, SiC(CH_3)$_3$, 9H), 0.17 (s, Si(CH_3)$_2$, 6H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 151.4, 149.0, 146.7, 134.0, 132.4, 121.8, 111.4, 27.7, 25.9, 21.6, 18.6, −4.5. HRMS-CI$^+$: calcd for $C_{15}H_{24}NOSi$, 262.1627, (MW+1), found 262.1613.

7-Bromo-6,7-dihydro-8(5*H*)-Quinolinone (8). To the oil **7** (85.0 mg, 0.325 mmol) was added 15 mL of glacial acetic acid and 108 mg (0.304 mg, 0.94 equiv) of technical grade (90%) pyridinium bromide perbromide. A large amount of yellow salt precipitated after 5−10 min. The reaction was allowed to stir for 12 h. Most of the acetic acid was removed under vacuum. The residue was neutralized with a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, and condensed via rotary evaporation to give an oil. The crude product **8** was purified via flash chromatography, eluted with 50:50 EtOAc:CH_2Cl_2. The band with an R_f of 0.46 was collected, and the solvent was removed by rotary evaporation to yield a yellow solid (54% yield). Mp: 76.0−77.0 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.72 (d, Pyr-*H*, 1H), 7.67 (d, Pyr-*H*, 1H), 7.42 (dd, Pyr-*H*, 1H), 4.80 (t, COC*H*Br, 1H), 3.38 (m, COCHBrC*H*H, 1H), 2.92 (dt, COCHBrCH*H*, 1H), 2.54 (m, COCHBrCH$_2$CH_2C, 2H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 188.6, 149.7, 145.7, 139.5, 137.5, 127.4, 50.0, 30.8, 25.4. MS: *m/z* 228 (MW + 2, 16.7), 226 (MW, 16.5), 148 (100). HRMS-CI$^+$: calcd for C_9H_8NOBr, 224.9789, found 224.9786. Anal. Calcd for C_9H_8NOBr: C, 47.82; H, 3.57; N, 6.20. Found: C, 47.93; H, 3.62; N, 6.12.

2-Amino-4,5-dihydro-1*H*-pyrrolo[3,2-*h*]quinoline-3-carboxylic Acid, Ethyl Ester (9). To a flame-dried flask were added 0.50 g (2.21 mmol) of **8**, 0.80 g (7.91 mmol, 3.57 equiv) of dry Et_3N, 0.34 g (2.66 mmol, 1.2 equiv) of **3**, and 8 mL of dry THF. Aluminum foil was used to protect the reaction from light. The reaction mixture was allowed to stir under a nitrogen atmosphere for 12 h. Most of the solvent was

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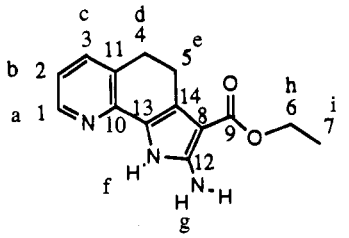

removed under vacuum, and the resultant thick paste was allowed to stand for an additional 12 h. All of the solvent was removed, and the residue was purified via flash column chromatography on silica gel (elution with EtOAc). The band with an R_f of 0.44 was collected and condensed at reduced pressure to a very concentrated solution which was placed in a −20 °C freezer. Bright yellow crystals of **9** formed in 82% yield. Mp: 145−147 °C dec. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.1(br s, f, 1H), 8.3 (d, a, 1H), 7.3 (d, c, 1H), 6.8 (t, b, 1H), 5.4 (s, g, 2H), 4.2 (q, h, 2H), 2.9 (m, d, and e, 4H), 1.3 (t, i, 3H). ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 166(C-9), 149.3 (C-12), 147.0 (C-10), 144.2 (C-1), 134.8 (C-3), 129.0 (C-13 or 14), 125.4 (C-14 or 13), 119.6 (C-11), 118.7 (C-2), 93.4 (C-8), 58.9 (C-6), 28.4 (C-4), 21.1 (C-5), 14.5 (C-7). HRMS-CI: calcd for $C_{14}H_{15}N_3O_2$, 257.1164, found 257.1162. Anal. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33. Found: C, 62.61; H, 5.77; N, 15.36.

General Procedure for Anion Synthesis. The anionic guests were synthesized by adding an equivalent of sodium ethoxide dropwise to the neutral starting material dissolved in absolute ethanol. Sodium ethoxide was freshly prepared from sodium metal in ethanol. Subsequent removal of the ethanol gave the anion sodium salt which was washed with dry acetonitrile in the drybox to remove any residual starting material. To impart solubility of the salt in acetonitrile, an equivalent of 15-crown-5 was added. Depending upon the solubility of the salt, this was carried out in dry acetonitrile, dry DMF, or mixtures of the two. Upon removal of the solvents under vacuum, the sodium 15-crown-5 anions were obtained. The sodium [2.2.1]cryptand was prepared in a similar manner to the 15-crown-5 version, while the bis(triphenylphosphoranylidene)ammonium counterion (PPN^+) was prepared by mixing PPN^+Cl^- with the sodium enolate salt in DMF. The solvent was then removed, and the oil dissolved in CH_3CN, which caused the NaCl to precipitate. The solution was filtered through dry Celite and condensed to a solid.

Sodium 15-Crown-5 1,3-Cyclohexanedionate (10a). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.06 (s, COC*H*NaCO, 1H), 3.68 (s, OCH_2CH_2O, 20H), 2.21 (t, COCH_2CH_2, 4H), 1.87 (qn, $COCH_2CH_2$, 2H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 195.7, 102.6, 69.1, 36.2, 22.6.

Sodium [2.2.1]Cryptand 1,3-Cyclohexanedionate (10b). ^{1}H NMR (CD_3CN, 250 MHz): δ 4.52 (s, COC*H*NaCO, 1H), 3.61 (m, OCH_2CH_2O, 8H), 3.50 (m, NCH_2CH_2O, 12H), 2.57 (m, NCH_2CH_2O, 12H), 2.00 (t, COCH_2CH_2, 4H), 1.76 (qn, $COCH_2CH_2$, 2H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 194.7, 102.5, 68.6, 67.0, 66.5, 53.6, 52.8, 36.3, 22.7.

PPN^+ 1,3-Cyclohexanedionate (10c). ^{1}H NMR (CD_3CN, 250 MHz): δ 7.58 (m, Ar-*H*, 30H), 4.55 (s, COC*H*NaCO, 1H), 1.97 (t, COCH_2CH_2, 4H), 1.74 (qn, $COCH_2CH_2$, 2H). ^{13}C{^{1}H} NMR (CD_3CN, 62.5 MHz): δ 193.4, 134.6, 133.2 (qn), 130.4 (m), 128.2 (d), 101.4, 37.4, 23.7.

Sodium 15-Crown-5 2,2-Dimethyl-1,3-dioxane-4,6-dionate (11). ^{1}H NMR (CD_3CN, 250 MHz): δ 3.62 (s, OCH_2CH_2O, 20H), 3.43 (s, COC*H*NaCO, 1H), 1.52 (S, $C(CH_3)_2$, 6H). ^{13}C{^{1}H} NMR (CD_3CN, 62.5 MHz): δ 168.1, 101.3, 69.6, 63.2, 26.2.

Sodium 15-Crown-5 Salt of 1,3-Di-*n*-butylbarbituric Acid (12). ^{1}H NMR (CD_3CN, 250 MHz): δ 4.11 (br s, COC*H*NaCO, 1H), 3.72 (t, N$CH_2CH_2CH_2CH_3$, 4H), 3.63 (s, OCH_2CH_2O, 20 H), 1.47 (qn, $NCH_2CH_2CH_2CH_3$, 4 H), 1.28 (sx, $NCH_2CH_2CH_2CH_3$, 4H), 0.90 (t, $NCH_2CH_2CH_2CH_3$, 6H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 165.1, 154.5, 76.6, 69.7, 40.3, 31.7, 21.1, 14.3.

Sodium 15-Crown-5 1,3-Cyclopentanedionate (13). ^{1}H NMR (CD_3CN, 250 MHz): δ 4.34 (s, COC*H*NaCO, 1H), 3.60 (s, OCH_2CH_2O, 20H), 2.04 (s, $COCH_2CH_2CO$, 4H). ^{13}C{^{1}H} NMR (CD_3CN, 62.5 MHz): δ 204.1, 101.1, 69.9, 35.2.

Sodium 15-Crown-5 Salt of 1,1-Dinitroethane (14). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.48 (s, OCH_2CH_2O, 20H), 1.79 (s, CH_3, 3H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 176.7, 69.3, 22.9.

Sodium 15-Crown-5 Salt of Cyanonitromethane (15). ^{1}H NMR (CD_3CN, 250 MHz): δ 5.60 (s, NCC*H*NaNO_2, 1H), 3.61 (s, OCH_2CH_2O, 20H). ^{13}C{^{1}H} NMR (CD_3CN, 62.5 MHz): δ 119.7, 82.0, 69.6.

Sodium 15-Crown-5 Salt of Malononitrile (16). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.67 (s, OCH_2CH_2O, 20H), 1.56 (br s, CNC*H*NaCN, 1H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 132.5, 77.2, 68.9.

Sodium 15-Crown-5 2-Nitrocyclohexanonoate (17). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.70 (s, OCH_2CH_2O, 20H), 2.77 (t, $CNaNO_2CH_2$-CH_2, 2H), 2.24 (t, $COCH_2CH_2CH_2$, 2H), 1.66 (m, $COCH_2CH_2CH_2$, 4H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 186.8, 121.0, 69.2, 39.8, 28.4, 23.7, 23.1.

Sodium 15-Crown-5 Salt of 2-Acetylcyclohexanone (18). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.69 (S, OCH_2CH_2O, 20H), 2.14 (br m, $COCH_3$, 2H), 1.91 (br m, $COCH_2CH_2$, 5H), 1.38 (br m, $COCH_2CH_2CH_2CH_2$, 4H). ^{13}C{^{1}H} NMR (CD_3CN, 62.5 MHz): δ 190.1, 187.2, 104.7 (br), 39.7, 29.4 (br), 28.7 (br), 25.9, 25.1.

Sodium 15-Crown-5 2,4-Cyclopentanedionate (19). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.04 (s, COC*H*NaCO, 1H), 3.65 (s, OCH_2CH_2O, 20H), 1.77 (s, $COCH_3$, 6H). ^{13}C{^{1}H} NMR ($CDCl_3$, 62.5 MHz): δ 188.4, 96.1, 69.1, 29.1.

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Supplementary Material Available: Titration curve for **10a** and **10b**, ^{13}C−^{13}C 2-D correlated NMR spectra of **2**, an X-ray summary of positions and thermal parameters, bond lengths and angles, listing of the H-bonding interactions, and a view showing the atom labeling and unit cell packing diagrams (27 pages); table of observed and calculated structure factors for **1** (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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