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The Advantages of Using Rigid Polyaza-Clefts for Hydrogen-Bonding Molecular Recognition.

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Abstract

Hydrogen bonding is an important driving force for molecular recognition. Herein, the advantages of developing rigid polyaza-clefts for hydrogen-bond-driven molecular recognition are discussed.

Introduction

The study of molecular recognition in synthetic and natural systems has had an impact on several fields of chemistry. First, it has given insight into the forces used by nature to bind substrates to enzymes,[1] haptens to antibodies,[2] cations to ionophores,[3] and proteins and drugs to DNA.[4] Second, templated synthesis[5] and artificial self-replicating molecules[6] have developed as a consequence of these studies. Third, synthetic catalysts with practical applications are widely being sought using the lessons gained from molecular recognition.[7] Finally, molecular devices based upon self-assembling subunits are actively being pursued due to recent developments in molecular recognition.[8]

Of all the binding forces used in the development of synthetic receptors and catalysts, hydrogen bonding is potentially the most powerful. Although hydrogen bond strengths are quite small (1 to 7 kcal/mol),[9] exceptionally strong complexation can be achieved when several act cooperatively. For example, the incorporation of six hydrogen bonds into a synthetic receptor for barbiturates resulted in 10^6 M^{-1} complexation constants for this important class of drugs.[10] Hydrogen bonds also allow for selectivity between competing guests. The addition or absence of just a single hydrogen bond in two otherwise identical enzyme inhibitors gives approximately a 4 kcal/mol difference in binding, reflecting a 40,000 to 1 preference.[11] Thus, the incorporation of multiple hydrogen bond donors and acceptors into a synthetic receptor can lead to both strong and selective binding.

Hydrogen Bonding with Preorganized Synthetic Clefts

One important concept in the development of synthetic receptors is preorganization.[12] It is well precedented that complexation strength is dependent upon the number of degrees of freedom frozen during a binding event: the fewer degrees of freedom lost in the host, the larger the complexation constant. This is valid given that the positions of the molecular recognition contacts on the host for the guest are nearly perfect. If the ideal binding conformation with a rigid host is not achieved, then a flexible host which can adopt a more favorable conformation will often better bind the guest even though there may be a loss of degrees of freedom.[13]

In the development of highly preorganized hosts, bond rotations are often restricted by the incorporation of fused rings. The entropy loss in reducing the degrees of freedom of a flexible host when binding a guest is paid in the synthesis of the host prior to study of complexation. Several examples of this strategy are shown in Figure 1.[14] In each case heterocycles are fused in such a manner as to create hydrogen bonding contacts in spatial arrays complementary to a target guest. The following sections explore this host design motif

with studies targeted at complexing cyclitols, phosphoesters, and enolates, complete with an analysis of the advantages of using rigid polyaza-clefts.

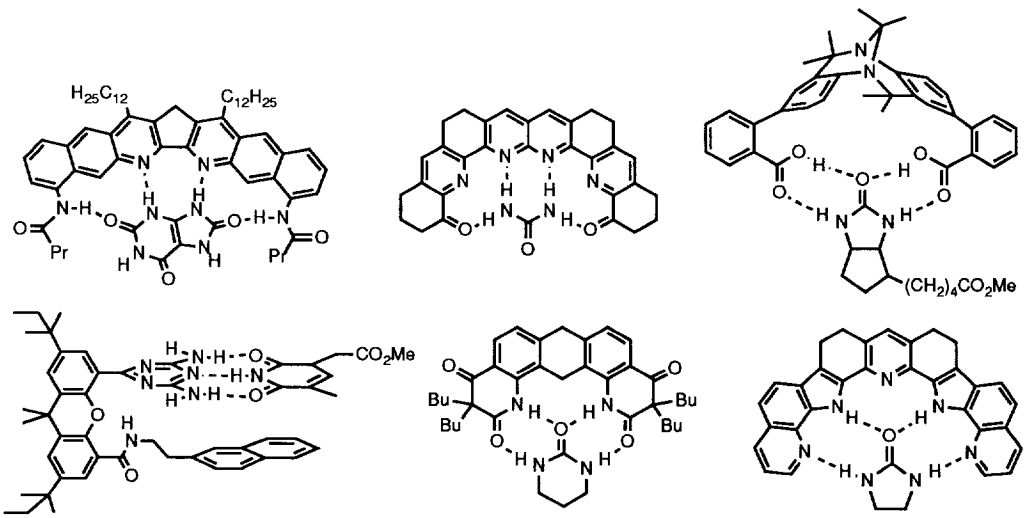


Figure 1: Examples of Rigid Polyaza-Clefts and Their Host-Guest Structures.[14]

Cyclitol Complexation

The three dimensional shape of a monosaccharide or cyclitol dictates that binding will be facilitated using a three dimensional receptor. Figure 2A displays a rigid polyaza-cleft **1** that can bind to a cyclitol from above and below the cyclohexane ring. Figure 2B displays the primary hydrogen bonding found by molecular mechanics for **1** with 1,3/2-cyclohexanetriol.

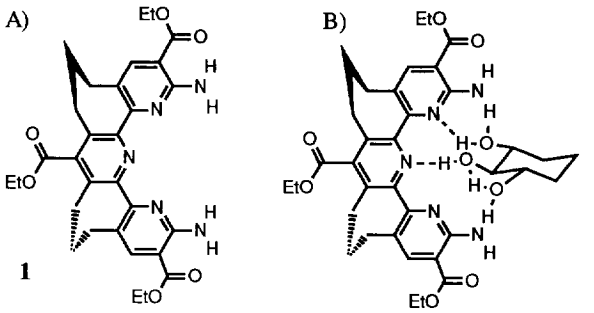


Figure 2: A) A three dimensional polyaza-cleft. B) Proposed binding mode to 1,3/2-cyclohexanetriol.

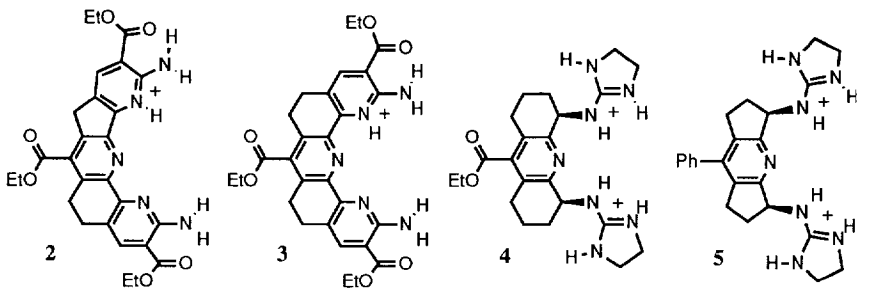
Compound **1** was found to bind 1,2,3-cyclohexanetriols with only moderate association constants (40 to 110 M^{-1})[15] with *trans* stereochemistry of the vicinal alcohols resulting in larger binding constants. Careful analysis of potential secondary destructive interactions, the complementarity of **1** to the triols, and the expected strength of hydrogen bonds between the guest's hydroxyls and the host's nitrogens, led to the conclusion that factors other than the above resulted in the small binding constants and the *cis/trans* selectivity.[16] It is known that

cyclitols possess intramolecular hydrogen bonds between vicinal alcohols, and that the *cis*-intramolecular hydrogen bond is stronger than the *trans*.[17] It was postulated that the complexation was inhibited by the intramolecular hydrogen bonds between the hydroxyls in the guests, and that these hydrogen bonds internally solvated the cyclitols. To support this postulate, the strengths of cyclitol intramolecular hydrogen bonds were measured by a variety of conformational analysis techniques. The *trans*- and *cis*-intramolecular hydrogen bond strengths were found to be 1.9 and nearly 2.3 kcal/mol, respectively. These values correlate well with the selectivity of triol binding. It was concluded that the energy paid to break the guest intramolecular hydrogen bonds results in low association constants.[16]

The conclusions drawn based upon the energetic consequences of breaking the guest intramolecular hydrogen bonds were possible due to the rigidity of the host. Since the host possessed no significant degrees of freedom, the conformation of the host was well known. A more flexible host would have complicated the analysis since the small binding constants could have been a consequence of the restriction of degrees of freedom. Similarly, the analysis of triol binding selectivity by molecular mechanics was facilitated due to the rigid nature of the host. In contrast, the conformational rigidity of 1 did not allow for any significant changes in host structure so as to better match the guest. Thus, a more flexible host may be a better approach to cyclitol and saccharide binding. This remains to be determined, however.

Phosphoester Binding

The ability to recognize, sequester, transport, and cleave phosphoesters has significant ramifications in the biological and medicinal sciences.[18] As a result, many receptors employing different design strategies have been synthesized and tested.[19] In specific, we have been concentrating on the transport and cleavage of phosphodiesters as a means of developing drug delivery systems and RNA affinity cleavage agents respectively. In the area of transport, our studies have focused upon binding phosphodiesters with rigid polyaza-clefts **2** and **3** in lipophilic solvents such as chloroform.[20] In the area of phosphodiester hydrolysis our studies have concentrated upon binding and cleavage of RNA with receptors **4** and **5** in both DMSO and water.[21]



The studies in chloroform revealed several important host design considerations. First, there is a high propensity for dimerization of phosphoesters in low dielectric solvents, yielding two-to-one guest-to-host complexes.[20] The dimerization constant between dibenzyl hydrogen phosphate and dinaphthyl hydrogen phosphate was determined to be $6.4 \times 10^4\ M^{-1}$. Transport is therefore expected to involve complexes with stoichiometry higher than one-to-one.[22] A similar situation has also been observed with less rigid polyaza-clefts.[23] Second, the studies also allowed for an analysis of the hydrogen bond strengths between the host and guest. For example, it was found that the strongest binding interaction between host and guest is the hydrogen bond formed between the acid hydrogen on a phosphoric acid diester and a basic pyridine nitrogen in the host. This interaction is worth more than 3.5 kcal/mol. The other

hydrogen bonds formed between the host and guest were equal in strength to those found in binding urea-like guests (1.2 kcal/mol).[24] The other hydrogen bonds between **2** or **3** and a guest range from 0.77 to 2.4 kcal/mol each. Finally, these studies revealed that subtle differences in host structure can have dramatic effects on the binding constants. For example, host **2** binds phosphodiesters with association constants near 8 x 10^4 M^{-1}, whereas host **3** binds phosphodiesters with association constants near 7 x 10^3 M^{-1}. This is nearly a 1.4 kcal/mol difference in binding strength imparted by the removal of one carbon in the fused ring spacer of **2**. Molecular mechanics energy calculations of the host-guest structures suggest that the cavity in **3** is too small for a phosphoric acid diester, and that the hydrogen bond angles deviate from linearity by an average of 10 to 12 degrees more in the complex with **3** than with **2**. Therefore clefts with wider cavities than that of **3** are optimum for the hydrogen bonding surface of phosphodiesters.

The conclusions relating to hydrogen bond strength and the optimal width of the cleft were made based upon the rigid nature of the hosts. For example, in the analysis of hydrogen bond strengths, the free energy of binding with a more flexible host would have included the energy required to freeze the host into the binding conformation. A more flexible host could bind to a guest more readily and form stronger bonds than **2** and **3**, but the energy to obtain this conformation would not be readily determined and easily factored into an equation giving the hydrogen bond strengths. Similarly, a more flexible host may better accommodate the size of the phosphodiester guest than **3**, but obtaining the information that the cavity size, as in **3**, is too small for phosphodiester guests would be difficult without a crystal structure. Therefore the use of a rigid cavity gives precise, though limited, knowledge as to the positions of the hydrogen bond donors and acceptors.

We tested whether a rigid and preorganized receptor can be well applied for the complexation of nucleotides in aqueous media. Compounds **4** and **5** exhibit binding constants with phosphodiesters in DMSO of 2.2 x 10^3 M^{-1} and 1.4 x 10^3 M^{-1} respectively, but the binding strength drops significantly to below 10 M^{-1} in pure water.[25] In contrast, phosphomonoesters exhibit significantly stronger binding in pure water. For example, uridine 5'-monophosphate associates with **5** at pH 9.4 in 3 mM 2-amino-2-methyl-1-propanol (AMPS) buffer with a binding constant of 9.6 ± 1.9 x 10^2 M^{-1} (Figure 3A). This is significantly stronger than that reported for the association of HPO_4^{-2} with a monoguanidinium host in water (below 16 M^{-1}).[26] Therefore, receptors such as **4** and **5** exhibit much better binding than that of the simple side chain of arginine.

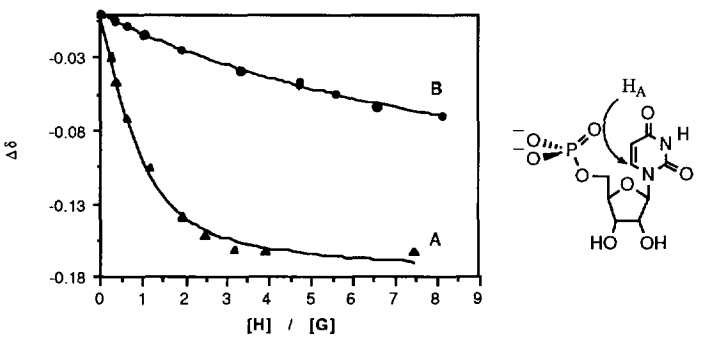


Figure 3: A) ^1H NMR isotherm for binding uridine 5'-monophosphate (pU) with **5**. Following H_A (δ = 5.775 ppm), [pU] = 3.55mM. B) ^1H NMR isotherm for binding uridine 5'-monophosphate with **6**. Following H_A (δ = 5.770 ppm), [pU] = 2.55 mM.

In order to test if the preorganization and convergence of the guanidiniums in **4** and **5** also lead to an increase in binding, compound **6** was synthesized as a comparison (Figure 4). Precursor **8** was made using a procedure involving the isothiocyanate **7**.[27] The initially formed *bis*-thiourea **8** was alkylated with EtBr, the Boc protecting groups were removed *via* treatment with TFA, and slightly basic conditions were effective at inducing cyclization to free base **6**. These three steps are all performed in the same flask with no purification. Purification of **6** was performed by formation of the *bis*-picrate salt, followed by ion exchange to the dichloride salt. Overall yield from 1,5-pentanediamine was 78 percent.

Figure 3B shows the binding isotherm generated with **6** at pH 8.7 ± 1.7 with 3 mM AMPS buffer. The computer modeling of the data resulted in a binding constant of 41 ± 8 M^{-1}. Therefore, the preorganization imparted by the spacer in **5** resulted in a 1.8 kcal/mol advantage in complexation of uridine 5'-monophosphate in water.

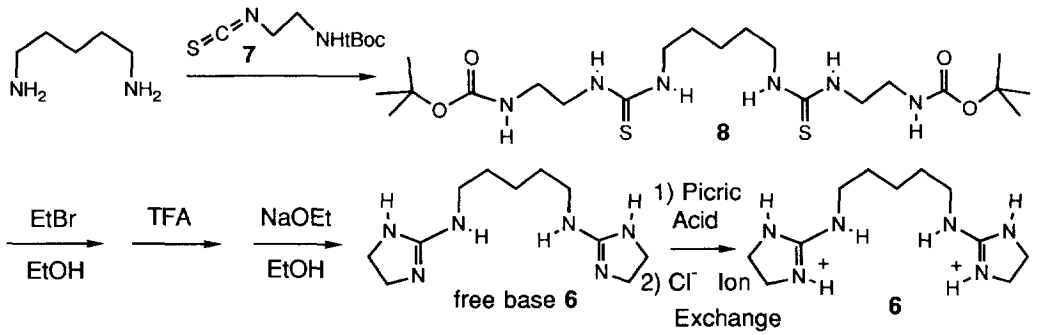


Figure 4: Synthetic route to **6**.

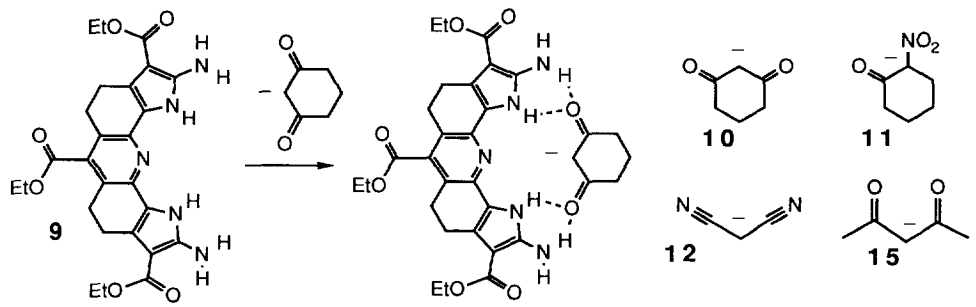
The preorganization and rigidity of **5** yield a significant energetic advantage for the complexation of phosphoesters in water. The preorganization of **5** is not only imparted by the spacer, but also by the restricted rotation about the guanidinium N - spacer C due to the repulsion between the positive charges. This repulsion keeps the cavity open and in the correct conformation for complementary hydrogen bonding and ion pairing to a bound phosphomono- or diester. The only disadvantage in using **5** as a phosphodiester receptor as compared to **6** is the relative synthetic difficulty.

Enolate Binding

The complexation of enolates with synthetic receptors has the potential for practical applications in organic synthesis. For example, chiral receptors could be used to template alkylation reactions resulting in enantioselective nucleophilic substitutions.[28] As will be discussed, the first generation chiral templates did not possess sufficient preorganization to allow examination of the template-alkylation method. However, the study of an achiral enolate receptor **9** that binds enolates of active methylene compounds (**10** - **12**) in acetonitrile has revealed a successful approach to the binding of such anions.

The associations between compound **9** and the enolates in the mildly competitive solvent acetonitrile are controlled predominantly by the shape of the enolate.[29] Sodium enolates with divergent 1,3-diketo-like moieties have the largest binding constants (K_{assoc}) to **9** of all the enolates studied; they range from 10^3 to 10^4 M^{-1}. A linear correlation between the pKa and the pK_{assoc} was found.[30] This linearity indicates that increased basicity within a series of structurally related guests directly correlates to an increase in association. In addition, the complexation strength is highly dependent upon the enolate counterion: the more "naked" the

enolate, the larger the association constant. For example, when the sodium counterion is encapsulated in 3,3,2-cryptand, the binding constant is 2.72 ± 0.68 x 10^4 M^{-1}, but when 15-crown-5 is used, the binding constant decreases to 1.35 ± 0.14 x 10^4 M^{-1}. Finally, enolates possessing nitrile groups have the lowest association constants with **9**. Although the malononitrile anion is the most basic guest examined, it has the lowest binding constant (175 ± 18 M^{-1}). The reduced number of hydrogen bonds that a CN group can accept relative to the oxygen containing enolates likely lowers the binding affinity.



The successful complexation of enolates with compound **9** in acetonitrile prompted an investigation of receptors with similar molecular shape and with the introduction of chirality. Figure 5 shows the first generation chiral enolate receptors that are based upon the molecular shape of compound **9**. These compounds were synthesized in one step from *d,l*- 3,5- diamino- 1,2,3,5,6,7- hexahydro- 8- phenyldicyclo- penta[*b,e*]pyridine[25] and either benzylisocyanate or benzylisothiocyanate.

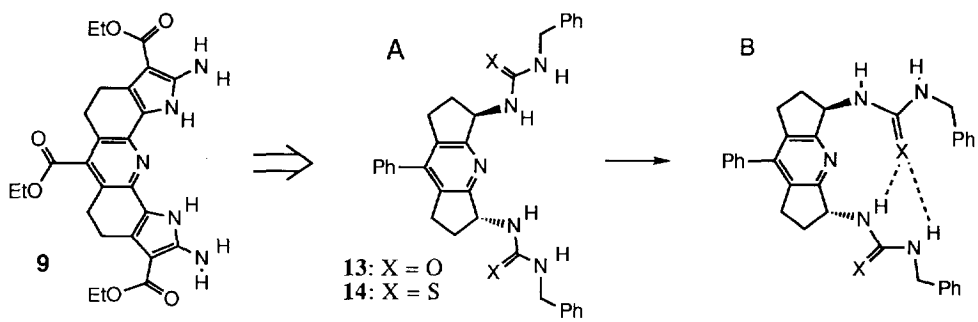


Figure 5: (A) The chiral design of enolate receptors **13** and **14** is patterned after that of **9**. (B) Intramolecular hydrogen bonding in **13** and **14** leads to the collapse of the cavity.

The molecular shapes of 9 and 13 are quite similar, but their ability to bind enolates is significantly different. As drawn in Figure 5A, compounds **13** and **14** both possess four hydrogen bond donors in spatial positions similar to those in **9**. Ureas and thioureas are used as the hydrogen bond donors since they are known to form hydrogen bond complexes in highly competitive solvents.[31] The only significant structural difference between **9** and the chiral receptors is flexibility. The N-C bond that links the ureas or thioureas with the 1,2,3,5,6,7-hexahydro-8-phenyldicyclopenta [*b,e*]pyridine spacer is essentially free to rotate in **13** and **14**. In contrast, similar rotations are frozen in **9**. This bond rotation is a severe detriment to binding, as was indicated by the lack of significant NH chemical shift movement during a ^1H

NMR titration experiment conducted in acetonitrile with **13**. Although **9** binds enolates quite strongly in acetonitrile, the chiral receptors do not. Furthermore, binding only becomes apparent in the noncompetitive solvent dichloromethane. In dichloromethane, compounds **13** and **14** bind **15** with association constants of 500 and 20 M^{-1} respectively. These are exceptionally low binding constants for an anion with hosts possessing good hydrogen bond donors, as well as being strikingly low in comparison to the 10^3 and 10^4 M^{-1} values determined for **9** with diketone enolates.

The reason for the low binding constants is apparent from examination of the ^1H NMR spectra of **13** and **14**. The chemical shifts of the urea NHs in **13** are 4.38 and 6.34 ppm, while in thiourea **14**, the NH resonances are at 4.87 and 8.05 ppm respectively. Typical urea and thiourea NH resonances in methylene chloride are near 4.5 and 5.3 ppm respectively. It is evident that the chemical shifts are significantly further downfield in **13** and **14** than normal. Such a result is indicative of strong intramolecular hydrogen bonding within the receptors. As shown in Figure 5B, rotation about the C-N bond leads to a conformation in which one urea oxygen and an adjacent urea NH can form a hydrogen bond. The effect is further accentuated with thioureas because they form stronger hydrogen bonds than ureas.[31] The stronger intramolecular hydrogen bonding with **14** is reflected in both the decreased intermolecular hydrogen bonding with the enolate **13**, and the very large downfield shifts of the NH resonances (8.0 ppm).

The advantage of a rigid receptor is well demonstrated in the complexation of enolates. A dramatic difference in binding ability results from restricting the degrees of freedom of the receptor. Intramolecular hydrogen bonds in the flexible receptor lead to a conformation which in essence completely blocks entry of the guest into the cavity.

Summary

There are several advantages to using a rigid polyaza-cleft for hydrogen-bond-driven molecular recogntion. Since the hydrogen bonding contacts on the receptors are fixed, the geometry of the host when binding the guest is known. With regards to cyclitol binding, this ability to predict the host-guest structure allowed for a careful analysis of binding energies and the influence of guest intramolecular hydrogen bonds. In addition, the rigidity of the polyaza-clefts allows for fewer potential conformations, and thereby simplifies molecular mechanics and dynamics calculations. In both cyclitol and phosphodiester binding motifs, molecular mechanics has been used to accurately predict the structures of the hosts and host-guest complexes. Also, since the positions of the hydrogen bonding contacts are well known, one can subtly change the position of these contacts and be confident in conclusions concerning how such changes effect complexation. With regards to phosphodiester binding, subtle changes in the distances between two host hydrogen bonding contacts significantly affected binding strength. This allowed for mapping of the hydrogen bonding surface of the guest. The preorganization of guanidiniums in **5** gave a 1.8 kcal/mol binding advantage over **6** to a nucleotide monoester in water. Finally preorganization is essential to achieving strong enolate binding in the moderately competitive solvent acetonitrile. In the absence of preorganization the cavity collapses, resulting in weak binding even in the noncompetitive solvent dichloromethane. Thus, in these different cases, we observe the advantages of preorganization in hydrogen-bond-driven molecular recognition.

Experimental

A) General Considerations.

^1H and ^{13}C NMR spectra were obtained in $CDCl_3$, DMSO-d_6, or D_2O, and recorded on a General Electric QE-300 or a Bruker AC-250 spectrometer. 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 and high resolution mass spectra were measured with Finnigan TSQ70 and VG Analytical ZAB2-E instruments respectively.

Solvents and reagents were purchased from Aldrich and used without further purification. Uridine 5'-monophosphate and 2-amino-2-methyl-1-propanol were purchased from Sigma Chemical Company and used without further purification. *Meso*-N,N'-(4,5-dihydro-1H-imidazol-2-yl) -1,2,3,5,6,7 -hexahydro -8 -phenyldicyclopenta -[*b,e*]-pyridine-3,5-diamine dihydrochloride (**5**) was obtained form the corresponding dihydropicrate salt using ion exchange as previously described.[25] Preparative flash chromatography was performed on Scientific Adsorbants Incorporated Silica Gel 40 μm. Analytical TLC was performed on precoated Silica Gel 60 F-254 plates.

B) Determination of Binding Constants.

Nucleotide Binding

All compounds were stored and weighed in a Vacuum Atmosphere MO-40 dry box. An aqueous solution of the nucleotide, henceforth called the stock solution, of about 3 mM concentration was made using 2-amino-2-methyl-1-propanol (AMPS) as buffer. The concentration of the stock solution was determined by UV-Vis absorbance measurements. The extinction coefficient and absorbance maximum of the nucleotide were taken from the literature.[32] An approximately 15 mM host solution (700 μL) was made using the nucleotide stock solution, and exactly 500 μL was transferred to a 5 mm NMR tube sealed with a rubber septum. 500 μL of the stock solution was transferred to another tube. Due to the relative intensity of the HOD resonance, the solvent suppression pulse sequence PRESAT was utilized. A standard proton spectrum of the stock solution was recorded, and then the pulse sequence was begun. Aliquots of the host-guest solution were removed from the NMR tube *via* syringe through the septum and replaced by an equal volume of the stock solution. In this manner, the host concentration was systematically reduced while the guest concentration remained constant. The binding studies monitored the chemical shift change of three protons of the nucleotide. The three protons consisted of a base proton, the anomeric proton, and a ribose proton. The binding constants were obtained by using a non-linear least squares curve-fitting program provided by Professor Whitlock of The University of Wisconsin.[33]

Enolate Binding

A CD_2Cl_2/DMSO-d_6 (93/7) solution (500 μL) of the host of approximately 5 mM concentration was added to a 5 mm NMR tube sealed with a rubber septum. The concentration of the solution was determined by an independent UV-Vis absorbance measurement. The NMR spectra were recorded. Incremental aliquots of a concentrated solution of the enolate in the same solvent system were added, followed by acquisition of a ^1H NMR spectrum. The concentration of the enolate was determined by NMR integration of the host and guest. In this manner, the host concentration was diluted slightly, while the guest concentration significantly increased. The binding studies monitored the chemical shift change of the host NHs. The binding constants were obtained using the aforementioned computer program.

C) Synthesis.

1,5-Bis[[[[2- [[(1,1-dimethylethoxy) carbonyl] amino] ethyl] amino] thioxomethyl] -amino] pentane (8).

[[(1,1-dimethylethoxy)carbonyl]amino]ethylisothiocyanate (**7**) (4.975 g, 24.63 mmol) dissolved in dry THF (55 mL) was added dropwise to a solution of 1,5-pentanediamine (1.2 mL, 10.25 mmol) in dry THF (50 mL). The solution was stirred for 24 hours under a positive pressure of nitrogen. The THF was removed by rotary evaporation. The product was isolated via flash chromatography using gradient elution from 100% dichloromethane to 5% ammonia-saturated methanol in dichloromethane, R_f = 0.225 in 5% NH_3 saturated CH_3OH/CH_2Cl_2. Yield: 1.1631 g (90%). mp 122-125°C. ^1H NMR (250 MHz, $CDCl_3$): δ 7.08 (br s, NH, 2H); 6.80 (br s, NH, 2H); 5.34 (br s, NH, 2H); 3.56 (br s, CH_2, 4H); 3.29 (br s, CH_2, 6H); 1.59 (t, CH_2, 4H); 1.40 (bs, $COC(CH_3)_3$, 22 H). ^{13}C {^1H} NMR (72.5 MHz, $CDCl_3$): δ 181.7, 157.2, 79.9, 43.8, 39.8, 28.3, 23.7 ppm. HRMS: *m/z* calcd for $C_{21}H_{42}N_6O_4S_2$ 507.2787, found 507.2782.

N, N'-Bis(4,5-dihydro-1*H*-imidazol-2-yl)-1,5-pentanediamine (free base 6, dihydropicrate and dihydrochloride).

Compound **8** (0.256 g, 0.506 mmol) was dissolved in a solution of bromoethane (5.0 mL) and absolute ethanol (5.0 mL). The mixture was heated at a mild reflux (oil bath 60 °C) under a nitrogen atmosphere for 24 hours. The solvent was removed by rotary evaporation. This

product was not isolated. The resulting oil was dissolved in trifluoroacetic acid (2.0 mL) and water (1.0 mL) and stirred at room temperature for 4 hours. The TFA was removed under vacuum overnight and the product lyophilized. This product was not isolated but taken directly to the next step. The pasty residue was dissolved in absolute ethanol (10.0 mL) and added dropwise over 2 hours to a solution of sodium ethoxide in ethanol (approximately 45 mg sodium metal in 5.0 mL of absolute ethanol). The system was stirred under an atmosphere of nitrogen for 24 hours, at which point the solvent was removed by rotary evaporation. Compound **6** was purified by conversion to the picrate salt in the following manner. The solid from above (0.12 g, 0.5 mmol) was dissolved in water (12.5 mL). Picric acid (0.40 g, 2.2 eq.: 60% suspension in water) was dissolved in water (27 mL). The solutions were heated to just below reflux, and then the free base **6** was added slowly to the picric acid. A precipitate formed, and the mixture was allowed to stand overnight at 0 °C. It was filtered and washed with cold water, ether, and hexanes. It was recrystallized from acetonitrile. Yield: 0.3282 g (91%). mp 223-225°C (dec). ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.57 (s, $C_6H_2O(NO_2)_3$, 4H); 8.13 (t, NH, 2H); 3.56 (s, NHCH_2CH_2NH, 8H); 3.07 (q, NHCH_2(CH$_2$)$_3$CH_2NH, 4H); 1.45 (m, (NHCH$_2$CH_2)$_2$CH$_2$, 6H); 1.26 (m, (NHCH$_2$CH$_2$)$_2$CH_2, 2H) ^{13}C {^{1}H} NMR (72.5 MHz, DMSO-d_6): δ 159.2, 141.8, 125.1, 123, 42.4, 42.0, 28.2, 23.0 ppm. Anal. Calcd for $C_{23}H_{28}N_{12}O_{14}$·(H_2O): C, 38.64; H, 4.23; N, 23.53. Found: C, 39.44; H, 4.09; N, 23.93. IRA-100 ion-exchange resin in the OH^- form was equilibrated in 0.2 M HCl overnight. Approximately 5 mL of resin was packed in a 1 cm diameter column and washed to neutrality with water. The dipicrate salt **6** (100 mg) was dissolved in a 50/50 mixture of THF/water (150 mL total volume). The solution was eluted slowly, and the effluent was collected in a 1 L round bottom flask, evaporated to dryness, transferred to a smaller flask, and lyophilized. The product is an oily yellow solid. Yield: 43.6 mg (95%). mp >181°C (dec). ^{1}H NMR (250 MHz, D_2O): δ 3.54 (s, NHCH_2CH_2NH, 8H), 3.06 (t, NHCH_2(CH$_2$)$_3$CH_2NH, 4H); 1.46 (m, (NHCH$_2$CH_2)$_2$CH$_2$, 6H); 1.24 (m, (NHCH$_2$CH$_2$)$_2$CH_2, 2H). ^{13}C {^{1}H} NMR (72.5 MHz, D_2O-dioxane standard): 159.7, 42.5, 42.1, 27.7, 22.7 ppm. Anal. Calc'd for $C_{11}H_{24}N_6Cl_2$·(H_2O): C, 40.22; H, 7.99; N, 25.6. Found: C, 41.16; H, 7.67; N, 24.99.

d,l -3,5 -Bis[[[benzylamino] oxomethyl] amino] -1,2,3,5,6,7 -hexahydro -8 -phenyldicyclopenta[*b,e*]pyridine - (13)

A 100 mL round bottom flask was charged with *d,l*- 3,5- diamino- 1,2,3,5,6,7- hexahydro-8-phenyldicyclo-penta[*b,e*]pyridine[29] (0.184 g, 0.546 mmol), benzylisocyanate (0.15 g, 1.12 mmol) and CH_2Cl_2 (50 mL). The reaction was stirred for 14 hours, at which point diethyl ether (50 mL) was added. The solution was filtered, and the precipitate was washed with ether (2 x 20 mL). The product was dried *in vacuo* to yield 0.26 g of a white solid (90% yield). mp 200-202 °C. ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.29 (m, 15H); 6.34 (s, 2H); 5.92 (d, 2H); 4.99 (q, 2H); 4.38 (d, 2H); 2.69 (m, 6H); 1.79 (m, 2H). ^{13}C {^{1}H} NMR (72.5 MHz, DMSO-d_6) δ 163.01, 162.88, 157.93, 140.84, 132.00, 128.52, 128.25, 127.21, 126.62, 72.31, 60.28, 55.11, 42.99, 34.63, 26.28. HRMS: m/z calcd. for $C_{33}H_{33}N_5O_2$ 531.6572, found 531.2612. Anal. calcd. for $C_{33}H_{33}N_5O_2$ C, 73.71; H, 6.30; N, 13.09. Found: C, 74.55; H, 6.26; N, 13.17.

d,l -3,5 -Bis[[[benzylamino] thioxomethyl] amino] -1,2,3,5,6,7 -hexahydro -8-phenyldicyclopenta[*b,e*]pyridine - (14)

The same procedure as for **13** was used. Yield 85%. mp 205-207 °C. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.05 (s, 2H); 7.93 (d, 2H); 7.35 (m, 13H); 5.71 (d, 2H); 4.87 (q, 2H); 4.58 (d, 4H), 2.91 (m, 2H); 2.62 (m, 4H); 1.67 (t, 2H). ^{13}C {^{1}H} NMR (72.5 MHz, DMSO-d_6) δ 182.89, 162.00, 139.22, 135.87, 133.52, 128.55, 127.70, 127.00, 59.22, 47.24, 34.00, 26.69. Anal. calcd. for $C_{33}H_{33}N_5S_2$ C, 69.15; H, 5.95; N, 12.18. Found: C, 70.30; H, 5.90; N, 12.42.

References:
1) Fersht, A. Enzyme Structures and Mechanism. 2nd Ed. W.H. Freeman and Company: New York, 1977.
2) Landsteiner, K. The Specificity of Serological Reactions. Harvard University Press: Cambridge, MA, 1945.
3) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev.* **1991**, *91*, 1721-2085.

4) Testa, T.; Kyburz, E.; Fuhrer, W.; Giger, R., Perspectives in Medicinal Chemistry. VCH: New York, 1993, pp 275-345.
5) Hoss, R.; Vögtle, F. *Angew. Chem. Int. Ed. Eng.* **1994**, *33*, 375-384.
6) Orgel, L. E. *Nature*, **1992**, *358*, 203-209.
7) Breslow, R. *Science*, **1982**, *218*, 532-537. Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170-177.
8) Lindsey, J. S. *New. J. Chem.* **1991**, *15*, 153-180.
9) Jeffrey, G. A.; Saenger, W. Hydrogen Bonding in Biological Structures. Springer-Verlag: New York, 1991.
10) Chang, S.-K.; Van Engen, D.; Fan, E.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 7640-7645.
11) Bartlett, P. A.; Marlowe, C. K. *Science*, **1987**, *235*, 569-571.
12) Pedersen, J. C. *Angew. Chem. Int. Ed. Eng.* **1988**, *27*, 1021-1027. Cram, D. J. *Angew. Chem. Int. Ed. Eng.* **1988**, *27*, 1009-1020.
13) Schmidtchen, F. P. *J. Am. Chem. Soc.* **1986**, *108*, 8249-8255. Schmidtchen, F. P. *Tetrahedron Lett.* **1989**, *30*, 4493-4496.
14) Kelly, T. R.; Maguire, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 6549-6551. Bell, T. W.; Liu, J. *J. Am. Chem. Soc.* **1988**, *110*, 3673-3674. Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem Soc.* **1989**, *111*, 8055-8057. Park, T. K.; Schroeder, J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 5125-5127. Goodman, M. S.; Rose, S. D. *J. Org. Chem.* **1992**, *57*, 3268-3270. Crego, M.; Marugàn, J. J.; Raposo, C.; Sanz, M. J.; Alcázar, V.; Caballero, M. C.; Morán, J. R. *Tetrahedron Lett.* **1991**, *32*, 4185-4188.
15) Huang, C.-Y.; Cabell, L. A.; Lynch, V.; Anslyn, E. V. *J. Am. Chem. Soc.* **1992**, *114*, 1900-1901.
16) Huang, C.-Y.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778-2793.
17) Kuhn L. P. *J. Am. Chem. Soc.* **1954**, *76*, 4323-4326. Kuhn, L.P. *J. Am. Chem. Soc.* **1952**, *74*, 2492-2499.
18) Christensen, H. N. Biological Transport. 2nd Ed. Benjamin Press: Reading, MA, 1975; Chap. 10.
19) For some representative examples see - Marecek, J. F.; Fischer, P. A.; Burrows, C. J. *Tetrahedron Lett.* **1988**, *29*, 6231-6234. Kimura, E.; *Top. Curr. Chem.* **1985**, *128*, 113-141. Mertes, M. P.; Mertes, K. B. *Acc. Chem. Res.* **1990**, *23*, 413-418. Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896-3904. Aoyama, Y.; Nonaka, S.-i.; Motomura, T.; Toi, H.; Ogoshi, H. *Chem. Lett.* **1991**, 1241-1244. Conn, M. M.; Deslongchamps, G.; de Mendoza, J.; Rebek, J. Jr. *J. Am. Chem. Soc.* **1993**, *115*, 3548 and references therein. Tabushi, I.; Kobuke, Y.; Imuta, J.-i. *J. Am. Chem. Soc.*, **1981**, *103*, 6152-6157. Furuta, H.; Cyr, M. J.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 6677-6678. Deslongchamps, G.; Galan, A.; de Mendoza, J.; Rebek, J. Jr., *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 61-63. Rudkevich, D.M.; Stauthamer, W. P. R. V.; Verboom, W.; Engbersen, J. F. J.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.*, **1992**, *114*, 9671-9673.
20) Chu, F.; Flatt, L. S.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 4194-4204. Flatt, L. S.; Lynch, V.; Anslyn, E. V. *Tetrahedron Lett.* **1992**, *33*, 2785-2788.
21) Smith, J.; Ariga, K.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 362-364.
22) Sessler, J. L.; Furuta, H.; Kral, V. *Supramolecular Chem.* **1993**, *1*, 209-220.
23) Hirst, S. C.; Tecilla, P.; Geib, S. J.; Fan, E.; Hamilton, A. D. *Isr. J. Chem.* **1992**, *32*, 105.
24) Vicent, C.; Fan, E.; Hamilton, A. D. *Tetrahedron Lett.* **1992**, *33*, 4269-4272. Schneider, H.-J.; Juneja, R. K.; Simova, S. *Chem. Ber.* **1989**, *122*, 1211-1213. Hung, C.-Y.; Hopfner, T.; Thummel, R. P. *J. Am. Chem. Soc.* **1993**, *115*, 12601-12602.
25) Kneeland, D. M.; Ariga, K.; Lynch, V. M.; Huang, C.-Y.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 10042-10055.
26) Schiessl, P.; Schmidtchen, F. P. *J. Org. Chem.* **1994**, *3*, 509-511.
27) Ariga, K.; Anslyn, E. V. *J. Org. Chem.* **1992**, *57*, 417-419.
28) Noyori, R. Asymmetric Catalysis in Organic Synthesis. John Wiley and Son, Inc.: New York, 1974, pp 328-332.
29) Kelly-Rowley, A. M.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1991**, *113*, 9687-9688.
30) Kelly-Rowley, A. M.; Anslyn, E. V. Manuscript in preparation.
31) Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369-370.
32) Dawson, R. M. C.; Elliott, W. H.; Jones, K. M. Data for Biochemical Research. 3rd Ed. Oxford University Press: New York, 1986, pp 97-113.
33) Friedrichsen, B. P.; Powell, D. R.; Whitlock, H. W. *J. Am. Chem. Soc.* 1990, *112*, 8931-8941.

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