General Effects of Binding Site Water Exclusion on Hydrogen Bond Based Molecular Recognition Systems: A "Closed" Binding Site Is Less Affected by Environmental Changes than an "Open" Site[1]

James C. Adrian, Jr. and Craig S. Wilcox*,[2]

Contribution from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260. *Received July 8, 1991*

Abstract: A general interpretative scheme for the effects of water on hydrogen bond based molecular recognition of neutral substrates in chloroform is presented. Diacid **1a** is shown to exist in wet or dry $CDCl_3$ in the intramolecular hydrogen bonded state. In comparison, diacids **2a** and **3a** are less tightly closed. As expected, the more tightly closed host binds more weakly to 2-aminopyrimidine in dry $CDCl_3$. A valuable feature of this self-enclosed host is that it is far less susceptible to hydration than the more easily opened hosts. Thus there is very little effect on the enthalpy and entropy changes associated with binding when water is introduced into the $CDCl_3$. This insensitivity of the binding site to limited environmental changes contrasts markedly with the behavior of hosts **2a** and **3a**. Thermodynamic measurements of binding for these latter hosts are quite sensitive to small amounts of water in the $CDCl_3$. It is suggested that the susceptibility of a host-guest system to environmental changes is variable and can be characteristic of induced-fit binding.

Water can act as a competitive inhibitor to the formation of hydrogen-bonded complexes.[3] We found that for one host-guest system the addition of water to dry chloroform leads to only a modest reduction in the free energy of binding but has very significant effects on the enthalpy and entropy changes for the binding process.[4a] This paper examines that phenomenon more closely. A new host which has a binding site that excludes water by forming internal hydrogen bonds is shown to be unaffected by the presence of water in chloroform. This new host binds to substrates through an induced-fit mechanism and illustrates a general strategy for reducing hydration of binding sites or active sites.

Introduction

The equilibria shown in Figure 1 summarize possible elementary effects of water on hydrogen bond based molecular recognition.[5] Two limiting cases can be considered. First consider a relatively rigid and preorganized receptor. For a rigid, preorganized hydrogen bonding receptor site, $\Delta G_{c/o}$ (the free energy change for closing the unhydrated binding site) will be positive. The receptor is "nonclosable". It will remain open, and in dry solvent it will be unoccupied until the substrate binds. In a wet solvent, the receptor will be open but hydrated. The free energy of hydration of this open site is represented as $\Delta G_{hyd/o}$. The observed association energy in wet solvent ($\Delta G_{assoc}^{o/wetted}$) will differ from that in dry solvent by an amount equal to the difference in the hydration free energy for the initial and final states involved.[6,7]

The second case to be considered is that of a receptor that is "closable" and favors the closed state when unoccupied. In this case $\Delta G_{c/o}$ will be negative. If $\Delta G_{c/o}$ is more negative than $\Delta G_{hyd/o}$, then $\Delta G_{hyd/c}$ (hydration of the closed receptor) will be positive, and the resting state of the receptor will be closed and not hydrated. In this case, the observed association energy in dry solvent ($\Delta G_{assoc}^{c/dry}$) will be smaller than that for binding to the unoccupied open host by an amount equal to $-\Delta G_{c/o}$. Importantly, the presence of water in this case will have a smaller effect than does the presence of water in the case of a rigid receptor. This is true because the effect of water is always equal to the total differences in the hydration free energy for the initial and final states involved, and the internal hydrogen bonds are formed when the receptor closes insure that hydration of the "closable" receptor is always less than that of the "nonclosable" receptor by an amount equal to $-\Delta G_{c/o}$.

A receptor that favors a closed form which excludes water can bind to substrates only through an "induced-fit" mechanism.[8,9] Binding to the closable receptor will still be affected by the presence of water, but the major part of this effect will be due only to hydration of the guest. Hydration of the separate host and the host within the complex will not differ by much if the binding site is closed when unoccupied.

These ideas suggest that the susceptibility of a host-guest system to water should be variable *and can be characteristic of induced-fit binding.* A host that binds through the induced-fit mechanism can bind with association constants that are insensitive to the presence of water or other inhibitory molecules.[9] With these thoughts in mind, the following studies are presented.

Results and Discussion

Host 1a Has a Closed Resting State in Wet or Dry $CDCl_3$. Diacid host **1a** is an example of a host that has a "closed" resting state in dry $CDCl_3$ and in wet $CDCl_3$.[10] To show this, varia-

(1) Nineteenth in a series on the Chemistry of Synthetic Receptors and Functional Group Arrays. Part 13: Smith, P. J.; Wilcox, C. S. *J. Org. Chem.* **1990**, *55*, 5675-5678.

(2) Fellow of the Alfred P. Sloan Foundation, 1988-1991.

(3) Pauling, L.; Pressman, D. *J. Am. Chem. Soc.* **1945**, *67*, 1003.

(4) (a) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678-680. (b) Sucholeiki, I.; Lynch, V.; Phan, L.; Wilcox, C. S. *J. Org. Chem.* **1988**, *53*, 98-104.

(5) Figure 1 has been simplified by leaving out considerations of guest hydration. Guest hydration is important and can be a dominant effect with some host-guest combinations. It is apparent that for hosts **2a** and **3a** most of the effect of water is due to host hydration.[7]

(6) The free energies of hydration of the hydrogen bonding functional groups far exceed the hydration energies of the nonpolar portions of the host and guest. The thermodynamic properties of nonionic solutes in dilute aqueous solutions and the free energies of hydration and functional group contributions listed in a valuable work by Cabani can be used to estimate the relative energies of hydration of each of these functional groups: (a) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. *J. Solution Chem.* **1981**, *10*, 563-595. (b) Butler, J. A. V.; Ramchandani, C. N. *J. Chem. Soc.* **1935**, 952. (c) Butler, J. A. V.; Ramchandani, C. N.; Thomson, D. W. *J. Chem. Soc.* **1935**, 280. (d) Christie, A. O.; Crisp, D. J. *J. Appl. Chem.* **1967**, *17*, 11.

(7) Carboxylic acids are more strongly hydrated than amines and aromatic imines in dilute aqueous solution. (For example, respective experimental free energies of hydration for butanoic acid, *n*-butylamine, and *n*-butanol are −26.59 kJ/mol[6b], −19.73 kJ/mol[6c], −17.97 kJ/mol.[6b,d]) This fact agrees with the data in this paper that show that it is hydration of these acid-containing hosts that dominates the effects of water on binding in these systems.

(8) Stryer, L. *Biochemistry*, 2nd ed.; W. H. Freeman: San Francisco, 1981; p 109.

(9) Koshland, D. F., Jr. *Adv. Enzymol.* **1960**, *22*, 45.

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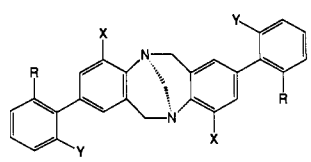

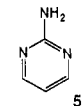

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	R	X	Y
1a	CO_2H	H	CH_3
1b	CO_2CH_3	H	CH_3
1c	$CONH_2$	H	CH_3
2a	CO_2H	H	H
2b	$CONH_2$	H	H
3a	CO_2H	CH_3	H
3b	$CONH_2$	CH_3	H
4a	CO_2H	H	CO_2H
4b	CO_2CH_3	H	CO_2CH_3

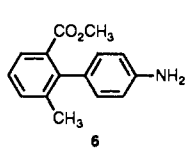
ble-temperature NMR experiments were carried out first with dimethyl ester **1b** in $CDCl_3$ and in DMF-d_7. Diesters and diacids of this general type can take one of three principal conformations: both functional groups can point out (the "out-out" isomer, Figure 2), one functional group can point out (the "in-out" isomer, Figure 2), or both functional groups can point in (the "in-in" isomer, Figure 2). In $CDCl_3$ at −20 °C, ester **1b** takes up all three possible shapes (Figure 3). Dynamic NMR studies reveal a barrier to aryl–aryl rotation of 15.6 ± 0.2 kcal/mol for this diester at the coalescence temperature of 47 °C in DMF-d_7.

Unlike diester **1b**, diacid **1a** has a simple NMR spectrum in $CDCl_3$ at −20 °C (Figure 4). It is not rapid interconversion of the three possible conformers that makes this spectrum simple—the ester has too high a barrier to rotation and the acid will not have a significantly lower barrier.[11] The diacid in $CDCl_3$ at −20 °C (or at 25 °C) must therefore give a simple NMR spectrum because only one conformer is present—the "in-in" conformer. Methanol should disrupt any intramolecular hydrogen bonds that are responsible for the folding of diacid **1a**. In fact, in methanol at −20 °C all three isomers of the diacid are present (Figure 4b).

To unequivocally prove that the differences in the spectra observed in $CDCl_3$ at −20 °C and CD_3OD at −20 °C are due to shifts in conformational equilibria and not due to changes in rotational rate constants, spectra were obtained for a series of intermediate solvent compositions (Figure 5). In all mixtures of chloroform and methanol, observed peak widths were narrow, and no evidence of a slow exchange process was found.

The data in Figure 5 dramatically show that the observed changes in spectra are due to solvent-induced changes in the relative standard free energies of the solutes. In pure chloroform the "out-out" and the "in-out" isomers are unstable relative to the "in-in" isomer, but as methanol is added, better solvation of these isomers results in greater stabilization of these isomers than the "in-in" isomer. As a result, as methanol concentration increases, the standard free energies of the three rotamers become nearly the same.[12,13]

(10) Host **1** was prepared from methyl 4′-amino-6-methylbiphenyl-2-carboxylate (**6**), which is available through standard methods.[24]



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(11) Variable-temperature NMR experiments show that, in a 1/1 (v/v) mixture of $CDCl_3$ and THF-d_8, the barrier to aryl–aryl rotation for tetraacid **4a** is 15 kcal mol^{-1} at 20 °C and the barrier for tetraester **4b** is 14.5 kcal mol^{-1} at a coalescence point of 37 °C. The barrier for the tetraester is unchanged when CD_3OD is the solvent. The barrier for the tetraacid drops to 14 kcal mol^{-1} in CD_3OD.[23]

(12) The less symmetrical isomer is favored by a factor of RT ln 2. (a) Benson, S. W. *J. Am. Chem. Soc.* **1958**, *80*, 5151.

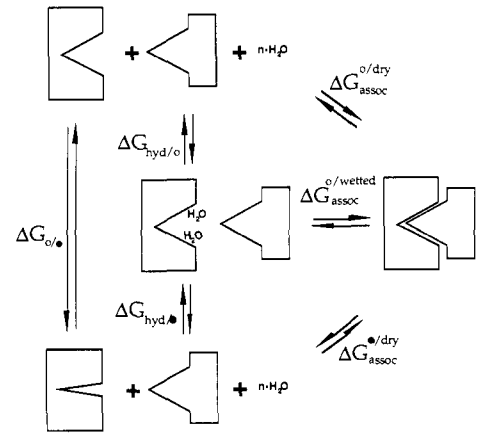

Figure 1. General scheme for water effects in hydrogen bond based binding.[5]

Table I. Association Constants for 2-Aminopyrimidine[16]

host	K_a, M^{-1} [a]	K_a, M^{-1} [b]
1a	190 ± 30	240 ± 40
2a	1060 ± 120	6200 ± 300

[a] 20 °C in wet $CDCl_3$. [b] 20 °C in dry $CDCl_3$.

Host 1a Is More Closed than Host 2a or Host 3a. The predominance of the in-in rotamer of diacid **1a** in $CDCl_3$ can be attributed to the presence of one or two intramolecular hydrogen bonds. The possibility of such hydrogen bonds is supported by NMR data for diamide **1c**. One of the amide protons in **1c** shows a large downfield shift.

Infrared data illuminate an important difference among the three diamides (**1c**, **2b**, and **3b**) that correspond to the three hosts. Data for four molecules are compared in Figure 6. The first molecule to be considered is 2-phenylbenzamide (2-PBA), a simple model for the more complex diamides. The spectrum of 2-PBA (Figure 6a) contains peaks at 3521 and 3404 cm^{-1}, which correspond to the antisymmetrical and symmetrical N–H stretching modes for a primary amide.[14] This spectrum should be compared with the spectrum of diamide **2b** (Figure 6c). Two absorbances for **2b** correspond well to the model compound 2-PBA: there are peaks at 3521 and 3405 cm^{-1} that can be assigned to the non-hydrogen-bonded antisymmetrical and symmetrical N–H stretching modes. In addition, however, the spectrum of diamide **2b** shows additional N–H stretching bands at 3485 and 3169 cm^{-1}. These undoubtedly represent absorbances due to H-bonded primary amides.[14] The concentration dependence of these spectra and of the NMR spectra show that these peaks are due to intramolecular hydrogen bonds. The two small peaks at 3341 and 3279 cm^{-1} also suggest the presence of intramolecularly hydrogen bonded primary amides.[14]

The infrared spectrum of diamide **2b** indicates that both intramolecularly hydrogen bonded and non-hydrogen-bonded solutes are present in chloroform. Diamide **3b** is similar to **2b**, but two additional methyl groups are present on the dibenzodiazocine portion of **3b**. In prior work it has been shown by crystallographic data that the presence of methyl groups at this position can lead to an increase in the dihedral angle formed by the intersection of the planes of the aromatic rings of the dibenzodiazocine. In other words, methyl groups at that position open the "hinge" region of the host to a wider resting state.[4b] Of course, for the present diamides, a more oblique hinge angle would make the intramolecularly hydrogen bonded state less likely. It was reasoned that if this earlier observation on the relationship between substituents

(13) As revealed by Figure 5, diacid **1a** is an intriguing example of a molecule that undergoes solvent-dependent conformational changes. Experiments are underway to further examine this aspect of diacid **1a**.

(14) (a) Jones, R. N.; Sandorfy, C. In *Chemical Applications of Spectroscopy*; West, W., Ed.; Interscience: New York, 1956; pp 510–519. (b) Avram, M.; Matescu, Gh. D. *Infrared Spectroscopy Applications in Organic Chemistry*; Wiley-Interscience: New York, 1978; pp 439–444.

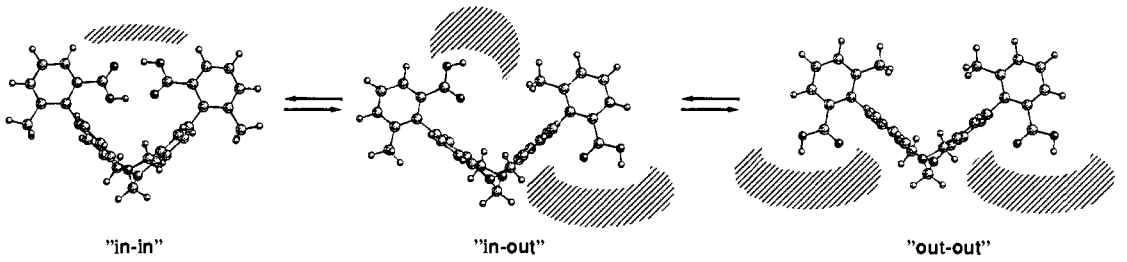

Figure 2. Representations of the three possible conformers for **1a**. The shaded areas indicate potentially strong hydration sites.

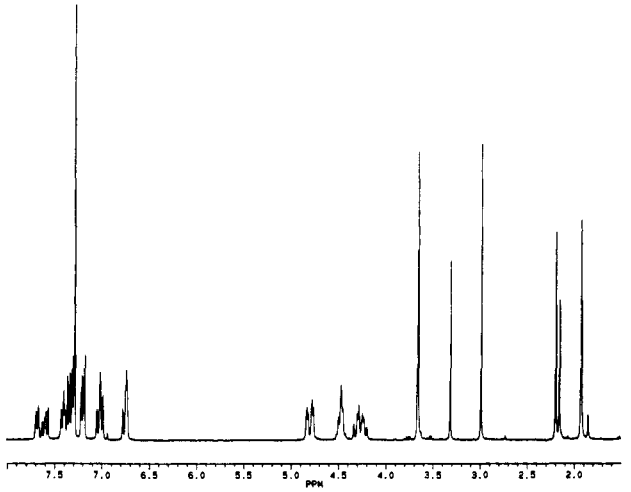

Figure 3. ^{1}H NMR spectrum of **1b** at −20 °C.

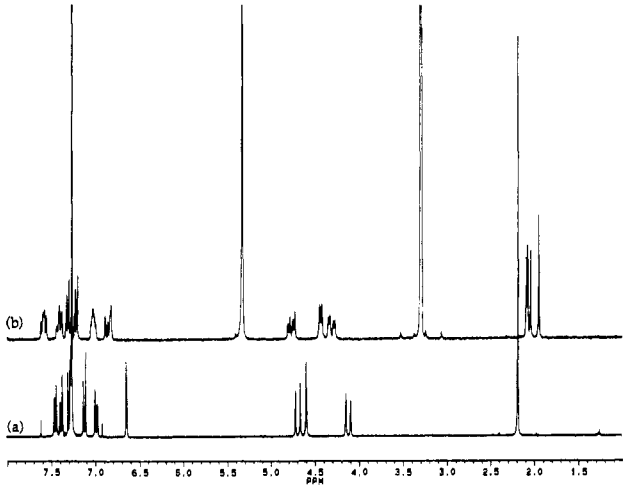

Figure 4. ^{1}H NMR spectra of **1a** in two solvents: (a) $CDCl_3$ at −20 °C; (b) CD_3OD at −20 °C.

and hinge angle is generalizable and applies to solvated dibenzodiazocines, then **3b** should be less intramolecularly hydrogen bonded than **2b**. In fact, the infrared spectrum of **3b** (Figure 6b) is consistent with this reasoning. The absorption bands that are attributed to intramolecularly hydrogen bonded amide species are less pronounced in the spectrum of **3b** than they are in the spectrum of **2b**. Diamide **3b** shows less propensity to form intramolecular hydrogen bonds than diamide **2b** and can be said to be "less tightly closed" than **2b** because of the effect the methyl groups have on the hinge angle.

With the above data available for comparisons, the infrared spectrum of diamide **1c** can be interpreted. The data clearly show that **1c** has a closed resting state in chloroform solution. The spectrum is relatively simple, because resonances that correspond to open, non-hydrogen-bonded primary amides are almost completely absent. Only the two medium bands (3490 and 3169 cm^{-1}) and two weak bands (3333 and 3278 cm^{-1}) expected for a hydrogen-bonded primary amide are present.

Table II. Thermodynamic Parameters for Binding of **1a**, **2a**, and **3a** to 2-Aminopyrimidine[16]

host	$-\Delta H$, "wet"	$-\Delta H$ "dry"	$-\Delta S$, "wet"	$-\Delta S$ "dry"
1a	10.7 ± 0.4	10.7 ± 0.6	26.1 ± 1.2	25.8 ± 2.0
2a	8.6 ± 1.0	15.4 ± 0.4	15.4 ± 3.1	35.3 ± 1.2
3a	9.3 ± 0.6	14.3 ± 0.6	16.0 ± 2.2	30.4 ± 1.7

It seems acceptable to expect that the above observations on amides can be extrapolated to the carboxylic acid hosts. Overall, these data leave little doubt that in wet or dry chloroform diacid **1a** is intramolecularly hydrogen bonded and is an example of a host that has a closed resting state. At high concentrations, methanol can disrupt the intramolecular hydrogen bond and "open" the binding site, but water in chloroform (up to the saturation point) has no observable effect.

Host 1a Binding to 2-Aminopyrimidine Is Unaffected by Water in Chloroform. In comparison with the analogous host **2a**, host **1a** displays a lower affinity for 2-aminopyrimidine (**5**) in both wet $CDCl_3$ and dry $CDCl_3$[15,16] (Table I). This difference indicates that the presence of the methyl group must be destabilizing the bound state of the system in comparison to the unbound state of the system.

An especially illuminating observation is that the difference between these two hosts depends strongly on the environment. In wet chloroform these hosts differ by only a factor of 5, but in dry chloroform they differ by a factor of 25. Host **1a** is almost unaffected by water in chloroform, but host **2a** binds 2-aminopyrimidine in wet chloroform 6 times more weakly than in dry chloroform. The effect of water on binding to host **2a** is similar to the effect we observed with **3a** and with other hosts, but the effect on **1a** is unusually small.

A van't Hoff plot for the binding of **1a** and **5** in dry and wet chloroform (Figure 7a) reveals that for the binding process in dry $CDCl_3$ $\Delta H = -10.7 \pm 0.6$ kcal mol^{-1} and $\Delta S = -25.8 \pm 2.0$ cal mol^{-1} deg^{-1}. In wet $CDCl_3$ the ΔH of binding was -10.7 ± 0.4 kcal mol^{-1}, and the ΔS of binding was -26.1 ± 1.0 cal mol^{-1} deg^{-1}.[16] These results further confirm the picture of a binding site which is closed and unaffected by water (Figure 8).

It is interesting to compare these thermodynamic studies with those obtained for hosts **2a** and **3a** and the same guest. The presence of water has a large effect on the enthalpy and entropy changes associated with binding to **2a** and **3a** (Table II). Water is an inhibitor to binding with these hosts. The effect of water on the binding of 2-aminopyrimidine to **2a** is such as to diminish the heat of association (ΔH) by a large amount: 7 kcal mol^{-1} (Figure 7b). The free energy of association (ΔG) is not so greatly affected due to a compensating entropic factor. These effects of water on the ΔH and ΔS of binding—striking effects in the case of hosts **2a** and **3a**—are completely absent with host **1a**.

Conclusions. Host **2a** binds to *water* much better than host **1a**. The results shown in Table II, considered in the light of the thermodynamic cycles shown in Figure 1, require this conclusion. We propose that the most likely reason for this difference is that

(15) Association constants were determined by direct analysis of NMR titration data. For leading references to this technique, see: Wilcox, C. S. In *Frontiers of Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, Germany, 1991; pp 123–143.

(16) The uncertainties in ΔH and ΔS are calculated for the 95% confidence levels.

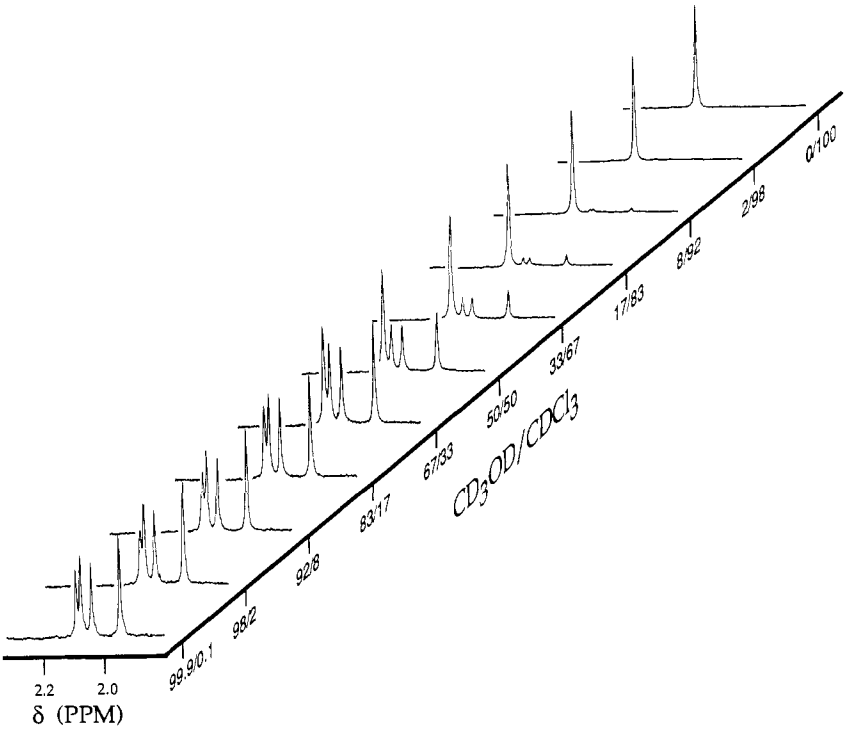

Figure 5. Effect of solvent changes on the aryl methyl group region of the ^1H NMR spectrum (25 °C) of diacid **1a**. In pure $CDCl_3$ one rotamer is present. As CD_3OD is added, the two other possible rotational conformers appear.

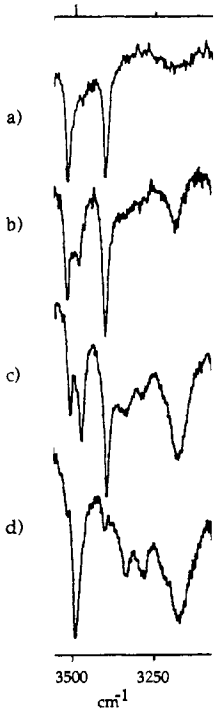

Figure 6. Portions of the infrared spectra of four amides in chloroform: (a) 2-phenylbenzamide; (b) amide **3b**; (c) amide **2b**; (d) amide **1c**. In each case the concentration was 20 mM.

the intramolecular hydrogen bond in host **1a** is stronger than that in **2a**; in other words, the closed resting state is more stable for **1a** than for **2a**. This would explain why **2a** but not **1a** is hydrated by water in chloroform.

The proposition that the intramolecular hydrogen bond in **1a** is stronger than that in **2a** is not incontestable, but infrared data on the diamides corresponding to these hosts leave no doubt that the diamides differ and that diamide **1c** is much more tightly closed than diamide **2b**. The acids **1a** and **2a** are expected to differ in an analogous fashion.

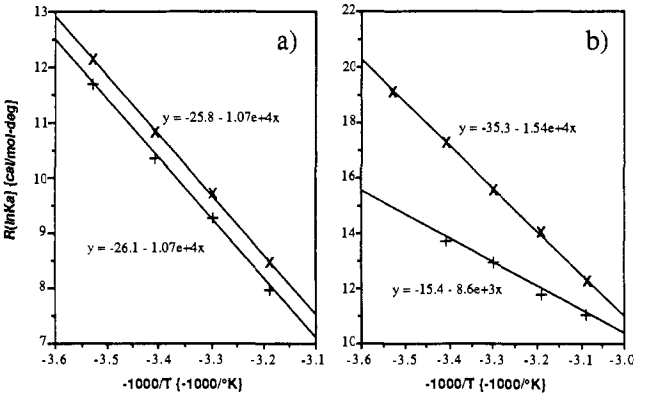

Figure 7. van't Hoff plots for the binding of 2-aminopyrimidine (**5**) by two hosts in dry chloroform (×) and wet chloroform (+): (a) **1a**; (b) **2a**. For host **1a** there is no significant change in the ΔH and ΔS of binding in going from dry chloroform (×) to wet chloroform (+).[10] For host **2a** the change to wet chloroform has an obvious effect.

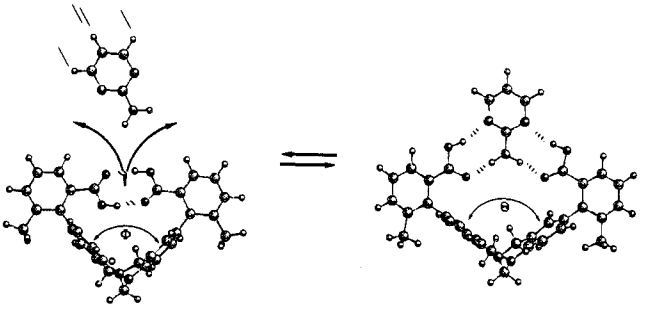

Figure 8. Illustration of the structural changes (induced fit) required when 2-aminopyrimidine binds to host **1a**. The angle formed by the intersection of the two dibenzodiazocine aryl units (the "hinge angle") is designated for the bound and unbound host. Note that Θ is greater than Φ.

The methyl groups must be responsible for the observed differences between **1a** and **2a**, but the mechanism of this effect remains obscure. In host **2a**, the methyl groups are absent and

aryl–aryl rotation is relatively fast. An exact measurement has not yet been achieved, but from NMR line broadening effects we can estimate that the barrier to rotation is less than 11 kcal mol^{-1}. Formation of the internal hydrogen bond requires the loss of two internal rotations and a consequent decrease in internal entropy. Host **1a** might have a stronger internal hydrogen bond because the decrease in internal entropy that attends hydrogen bond formation will be less for this more conformationally restricted molecule. However, solvation phenomena may also contribute to the observed difference. The "in-in" isomer of **1a** presents more lipophilic surface area to the solvent (chloroform) than the "in-in" isomer of **2a**. In addition, the added methyl groups in **1a** will cause changes in bond angles that are expected to decrease the acid–acid distance. Other more complex causes may be imagined, but the available data do not allow an evaluation of the validity or relative importance of these potential explanations.

A simple comprehensive explanation of the binding data and the effects of water on binding can be based on the general scheme that was presented in the introduction. The compensating effects of water on hydrogen bond based host–guest systems in chloroform are critically dependent on the susceptibility of the solvated species to hydration. The results presented are consistent with the picture of **1a** as a host with a binding site which excludes water and thereby requires the guest to induce a favorable binding conformation. This host has a closed resting state and binds through an "induced-fit" type mechanism. It is not affected by the presence of water in chloroform. Hosts **2a** and **3a** are more affected by water because the intramolecular hydrogen bonds possible for these hosts are not as stable as the intramolecular hydrogen bond in host **1a**.

This paper illustrates an interesting difference between a preorganized and complementary binding site and a binding site that has a self-enclosed resting state and binds through an induced-fit mechanism. Closed binding sites cannot associate with a given substrate with the highest possible affinities because of the energetic costs that must be paid to open the cavity; nevertheless, closed binding sites will be advantageous in some contexts because they will be less sensitive to environmental changes than are open and more preorganized binding sites.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs of Norcross, GA. Infrared spectra were recorded on a Matteson Cygnus 100 spectrophotometer. Proton and carbon magnetic resonance spectra were obtained on a Bruker QM-300 MHz or Bruker QM-500 MHz spectrometer. Chemical shifts (^1H NMR and ^{13}C NMR) are expressed in parts per million (δ units) downfield from tetramethylsilane (TMS) used as an internal reference. For ^{13}C NMR, peak assignments were made relative to TMS (77.00 ppm) in $CDCl_3$. Low- and high-resolution mass spectra were obtained on a VG 7070 mass spectrometer. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60F-254 (0.25 mm) analytical glass plates. E. Merck silica gel 60 (230–400 mesh) was used for flash chromatography, which was performed according to the method of Still.[17] Alumina thin-layer chromatography (ATLC) was performed using E. Merck alumina 150 F_{254} (Type T, 0.25 mm) analytical glass plates. E. Merck neutral alumina activity I (70–230 mesh ASTM) was used for open column chromatography. Solvents were dried by distillation from the appropriate drying agent under a dry nitrogen atmosphere. Tetrahydrofuran (THF) and diethyl ether (ether) were distilled from sodium and benzophenone. Benzene was distilled from sodium. Ethyl acetate used for chromatography was dried over 4-Å molecular sieves for at least 24 h prior to use. All other commercially available reagents and solvents were reagent grade and used without further purification.

Where indicated, reactions run under a nitrogen atmosphere were arranged with a mercury or oil bubbler so that the system could be alternately evacuated and filled with nitrogen and left under positive pressure. Syringes and reaction flasks were dried for at least 12 h in an oven at ≥120 °C and cooled in a dessicator over calcium sulfate prior to use. "Room temperature" refers to ambient laboratory conditions: T = 20–27 °C, P = 720–770 mmHg. "Concentration in vacuo" refers to concentration on a rotary evaporator equipped with a heating bath (T ≤ 70 °C) sometimes followed by further concentration using a high vacuum (<2 mmHg) pump. $ZnCl_2$ was fused over an open flame under a stream of nitrogen.

Preparation of Wet and Dry $CDCl_3$. $CDCl_3$, when used in experiments for the determination of association constants, was prepared in one of two ways: (1) Dry $CDCl_3$ was prepared by refluxing over $CaCl_2$ for a minimum of 5 h and then distilling[18] over molecular sieves and storing under a dry nitrogen atmosphere not more than 2 days before use. (2) Wet $CDCl_3$ was prepared by filtering $CDCl_3$ through basic alumina and stirring the eluate with distilled water for at least 1 h. The resultant water-saturated $CDCl_3$ was used immediately.

Evaluation of Association Constants: Titrations. The procedures for recording ^1H NMR spectra for the determination of association constants are of two types. (1) The substrate was maintained at constant concentration in the presence of increasing concentrations of a receptor. The substrate was weighed and placed in a dry vial, and solvent was added by microanalytical syringe to bring the concentration of the substrate to the desired level. The stock solution was sonicated if necessary to accelerate dissolution. An aliquot of the substrate stock solution was added, using a microanalytical syringe, to a weighed amount of the receptor in an NMR sample tube to give a solution of the receptor and substrate, which was then sonicated if necessary. The solution of receptor and substrate was diluted with aliquots of the substrate stock solution using a microanalytical syringe. In this way, the concentration of the receptor decreased while the concentration of the substrate remained constant. (2) The substrate and receptor were maintained at equimolar concentrations through subsequent dilutions. The substrate was weighed and placed in a dry vial, and solvent was added by microanalytical syringe to bring the concentration of the substrate solution to the desired level. The solution was sonicated if necessary. The receptor was weighed and placed in an NMR sample tube; the amount of substrate solution required to bring the receptor to an equal concentration to that of the substrate was added via microanalytical syringe, and the resulting solution was sonicated if necessary. The receptor/substrate solution was subsequently diluted by the addition of aliquots of solvent via a microanalytical syringe.

Variable-Temperature Studies. Stock solutions of the receptor and substrate and the substrate in $CDCl_3$ (either wet or dry) were prepared in separate vials. In eight separate NMR sample tubes mixtures of each solution were added such that the concentration of the receptor decreased while the concentration of the substrate remained constant. Titrations were then conducted at variable temperatures generally in the range from 0 to 60 °C.

Methyl 4′-Amino-6-methylbiphenyl-2-carboxylate (6).[19] To a stirred solution of 20.2 g (63.8 mmol) of *N,N*-bis(trimethylsilyl)-4-bromoaniline[20] in 40.0 mL of ether at −78 °C under nitrogen was added dropwise, over 45 min, 83.0 mL (1.7 M, 141.1 mmol) of a solution of *tert*-butyllithium in pentane. The resulting mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature. The reaction mixture was concentrated under reduced pressure (~1.5 mmHg, care must be taken to avoid undue bumping) affording a yellow solid, which was subsequently redissolved in 40.0 mL of THF. The organolithium solution was then transferred via cannula to a stirred solution of 9.13 g (67.0 mmol) of fused $ZnCl_2$ in 40.0 mL of THF and stirred for 1 h at room temperature. The organozinc chloride solution was then transferred via cannula to a stirred solution of 19.3 g (70.0 mmol) of methyl 3-methyl-2-iodobenzoate[21] and $Ni(PPh_3)_4$ catalyst (prepared in situ by the reaction of 1.62 g (6.3 mmol) of $Ni(acac)_2$, 6.62 g (52.2 mmol) of PPh_3, and 6.3 mL (6.3 mmol) of diisobutylaluminum hydride) in 40.0 mL of THF and stirred overnight. The reaction mixture was poured into a separatory funnel containing 75 mL of 2 N HCl and 250 mL of ether and shaken, and the layers were separated. The ether layer was further extracted with two 50-mL portions of 2 N HCl. The combined aqueous portions were then allowed to stand. After 1 h, a red-brown "oil" was separated, and the remaining aqueous portion was concentrated under reduced pressure to 50 mL. After standing for 1 h, additional red-brown "oil" was separated and combined with the previously obtained material. The "oil" was then poured into a separatory funnel containing 100 mL of concentrated NH_4OH and 75 mL of CH_2Cl_2 and shaken. The organic layer was separated, and the remaining aqueous layer was further extracted with two 75-mL portions of CH_2Cl_2. The combined organics

(17) Still, W. C.; Khan, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(18) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1988; pp 121.

(19) According to the method of Negishi and co-workers: Negishi, E.; Takahashi, T.; King, A. O. *Org. Synth.* **1987**, *66*, 67–74.

(20) *N,N*-Bis(trimethylsilyl)-4-bromoaniline was prepared according to Pratt, J. R.; Massey, W. D.; Pinkerton, F. H.; Thames, S. F. *J. Org. Chem.* **1975**, *40*, 1090–1094.

(21) Methyl 3-methyl-2-iodobenzoate was prepared according to Mayer, F. *Chem. Ber.* **1911**, *44*, 2298–2305.

were dried ($MgSO_4$), filtered and concentrated in vacuo to afford 8.37 g (54%) of a dark red oil. ^1H NMR analysis of the product oil indicated ≥95% purity; therefore, no further purification was done: bp 212–218 °C (1.5 mmHg); R_f = 0.13 (SiO_2, 5%, ethyl acetate/CH_2Cl_2); IR ($CHCl_3$) 3451, 3374, 3009, 2953, 2863, 1722, 1622, 1520, 1459, 1436, 1294, 1179, 1141, 1121, 1003, 828 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$) δ 7.58 (d, 1 H, J = 7 Hz), 7.36 (d, 1 H, J = 7 Hz), 7.25 (t, 1 H, J = 7 Hz, J = 7 Hz), 6.95 (d, 2 H, J = 8 Hz), 6.72 (d, 2 H, J = 8 Hz), 3.78 (bs, 2 H), 3.57 (s, 3 H), 2.13 (s, 3 H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 169.3 (1C), 144.7 (1C), 141.5 (1C), 137.6 (1C), 132.8 (1C), 132.2 (1C), 130.4 (1C), 129.5 (2C), 126.6 (2C), 114.9 (2C), 51.8 (1C), 20.7 (1C); MS m/e calcd for $C_{15}H_{15}NO_2$ (M^+) 241.1103, measured 241.1102. Anal. Calcd for $C_{15}H_{15}NO_2 \cdot 0.1H_2O$: C, 74.11; H, 6.30; N, 5.76. Found: C, 74.02; H, 6.36; N, 5.71.

Dimethyl 6′,6″-Dimethyl-2,8-diphenyl-6*H*,12*H*-5,11-methanodibenzo-[*b*,*f*][1,5]diazocine-2′,2″-dicarboxylate (2).[22] A solution of 5.00 g (20.7 mmol) of biphenyl amine **6** and 2.90 g (20.7 mmol) of hexamethylenetetraamine in 40 mL of trifluoroacetic acid (TFAA) was stirred at room temperature. After 24 h, the TFAA was removed by distillation. The pot residue was taken up in 20.0 mL of water, poured into a separatory funnel, and basified by the addition of 50 mL of concentrated NH_4OH. The aqueous layer was then extracted with three 100-mL portions of CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 6.24 g of a yellow-brown glass foam. Preliminary purification by flash chromatography (75 mm × 40 mm column of SiO_2, eluted with 5/95% CH_3OH/CH_2Cl_2) afforded a yellow glass foam. Purification by flash chromatography (178 mm × 50 mm column of SiO_2, eluted with 2.5/97.5% CH_3OH/CH_2Cl_2) afforded 2.76 g (51%) of diester dibenzodiazocine **1b** (purity ≈ 90%) as a yellow glass foam, which was then crystallized from 95% ethanol (~40 mL) to afford a first crop of 1.47 g as florets of fine yellow needles and a second crop (~20 mL) of 0.43 g: mp 158.0–162.0 °C; R_f = 0.07 (SiO_2, 2.5/97.5%, CH_3OH/CH_2Cl_2); IR ($CHCl_3$) 3010, 2952, 2907, 2853, 1724, 1613, 1578, 1497, 1461, 1453, 1406, 1296, 1195, 1142, 1097, 1013, 965, 942, 967, 840 cm^{-1}; 300 MHz ^1H NMR (DMF-d_7, 90 °C) δ 7.50 (d, 2 H, J = 8 Hz), 7.41 (d, 2 H, J = 7 Hz), 7.30 (t, 2 H, J = 8 Hz, J = 7 Hz), 7.16 (d, 2 H, J = 8 Hz), 6.95 (d, 2 H, J = 8 Hz), 6.77 (s, 2 H), 4.73 (d, 2 H, J = 17 Hz), 4.36 (s, 2 H), 4.25 (d, 2 H, J = 17 Hz), 3.32 (bs, 6 H), 2.05 (s, 6 H); 300 MHz ^1H NMR ($CDCl_3$, −20 °C) δ 7.62 (m, 2 H), 7.36 (m, 4 H), 7.19 (m, 2 H), 7.04 (m, 2 H), 6.75 (m, 2 H), 4.79 (m, 2 H), 4.47 (m, 2 H), 4.26 (m, 2 H), 3.66 (s), 3.65 (s), 3.31 (s), 2.98 (s), 2.19 (s), 2.15 (s), 1.92 (s), 1.91 (s); 75 MHz ^{13}C NMR ($CDCl_3$, −20 °C) δ 169.5, 168.8, 168.7, 146.4, 141.1, 140.9, 140.8, 140.5, 137.2, 137.1, 136.8, 135.8, 135.5, 132.9, 132.1, 131.6, 131.4, 131.1, 127.8, 127.6, 127.2, 126.8, 126.5, 126.4, 124.4, 124.3, 66.7, 58.8, 58.6, 58.4, 51.9, 51.7, 51.0, 20.8, 20.7, 20.4; MS m/e calcd for $C_{33}H_{30}N_2O_4$ (M^+) 518.2206, measured 518.2208. Anal. Calcd for $C_{33}H_{30}N_2O_4$: C, 76.43; H, 5.83; N, 5.40. Found: C, 76.17; H, 5.87; N, 5.42.

6′,6″-Dimethyl-2,8-diphenyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine-2′,2″-dicarboxylic Acid (1a). To a stirred heterogeneous mixture of 842 mg (35.1 mmol) of anhydrous lithium hydroxide in 6.0 mL of 4/1 (v/v) CH_3OH/water at room temperature was added a hot (50 °C) solution of 523 mg (1.0 mmol) of **1b** in 8.0 mL of 4/1 (v/v) CH_3OH/water and 2.0 mL of CH_2Cl_2. The flask was sealed with a wired septum, and the stirred mixture was heated at 50 °C. After 24 h, the excess solid LiOH was removed by filtration, and the filtrate was concentrated in vacuo. The concentrate was diluted with 5 mL of water to afford a basic yellow homogeneous solution (pH ≥ 13.0). The basic solution was then acidified by the careful addition of 14.5 mL of 2 N HCl (final pH ≈ 2.0). The white precipitate was extracted with three 20-mL portions of $CHCl_3$. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 560 mg of host **1a** as fine white needles. Recrystallization from ~20 mL of $CHCl_3$ afforded 350 mg (1st crop) followed by a second crop (~10 mL) of 110 mg (total 92%) as fine colorless needles, which become opaque white when dry: mp 294–296 °C dec; IR ($CHCl_3$) 3250 (b), 2957, 2929, 2856, 2556, 1700, 1479, 1465, 1404, 1294, 1202, 941, 842, 750, 730, 662 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$, 23 °C) δ 7.44 (d, 2 H, J = 8 Hz), 7.38 (d, 2 H, J = 7 Hz), 7.27 (t, 2 H, J = 8 Hz, J = 7 Hz), 7.11 (d, 2 H, J = 8 Hz), 6.98 (dd, 2 H, J = 8 Hz, J = 2 Hz), 6.63 (d, 2 H, J = 2 Hz), 4.68 (d, 2 H, J = 16 Hz), 4.59 (s, 2 H), 4.17 (d, 2 H, J = 16 Hz), 2.18 (s, 6 H); 125 MHz ^{13}C NMR ($CDCl_3$, 23 °C) δ 174.3, 146.3, 141.1, 137.0, 135.3, 132.7, 132.4, 128.8, 127.7, 127.4, 127.1, 125.5, 123.8, 67.9, 60.2, 20.5; MS m/e calcd for $C_{31}H_{26}N_2O_4$ (M^+) 490.1893, measured 490.1894. Anal. Calcd for $C_{31}H_{26}N_2O_4 \cdot 1.0CHCl_3$: C, 63.08; H, 4.45; N, 4.58; Cl, 17.41. Found: C, 62.96; H, 4.59; N, 4.78; Cl, 17.04.

6′,6″-Dimethyl-2,8-diphenyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine-2′,2″-dicarboxamide (1c).[23] To a stirred, saturated solution of ammonia in 6.0 mL of benzene at room temperature was slowly added 1.2 mL (2.0 M, 2.4 mmol) of a solution of trimethylaluminum. After 15 min, a solution of 601 mg (1.2 mmol) of **1b** in 4 mL of CH_2Cl_2 was added. The stirred reaction mixture was heated to 40 °C. After 8 days, the reaction was quenched by the dropwise addition of 2 N HCl (caution should be taken here to avoid excess foaming). The reaction mixture was then poured into a separatory funnel with ~25 mL of water and ~25 mL of CH_2Cl_2 and shaken. The organic layer was separated, and the aqueous layer was further extracted with three 25-mL portions of CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford a yellow foam. Purification by open column chromatography (neutral alumina, activity I; 2/4/94% CH_3OH/ethyl acetate/CH_2Cl_2) afforded 110.0 mg of a white foam. The foam was crystallized from ~15 mL of ethyl acetate to afford 80.0 mg (14%) of **1c** as fine white needles: mp 192–194 °C dec; R_f = 0.13 (neutral alumina, 2/4/94% CH_3OH/ethyl acetate/CH_2Cl_2); IR ($CHCl_3$) 3490, 3333, 3278, 3169, 3007, 2976, 2914, 1731 (ethyl acetate), 1675, 1605, 1589, 1496, 1458, 1386, 1249, 1116, 964, 941, 844 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$, 23 °C) δ 7.81 (bs, 2 H), 7.31 (m, 6 H), 7.14 (d, 2 H, J = 9 Hz), 7.04 (dd, 2 H, J = 9 Hz, J = 2 Hz), 6.63 (d, 2 H, J = 2 Hz), 5.02 (bs, 2 H), 4.69 (d, 2 H, J = 16 Hz), 4.56 (s, 2 H), 4.11 (d, 2 H, J = 16 Hz), 2.16 (s, 6 H); 75 MHz ^{13}C NMR ($CDCl_3$, 23 °C) δ 173.0 (2C), 147.0 (2C), 1138.4 (2C), 137.2 (2C), 136.5 (2C), 135.0 (2C), 131.0 (2C), 128.3 (2C), 128.2 (2C), 127.8 (2C), 127.3 (2C), 124.4 (4C), 67.7 (1C), 59.9 (2C), 20.5 (2C); MS m/e calcd for $C_{31}H_{28}N_4O_2$ (M^+) 488.2212, measured 488.2212. Anal. Calcd for $C_{31}H_{28}N_4O_2 \cdot 0.5C_4H_8O_2 \cdot 0.6H_2O$: C, 72.95; H, 6.16; N, 10.31. Found: C, 72.99; H, 6.07; N, 10.23.

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(22) Johnson, R. U.S. Patent 4447607, 1984.

(23) Basha, A.; Lipton, M.; Wienreb, S. M. *Tetrahedron Lett.* **1977**, 4171–4174.

(24) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055–8057.