Binding of 9-*N*-Butyladenine by Carboxylic Acids: Evidence that Hoogsteen Binding Can Dominate in Solution

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^{1}H NMR titration of 9-*N*-butyladenine (**1**) with a series of 11 representative carboxylic acids has been carried out in $CDCl_3$ by following the chemical shifts of δ_{H-2} and δ_{H-8} of **1** to determine the association constants for the Watson−Crick and the Hoogsteen modes of binding. Compound **1** has been found to bind carboxylic acids through the Watson−Crick (WC) site or the Hoogsteen (HG) site. The binding of carboxylic acids from the WC site shifts the δ_{H-2} signal upfield, whereas the binding from the HG site shifts the δ_{H-8} signal downfield. Analyses of binding-induced shifts of δ_{H-2} and δ_{H-8} of **1** in the presence of the carboxylic acids have indicated a *distinct preference for the HG site* by aromatic carboxylic acids, such as benzoic acid and monobenzyl isophthalate. On the other hand, aliphatic acids such as 4-nitrophenylacetic acid and propanoic acid are found to prefer the WC site for complexing **1**. Binding affinities of a few alkenoic and alkynoic acids were also determined. In addition to the complexes (**1**/acid**)** of 1:1 stoichiometry, the possibility of a 1:2 complexation between **1** and the carboxylic acids is addressed. A possible rationale for the *upfield* shift of H-2 (of **1)** upon binding by a carboxylic acid is discussed. The K_a's were found to increase in general with enhanced acidity of the carboxylic acids. However, the pK_a values of the acids do not appear to determine the site-specificity of the binding of **1**.

Introduction

The hydrogen-bonding surface of the carboxyl group has been used extensively in synthetic receptors,[1] and in many of these receptors this functionality has been used to bind adenine derivatives, which are important targets in molecular recognition.[2−4] In the binding of adenine the determination of site specificity has been considered to be an important aspect. For example, in 9-*N*-butyladenine (**1**) there are two different sites of binding: the Watson−Crick (WC) and the Hoogsteen (HG) sites (Figure 1). In the early studies of the complexation of nucleobases by Rich and co-workers the possibility of WC/HG binding was not considered.[5] Katz established through ^{1}H NMR measurements in $CDCl_3$ that for the A−U pair the remote H-2 and H-8 signals of 9-*N*-ethyladenine (**2**) underwent shifts during binding.[6] The chemical shifts of H-2 and H-8 were shown to indicate binding through the WC and HG sites, respectively. Carboxylic acids may complex **1** from either of these two sites. Using butanoic acid and adenine derivative **2** in $CDCl_3$, Lancelot showed that WC binding caused the adjacent H-2 to move *upfield*, whereas HG binding induced *downfield* shifts to the adjacent H-8.[7] An experimental differentiation between WC and HG binding using 6-*N*-methyl-9-*N*-ethyladenine (**3**) was reported by Engel et al.[8]

Considerable attention has been paid in recent years to understand HG binding in solution using the nuclear Overhauser effect (nOe) as the probe. Rebek and co-workers had performed a detailed study on the site specificity of the binding of **2** by a synthetic receptor.[9] Both the WC and HG binding modes were shown to be operative. This method has been extended to other base-pairs by Schneider and co-workers.[10] Zimmerman's molecular tweezer was also shown to bind **2** from the HG site.[11] In relation to our work on the development of a bile acid based dicarboxyl functionalized molecular tweezer for binding **1**[12] we examined the binding of **1** by various carboxylic acids.

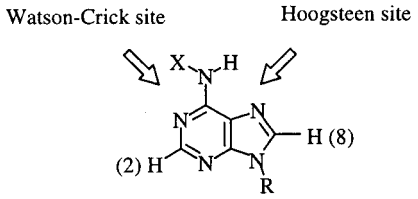

Figure 1. Watson−Crick and Hoogsteen sites. **1**: R = Bu; X = H. **2**: R = Et; X = H. **3**: R = Et; X = Me.

We realized that the remote-proton shifts (H-2 and H-8) of **1** observed by ^{1}H NMR were a powerful tool to examine the site-specific binding of **1** by carboxylic acids. In this paper we present a detailed account of our observations regarding the recognition between different carboxylic acids and **1** in $CDCl_3$ by following the ^{1}H NMR shifts of the remote H-2 and H-8 protons.

Results

When ^{1}H NMR titrations[12] between monobenzyl isophthalate (as a model monocarboxylic acid, 3.5−18 mM) and adenine derivative **1** (5.7 mM) were carried out in $CDCl_3$, an interesting observation was made: *an almost exclusive downfield shift of H-8* by 0.10 ppm (K_a = 250 M^{-1}) was observed. A similar result was also obtained from the titration of **1** with benzoic acid (3.0−26 mM). There was a downfield shift *only* for H-8 ($\Delta\delta_{obs}$ = 0.1),[13] yielding a K_a of 150 M^{-1}.

Lancelot had indicated that for the binding between a carboxylic acid and compound **2** three competitive complexations could be observed: WC binding, HG binding, and a

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TABLE 1: Binding Data from the Titration Experiments between 1 and Various Carboxylic Acids

entry	acid	acid (mM)	**1** (mM)	$\Delta\delta_{obs}$ ($\Delta\delta_{max}$[a]) H-8 downfield (ppm)	$\Delta\delta_{obs}$ ($\Delta\delta_{max}$[a]) H-2 upfield (ppm)	$K_a(M^{-1})$[b,c] (site)	pK_a[d]
1	2-iodobenzoic acid	5.0−35	20	0.179 (0.31)	0.008[e]	15 (HG)	2.85
2	monobenzyl isophthalate	3.5−18	5.7	0.12 (0.16)	0.00	250 (HG)	3.54[f]
3	benzoic acid	3.0−26	5.0	0.12 (0.15)	0.00	150 (HG)	4.19
4	4-methoxybenzoic acid	4.0−58	8.0	0.10 (0.14)	0.02[e]	53 (HG)	4.47
5	4-nitrophenylacetic acid	6.6−22	10.5	0.06 (0.10)	0.15 (0.18)	263 (WC); 53 (HG)	3.85
6	propanoic acid	2.6−26	21	0.02[g]	0.07 (0.13)	55[h] (WC)	4.87
7	dimethylacetic acid	4.0−26	7.2	0.02[g]	0.05 (0.15)	19 (WC)	5.03
8	*E*-3-phenylpropenoic acid	5.0−35	10	0.045 (0.050)	0.068 (0.13)	198 (WC); 51 (HG)	4.44
9	*E*-2-butenoic acid	5.0−35	10	0.066(0.089)	0.044 (0.07)	100 (WC); 67 (HG)	4.69
10	3-phenylpropynoic acid	5.0−35	10	0.205 (0.28)	*e*	96 (HG)	2.25[h]
11	2-butynoic acid	5.0−35	20	*g*	0.100 (0.150)	158 (WC)	2.66[h]

[a] Value obtained from a nonlinear curve-fitting program. [b] Average value of at least two experiments (25 °C). The K_a's were obtained within ±20% even while employing different concentrations of **1** for the same carboxylic acid. [c] Association constants between **2** and a number of substituted benzoic acids were reported in ref 11. [d] *CRC Handbook of Chemistry and Physics*, 61st ed.; Weast, R. C., Ed.; CRC Press Inc.: Boca Raton, FL, 1980. [e] The δ_{H-2} moved upfield initially, and then the direction of shift changed. As a result, the K_a could not be evaluated. [f] First pK_a of isophthalic acid. [g] δ_{H-8} underwent a downfield shift and then remained constant. K_a could not be evaluated. [h] Krygowski, T. M.; Guillemé, J. *J. Chem. Soc., Perkin Trans. 2* **1982**, 531.

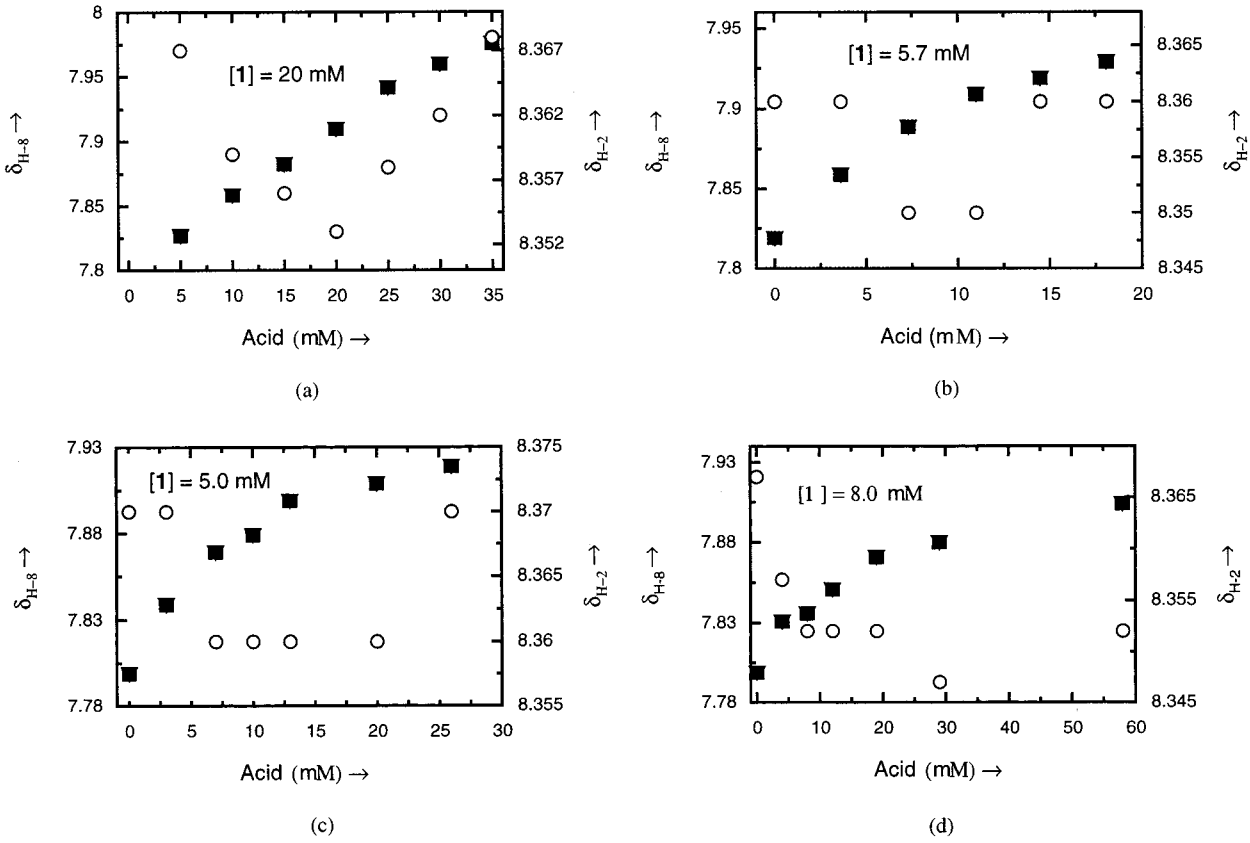

Figure 2. Plots of chemical shift (δ) vs concentration (mM) of aromatic carboxylic acids: (a) 2-iodobenzoic acid; (b) monobenzyl isophthalate; (c) benzoic acid; (d) 4-methoxybenzoic acid. ■ indicates δ_{H-8}; ○ indicates δ_{H-2}.

single-point binding at N-3. Binding with butanoic acid produced a large *upfield* shift ($\Delta\delta_{max} = 0.17$)[14] of H-2 (which was in itself an unusual finding considering that protons adjacent to an H-bonded atom always shift downfield!) and a smaller *downfield* shift ($\Delta\delta_{max} = 0.05$) of H-8. Evidence provided by Lancelot suggested that the major site of binding was the WC site with a 3-fold preference over the Hoogsteen site. He also noted that for **3**, HG binding will produce a predominant downfield shift of H-8 with a negligible upfield shift of H-2.

A titration between propanoic acid and **1** was performed in order to understand the HG vs WC binding under Lancelot's conditions. Following the concentrations prescribed by him, a solution of **1** (20 mM) was titrated with propanoic acid (5−26 mM). In this case we observed that the nature of the shifts was similar to those reported by Lancelot, i.e., a predominant upfield shift of H-2 ($\Delta\delta_{obs} = 0.06$) and a small downfield shift of H-8 ($\Delta\delta_{obs} = 0.02$), indicating a preference for the WC mode of binding. The K_a calculated from the shifts of H-2 was 55 M^{-1}. Incorporation of the dimerization data[15] for propanoic acid raised the K_a to 180 M^{-1}, compared to the value of 160 M^{-1} reported by Lancelot. To probe the binding behavior of a "stronger" aliphatic carboxylic acid, compound **1** (10.5 mM) was again titrated with 4-nitrophenylacetic acid (5.5−18 mM). Once more, a predominant upfield shift of H-2 ($\Delta\delta_{obs} = 0.16$, $K_a = 263$ M^{-1}) and a minor downfield shift of H-8 ($\Delta\delta_{obs} = 0.06$) were observed.

Further experiments involving a number of aromatic, aliphatic, alkenoic, and alkynoic carboxylic acids were performed and the results are listed in Table 1.

The titration of **1** with 2-iodobenzoic acid resulted in a downfield shift of H-8 ($\Delta\delta_{obs} = 0.18$, $K_a = 15\ M^{-1}$), whereas the shift for H-2 was negligible (entry 1). The results obtained from the titration employing monobenzyl isophthalate and benzoic acid have already been discussed (entries 2, 3). When 4-methoxybenzoic acid was used, a small upfield shift was observed for H-2 ($\Delta\delta_{obs} = 0.02$, entry 4). However the major shift encountered was that of H-8 ($\Delta\delta_{obs} = 0.10$) with a calculated K_a of 53 M^{-1}.

Among the aliphatic carboxylic acids studied, 4-nitrophenylacetic and propanoic acids showed a pronounced upfield shift of the δ_{H-2} and a minor downfield shift of the δ_{H-8} as discussed earlier (entries 5, 6). The trend observed for the aliphatic carboxylic acids continued for the titration involving dimethylpropanoic acid (entry 7). The signal for H-2 underwent a larger upfield shift ($\Delta\delta_{obs} = 0.05$, $K_a = 19\ M^{-1}$) compared to that observed for H-8 ($\Delta\delta_{obs} = 0.02$).

For the two alkenoic acids examined, it was observed that both *E*-3-phenylpropenoic acid and *E*-2-butenoic acid caused comparable upfield shifts of H-2 and H-8 of **1** (entries 8, 9). The association constants could be determined from both δ_{H-2} as well as δ_{H-8} but were larger for those determined from the shifts of δ_{H-2}.

For titrations involving the two alkynoic acids, it was observed that 3-phenylpropynoic acid resulted in a predominant shift of H-8 ($\Delta\delta_{obs} = 0.21$, $K_a = 96\ M^{-1}$, entry 10). The binding of 2-butynoic acid proceeded to elicit a major shift of H-2 ($\Delta\delta_{obs} = 0.10$, $K_a = 158\ M^{-1}$, entry 11).

Discussion

(a) Site Specificity of Binding. Our observations indicate that *aromatic carboxylic acids prefer binding from the HG site, whereas aliphatic carboxylic acids show a binding preference from the WC site*. This result is based on the predominant shift of the H-8 signal caused by aromatic carboxylic acids during the titration of **1** (Table 1, entries 1−4; Figure 2a−d). Moreover, the changes in δ_{H-8}, and not δ_{H-2}, could be fitted, yielding the K_a's for HG binding. Despite possessing a carboxyl group tilted out of the aromatic plane by 17°,[16] the preference for the HG site remained unaltered for 2-iodobenzoic acid (entry 1). In contrast, for aliphatic carboxylic acids, the upfield shift of the H-2 signal was dominant (entries 5−7; Figure 3a−c). Here, the shifts in δ_{H-2}, and not δ_{H-8}, could be fitted for the determination of the K_a for WC binding (entries 6, 7). For 4-nitrophenylacetic acid K_a(WC) is much larger than K_a(HG) (entry 5).

(b) Downfield Shift of H-8 and the Upfield Shift of H-2 Signals. Even though the phenomenon of an upfield shift of the H-2 signal in **2** upon the binding of carboxylic acids was noticed by Lancelot, its origin was not clear. Lancelot did not offer an explanation for his observations vis-à-vis Katz's, who observed a downfield shift of the H-2 signal of **2** when uracil was bound to it via the WC mode. We suggest that the upfield shift of the H-2 results from a binding-promoted π-polarization, inducing a partial negative charge on the adjacent N-1 (Figure 4).[17] No such polarization occurs during the HG binding. As an indirect support for our hypothesis, the work done by Fish and others may be cited.[18] Fish observed that a "Hoogsteen" type chelate between 9-*N*-methyladnine (with a formal negative charge on N-6) and a rhodium fragment showed a pronounced upfield shift of H-2 and yet a downfield shift of H-8 (Figure 5).

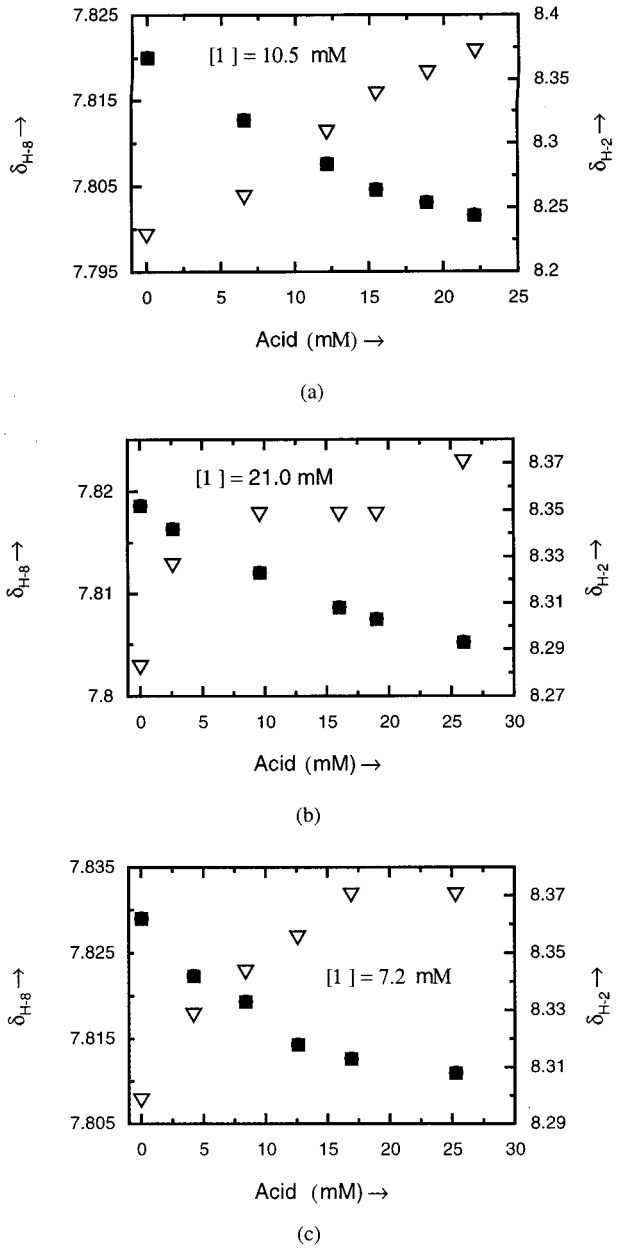

Figure 3. Plots of chemical shift (δ) vs concentration (mM) of aliphatic carboxylic acids: (a) 4-nitrophenylacetic acid; (b) propanoic acid; (c) dimethylpropanoic acid. ▽ indicates δ_{H-8}; ■ indicates δ_{H-2}.

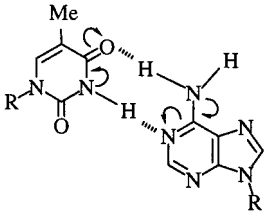

Figure 4. π-Polarization in adenine−thymine pairing. Arrows indicate direction of the π-polarization.

In the case of AU pairing in the WC mode, the proximal positioning of a uracil oxygen and the H-2 causes the downfield shift. This type of CH···O H-bonding has been termed a *spectator H-bond* by Rebek[19] and has recently been invoked as a duplex stabilizing interaction (Figure 6).[20]

(c) Equilibria Assessed in an Isotherm. To evaluate the association constants between the carboxylic acids and **1**, a number of equilibria in $CDCl_3$ need to be examined as shown in Figure 7. The carboxylic acids are always in equilibrium with

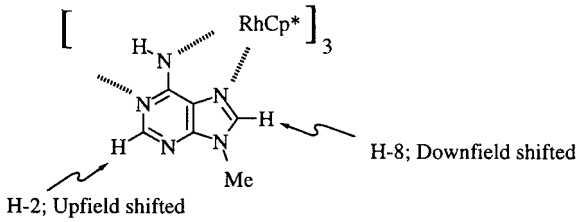

Figure 5. Nature of chemical shifts of H-2 and H-8 in an adenine derivative with a formal negative charge on N-6.

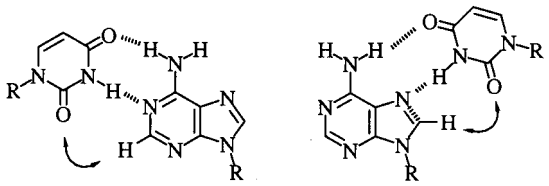

Figure 6. Proximal positioning of H-2 and H-8 with uracil oxygen.

the dimer, and this process is strongly influenced by residual water. Compound **1** can also self-associate and get solvated by water. The complexes formed may involve 1:2 species as well. In our analysis of the K_a's, however, only the process involving the unassociated acid, and unassociated **1**, through a 1:1 complex has been considered. Because of the assumption that all the acid stays in the monomeric form, the observed K_a's will be reduced in magnitude.[21] However, the relative K_a's and other comparative information, e.g., the site-specificities of binding, can be assessed instructively.[22]

(d) Stoichiometry of Complexes. A careful analysis of the shifts of the H-2 and H-8 signals of **1** revealed that with increasing concentration of the carboxylic acid the shift in H-8 was *always* downfield (Figure 2a−d). However, with the aromatic carboxylic acids the H-2 shift was observed to move downfield beyond a certain concentration of the acid (Figure 2a−d). This apparent anomaly can be explained as follows. It appears that such a turnaround in the H-2 shift is indicative of the formation of a complex of 1:2 stoichiometry (vide supra, Figure 7) between the adenine derivative and the acid, respectively, at higher concentrations of the acid. A downfield shift of the H-2 proton has been documented by us previously in the case of "ditopic" binding of **1** by a dicarboxyl receptor.[12] During concomitant binding by two strong H-bond donors such as the carboxyl group, the purine moiety is polarized to overcome the effects of π-polarization arising out of a simple WC binding.

TABLE 2: Effect of Concentration on the Stoichiometry of 1/Acid

entry	acid	(∑mM)[a]	stoichiometry[b]
1	propanoic acid	35	1:1
2	benzoic acid	35	1:1
3	benzoic acid	50	1:2
4	2-iodobenzoic acid	50	1:2
5	2-iodobenzoic acid	70	1:2

[a] ∑mM indicates the sum of the concentrations (in mM) of the two components. [b] Analysis of ^{1}H NMR signals in $CDCl_3$.

To verify our hypothesis regarding the stoichiometry of the complex, Job plot analyses were performed with three carboxylic acids, keeping the sum of the concentrations constant at different values (Figure 8a−c) The results are summarized in Table 2. The Job plots clearly provide evidence for the formation of a 1:2 complex at higher acid concentrations (Table 2, entries 3−5).

At lower concentrations of the acid the 1:1 complex *is* the species formed (entries 1, 2). This fact explains the observed reversal of shift (upfield to downfield) for H-2 at higher concentration of the acid.[23] During the titration experiments saturation was not attained in some cases (e.g, Figure 2a: δ_{H-8}), but owing to the possibility of the formation of 1:2 complexes, high concentrations of the acid was avoided.

(e) K_a versus pK_a. The pK_a of the carboxylic acids along with the observed K_a's are shown in Table 1. The general trend appears to be an increase in the K_a with decreasing pK_a (stronger acidity) of the acids (separately) among the aromatic and aliphatic acid groups. However, 2-iodobenzoic acid showed an unusually low K_a (20 M^{-1}) despite its very low pK_a (2.85). Such a discrepancy can be taken into account if we consider the "solvent-perturbing influence" of the bulky ortho iodo group in lowering the pK_a.[24]

(f) ^{1}H NMR Shifts (δ_{H-2} and δ_{H-8}) and Corresponding K_a's. The K_a's for association of butanoic acid and **2** determined by Lancelot following the shifts δ_{H-2} and δ_{H-8} independently were identical. At the same time it was proven, following the technique of Engel and others, that WC binding had an almost 3-fold preference over HG binding. However, Engel et al. had used the *lack of shift* of the H-2 signal of **3**, upon titration with uracil, as the method to prove the preference for the HG mode of this purine derivative. Our experiments too show that wherever it was possible to analyze the data the K_a's determined for the WC and HG sites were different in magnitude (entries 5, 8, 9 of Table 1). We feel that since the *H-bond-donor abilities*

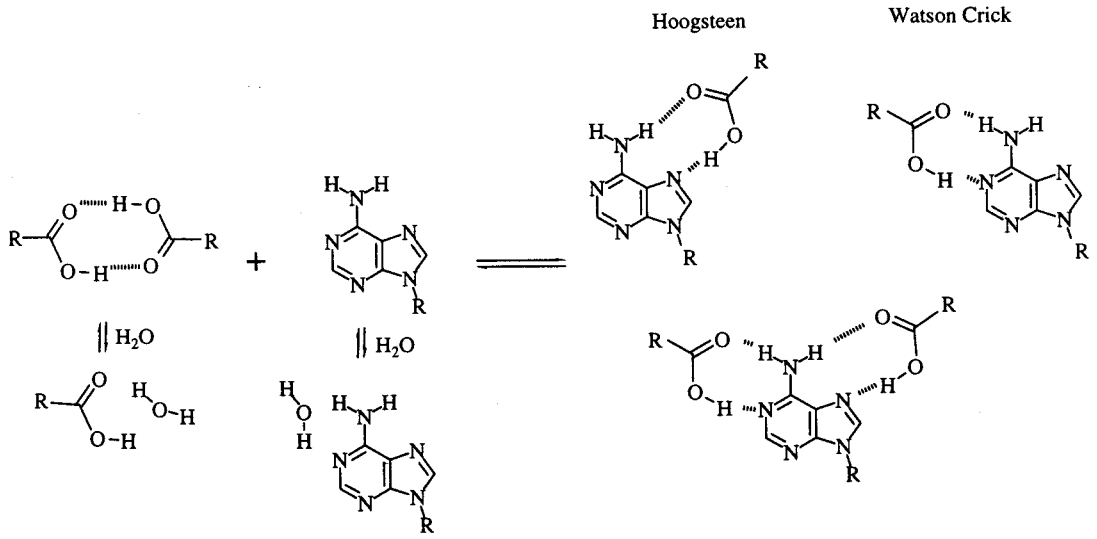

Figure 7. Different equilibria present during titration of carboxylic acids with 9-*N*-butyladenine in $CDCl_3$.

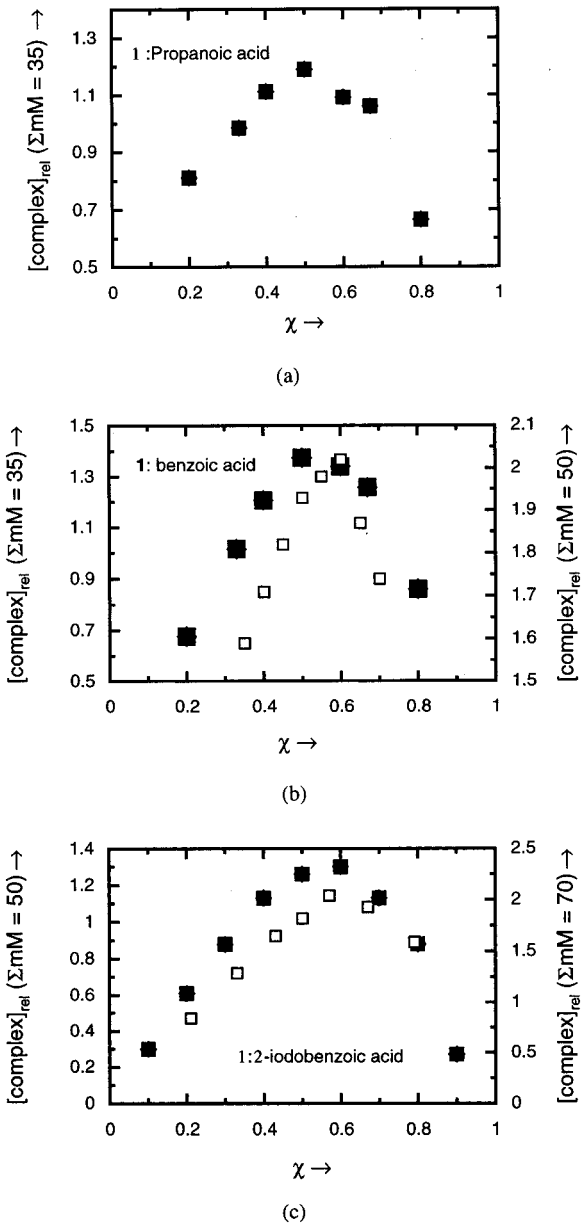

Figure 8. Job plots [relative concentration of the **1**/acid complex vs mole fraction (χ) of the acid]: (a) **1**/propanoic acid ($\sum$mM = 35); (b) **1**/benzoic acid (■ indicates $\sum$mM = 35, and □ indicates $\sum$mM = 50); (c) **1**/2-iodobenzoic acid (□ indicates $\sum$mM = 50, and ■ indicates $\sum$mM = 70)

of **1** and **3** are different, the site-specificity results from **3** may not be directly used for **1**. The method of independent determination of K_a's reported here possibly offers an improved method for the evaluation of site specificity.

Conclusion

We have shown that the analysis of the data (shifts in δ_{H-2} and δ_{H-8}) from a *direct* titration between carboxylic acids and compound **1** can yield reliable values of K_a for comparison between WC and HG binding. It has been shown that aromatic carboxylic acids studied *preferred the HG site* for binding **1** in $CDCl_3$ solution. In comparison, the aliphatic carboxylic acids examined preferred the WC site. The findings are based on the remote-proton shifts (H-2 and H-8) of **1** upon titration with a carboxylic acid.[25] The association constants of carboxylic acid binding to **1** increase with increasing acidity of the acid. However, the preference for a particular site over the other is *not* governed by pK_a. The WC site of **1** is geometrically preferred over the HG site, and yet geometry alone fails to determine the binding propensity.[26] The pK_a of N-1 (ca. 4) is much higher than that of N-7 (<1).[27] Therefore, the nitrogen lone-pair basicities do not seem to determine the site specificity either.

Even though our experiments provide evidence that *carboxylic acids can bind **1** in $CDCl_3$ via both WC and HG sites, with a distinct preference for one site over the other*, a number of questions still remain to be answered including what really causes the selective binding. A lot of experimental [26,28,29] and theoretical studies have been carried out to understand the principles behind WC and HG binding in AT pairs.[30] Further experiments along with theoretical studies will possibly elucidate the underlying principles of adenine binding by carboxylic acids.[31]

Experimental Section

General. Melting points were recorded in open capillaries and are uncorrected. Proton spectra were recorded on 90 and 300 MHz spectrometers. Unless otherwise stated, ^{1}H NMR were recorded in $CDCl_3$ using $CHCl_3$ as the internal standard (δ = 7.270). All reactions were conducted under dry nitrogen and stirred magnetically unless otherwise stated. Thin-layer chromatography was performed using precoated plates (silica gel 60F-254) purchased from Sigma.

Solvents. All solvents were purified and distilled before use. DMF was dried over BaO. Ethyl alcohol was dried over CaO. $CDCl_3$ used for recording proton NMR was purchased from Aldrich and was kept over freshly activated 4 Å molecular sieves before titration.

Synthesis. Compound **1**,[32] 2-butynoic acid,[33] 4-nitrophenylacetic acid,[34] 2-iodobenzoic acid,[35] 3-phenylpropynoic acid,[36] and monobenzyl isophthalate[12] were prepared and purified according to literature procedures.

Preparation of Samples for ^{1}H NMR Titrations. Stock solutions of the acid (50−80 mM) and **1** (50 mM) were prepared in $CDCl_3$. Appropriate volumes of the solutions of the acid and **1** were mixed according to the concentration desired in the NMR tubes and diluted with $CDCl_3$ to make the final volume 400 μL. After recording the ^{1}H NMR of these samples (25 °C), the shifts for H-2 and H-8 were determined and the data were analyzed using a nonlinear curve-fitting program.

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(13) The largest *observed* change in the chemical shifts during the course of the titration is expressed as $\Delta\delta_{obs}$.

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(25) During these titrations it was observed that the NH_2 signal of **1** underwent large downfield shifts (from δ 5.85 to ~7.00) and becomes broader. The residual water present in $CDCl_3$ also underwent a large downfield shift (from δ 2.00 to δ 6.00). But the K_a estimated between acid and water following the water signal was very small (1−2 M^{-1}).

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