Effects of Hydrogen Bonding on the Acidity of Uracil Derivatives

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The present study uses density functional theory to investigate the effects of hydrogen bonding on the acidity of C5- and C6-substituted uracil derivatives. The proton affinities and acidities of uracil donor and acceptor sites generally decrease and increase, respectively, with an increase in the electronegativity of the uracil substituent. Despite these substituent effects, the binding strengths of small molecules (NH_3, H_2O, or HF) to the uracil derivatives are relatively independent of the substituent, which indicates that the changes in the uracil proton affinity and acidity effectively cancel. The acidities of substituted uracil complexes increase not only with the electronegativity of the substituent, but also with the acidity of the small molecule bound to the uracil ring. However, the magnitude of the effect of hydrogen bonding on the acidity of uracil derivatives is not dependent on the nature or position of the substituent. Our results lead to a greater fundamental understanding of the effects of substituents on the hydrogen-bonding properties of uracil, which may have implications for understanding biological applications and processes that involve these modified nucleobases.

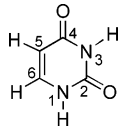
Introduction

Various noncovalent interactions, such as electrostatic attraction and repulsion, hydrophobic forces, and hydrogen bonding, stabilize proteins and prevent decomposition.[1] Hydrogen bonds are particularly important since they are relatively easily broken and reformed, and therefore they provide the delicate balance required by life molecules, such as proteins and DNA. Indeed, hydrogen bonds are responsible for the folding of enzymes and DNA duplex formation.

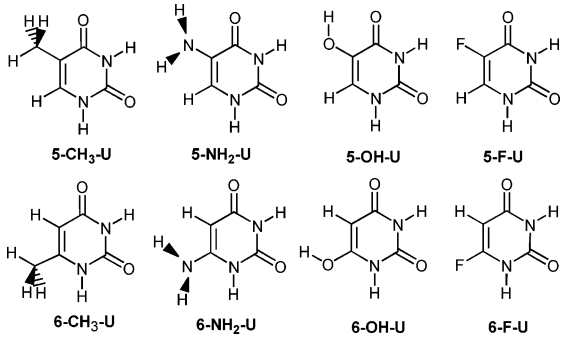
Hydrogen bonds sometimes also play an important role in substrate binding at active sites and enzyme selectivity. We are particularly interested in the role of hydrogen bonds in the mechanism of action of uracil DNA glycosylase (UDG), which is an enzyme that removes uracil from DNA in the base excision repair process.[2−5] The first step in the proposed mechanistic pathway involves scission of the glycosidic bond and production of a uracil (N1) anion.[2−6] Although the structure of UDG shows that amino acids in the enzyme active site interact with the uracil base so that every hydrogen bond donor and acceptor is utilized,[2,7,8] it is unknown which interactions, if any, lower the activation barrier for glycosidic bond cleavage or raise the (N1) acidity of uracil. Additionally, UDG has been shown to excise some uracil derivatives at varying rates,[9−11] while others act as inhibitors.[3,12] Since hydrogen bonding is believed to play an important role at the active site,[2,9−15] studies that examine the hydrogen-bonding properties of uracil and its derivatives, as well as the effects of hydrogen bonds on the properties of these molecules, are extremely important.

In addition to the implications for the mechanism of UDG, studies that investigate hydrogen bonding with uracil may address fundamental questions regarding hydrogen-bonding interactions involving biomolecules. Since computational studies allow examination of interactions and properties that are difficult to study experimentally, much of the current research on hydrogen bonding with uracil has implemented computational techniques.[16−44] Recent computational studies have investigated the properties of isolated uracil[28,34,41,42,44−47] and substituted uracil derivatives,[49] as well as their complexes formed with small molecules.[24,28,37,39,41,42,46,48,49]

SCHEME 1: Structure and Atomic Numbering of Uracil



SCHEME 2: Uracil Derivatives Considered in the Present Study



We extend upon previous computational work[42] on uracil (Scheme 1) in the present study by considering the hydrogen bonding properties of C5- and C6-substituted uracil derivatives (see Scheme 2 for structure and notation). The substituents examined were chosen from the second row of the periodic table due to the systematic increase in their electronegativity. The binding properties of ammonia, water, and hydrogen fluoride are investigated at various positions with respect to each uracil derivative (Scheme 3). Furthermore, we consider the effects of hydrogen bonds on the (N1) acidities of uracil derivatives.

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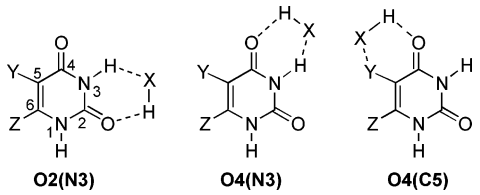
SCHEME 3: Hydrogen-Bonded Complexes between Small Molecules (X = NH_2, OH, F) and Various 5-Substituted (Y = CH_3, NH_2, OH, F; Z = H) and 6-Substituted (Y = H; Z = CH_3, NH_2, OH, F) Uracil Derivatives



From the present study, we hope to gain a greater understanding of interactions between small molecules and biologically important uracil derivatives. Our findings may aid the understanding of hydrogen-bonding interactions involving these molecules, which have important implications in biochemical applications of modified nucleobases, as well as the mechanism of action of various enzymes, such as the DNA glycosylases.

Computational Details

GAUSSIAN 98[50] was employed for all calculations. As in our previous study of (unsubstituted) uracil,[42] geometries were fully optimized in C1 symmetry by using the B3LYP functional in combination with the 6-31+G(d,p) basis set. Diffuse functions are required to adequately model anionic and hydrogen-bonded systems. Frequency calculations were performed at the same level of theory and all reported energies include scaled (0.98) zero-point vibrational energy corrections.

Single-point energies, which were used to evaluate the acidities and binding energies, were computed on the optimized geometries with use of the 6-311+G(2d,p) basis set. This basis set was previously shown to yield results for (unsubstituted) uracil complexes of similar accuracy to those obtained with larger basis sets.[42] All energies for computed complexes include corrections for basis set superposition error (BSSE), which was calculated with use of the Boys and Bernardi counterpoise method.[51] Previous studies have shown that BSSE corrections change binding energies for uracil−water complexes by approximately 3%.[31,36]

Despite reservations expressed about DFT methods,[52] DFT has successfully modeled hydrogen-bonded species,[53,54] including even the most weakly bound systems.[55] Furthermore, B3LYP has been used to study uracil−water complexes,[28,42] and similar structures and energies as the more computationally expensive MP2 method were obtained.[24] It is also important to note that the goal of the present investigation is to examine trends in hydrogen bond strengths, as well as the effects of hydrogen bonds on other uracil properties. Furthermore, the trends in our data for uracil[42] and 5-substituted uracils are in good agreement with previous studies.[31,32,49]

Results and Discussion

Proton Affinity. Since previous work identified a correlation between the proton affinities and acidities of uracil sites and the binding strengths in uracil−water complexes,[49] understanding the properties of the hydrogen bond donor and acceptor sites in uracil derivatives is important for understanding their hydrogen-bonding interactions with small molecules. In the present study, we consider the proton affinities of the uracil carbonyl groups at C2 and C4 (denoted as O2 and O4, respectively), where the proton is added in the molecular plane.

TABLE 1: Proton Affinities of Various Uracil Derivatives (kJ mol^{-1})[a]

compd	O2(N1)	O2(N3)	O4(N3)	O4(C5)
U[b]	812.1	817.3	844.3	855.6
5-CH_3-U	829.2	833.6	852.0	862.9
5-NH_2-U	850.3	853.3	843.4	869.3
5-OH-U	821.0	823.7	818.6	—[c]
5-F-U	798.7	803.1	815.9	836.6
6-CH_3-U	826.9	832.6	865.1	876.2
6-NH_2-U	831.1	841.1	894.4	906.8
6-OH-U	817.1	822.2	861.7	871.0
6-F-U	787.7	793.8	829.0	840.3

[a] See Schemes 1 and 2 for the structure, chemical numbering, and notation used for the uracil derivatives. [b] Reference 42. [c] Geometry could not be located due to close contact distances between the O4 proton and the C5 hydroxyl hydrogen.

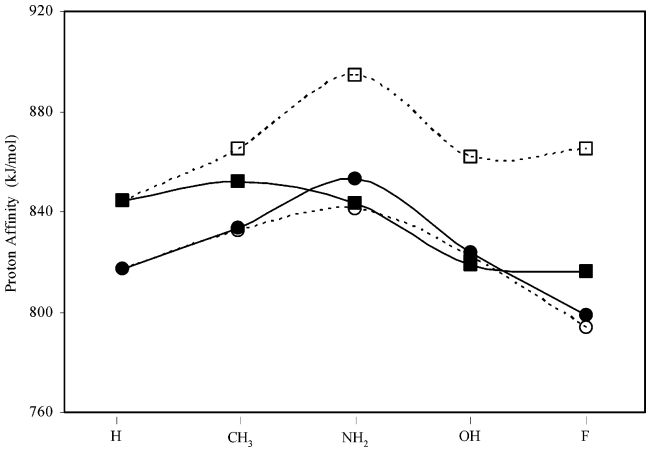


Figure 1. The O2(N3) (●) and O4(N3) (■) proton affinities of C5 (solid line) and C6 (dashed line) substituted uracil derivatives.

At each position, the proton can be oriented in two directions, which is specified in brackets in our notation.

Table 1 displays the calculated proton affinities for the uracil derivatives considered in the present work. The proton affinities of various positions in uracil, as well as some of its C5 derivatives, have been previously studied experimentally[56,57] and computationally.[28,42,47,49] Our calculated proton affinities for the uracil derivatives considered in the present work are in agreement with previously reported values.[49]

(i) C5-Substituted Uracil Derivatives. 5-CH_3-U (thymine) has a larger proton affinity than uracil (by approximately 17 kJ mol^{-1} at O2 and 8 kJ mol^{-1} at O4), which is due to the electron-donating properties of the methyl group. The proton affinity of 5-NH_2-U is generally larger than that of thymine due to the improved electron-donating properties of the amino group. The trend in the proton affinities of the amino, hydroxyl, and fluoro C5-substituted uracils parallels the electronegativities of the substituents (Table 1). Specifically, since fluorine has the greatest electronegativity, 5-fluorouracil has a smaller proton affinity than the hydroxyl derivative, which in turn has a smaller proton affinity than the amino derivative.

The trend in the O4 proton affinities is complicated by internal hydrogen-bonding interactions between the C5 substituent and O4. For example, hydrogen bonding between the C5 substituent and O4 reduces the proton affinity at the O4(N3) position in the amino- and hydroxyl-substituted uracils. As a representative example of the observed trends in the calculated proton affinities, the O2(N3) and O4(N3) proton affinities for C5-substituted uracil derivatives are compared in Figure 1. The reduced O4(N3) proton affinities for 5-NH_2-U and 5-OH-U can be clearly seen from this comparison.

The trend in the proton affinity at O4(C5) is complicated by steric hindrance due to the C5 substituent. For example, the O4(C5) minimum does not exist for 5-OH-U due to a close contact distance between the hydroxyl hydrogen and the proton at O4. Furthermore, the O4(C5) conformer for 5-NH_2-U involves a puckered and staggered amino group with respect to the C5−C6 double bond, which allows the formation of a N···H−O4 internal hydrogen bond.

The proton affinity was previously calculated to be greater at O4 than at O2 in uracil.[28,42,47] This trend prevails regardless of the C5 substituent except when the substituent forms a hydrogen bond with the O4 carbonyl. Specifically, hydrogen bonding between the C5 substituent and O4 reduces the proton affinity at the O4(N3) position in the amino- and hydroxyl-substituted uracils (see Figure 1).

As previously reported for uracil,[42] the O4(C5) position has the largest proton affinity for all substituted uracils. The difference between the O4(N3) and O4(C5) proton affinities is approximately 11 kJ mol^{-1} for uracil and thymine where interactions with the carbonyl group are minimized. However, this difference becomes larger for 5-NH_2-U and 5-F-U, where interactions between the substituent and the O4 position are possible. Specifically, protonation at O4(C5) is stabilized by Y···H−O4 interactions, while Y−H···O4 interactions reduce the proton affinity at O4(N3).

Similar to uracil,[42] the difference between the O2(N1) and O2(N3) proton affinities is 2−5 kJ mol^{-1} for all substituted uracils. The smaller difference noted between O2(N1) and O2(N3) relative to that discussed between O4(N3) and O4(C5) for all derivatives indicates that the substituent does not change the dependence of the O2 proton affinity on the orientation of the proton.

(ii) C6-Substituted Uracil Derivatives. Hydrogen bonds between the C5 substituent and the O4 carbonyl that affect the proton affinities in C5-substituted uracils are eliminated in the C6-substituted derivatives. Therefore, the trend in the proton affinities of C6-substituted uracils is mainly dependent on the nature of the substituent and the protonation site.[58] Indeed, the trends in the O2(N3) and O4(N3) proton affinities for the C6-substituted uracils with respect to the nature of the substituent are very similar to the trend discussed for the O2(N3) proton affinities of the C5 derivatives (see Figure 1). Furthermore, the trends in O2(N1) and O4(C5) proton affinities for the C6-substituted uracils are also the same (Table 1).

The trend with respect to the protonation site is similar for C5- and C6-substituted uracils, where the proton affinity is larger at O4 than O2. Exceptions for C5 derivatives arise when the C5 (amino or hydroxyl) substituent forms a hydrogen bond with the O4 carbonyl. These interactions, and therefore these exceptions, are absent for the C6 substituents.

Although 6-CH_3-U has a larger proton affinity than uracil by 15−21 kJ mol^{-1} (Table 1), the proton affinity of 6-CH_3-U is marginally smaller than 5-CH_3-U (by approximately 2 kJ mol^{-1}) for protonation at O2, but larger than 5-CH_3-U (by approximately 13 kJ mol^{-1}) for protonation at O4. The larger difference in the O4 proton affinities arises since hydrogen bonding and steric interactions with the C5-methyl substituent decrease the O4(N3) and O4(C5) proton affinities, respectively, in the C5 derivative.

Interestingly, all C6 derivatives have larger O4 proton affinities, but smaller O2 proton affinities, than the corresponding C5 derivatives. Thus, there is a larger difference between the O4 and O2 proton affinities for the C6 derivatives. This arises due to a combination of factors including a decrease in the O4 proton affinity due to internal hydrogen bonding with C5 substituents, an increase in the O4 proton affinity due to stabilization of resonance structures with a negative charge on O4 by electronegative C6 substituents, and a decrease in the O2 proton affinity due to destabilization of resonance structures with a negative charge on O2 by electronegative C6 substituents.

TABLE 2: Acidities of Various Uracil Derivatives (kJ mol^{-1})[a]

compd	N1 acidity	N3 acidity
U[b]	1389.4	1441.5
5-CH_3-U	1396.5	1443.7
5-NH_2-U	1400.8[c,d]	1437.3[c,e]
5-OH-U	1388.4	1410.9
5-F-U	1367.4	1413.0
6-CH_3-U	1395.5	1447.6
6-NH_2-U	1380.6	1455.0
6-OH-U	1375.9	1440.0
6-F-U	1337.0	1416.0

[a] See Schemes 1 and 2 for the structure, chemical numbering, and notation used for the uracil derivatives. [b] Reference 42. [c] The neutral molecule has a slightly puckered amino group. [d] The amino group in the anion is puckered and staggered with respect to the C5−C6 bond. [e] In the anion, one amino hydrogen is located in the molecular plane and directed toward O4, while the other amino hydrogen is located out of the molecular plane.

Similar to uracil and its C5-substituted derivatives, the O4(C5) position has the largest proton affinity for all C6-substituted uracils. The difference between the O4(C5) and O4(N3) proton affinities for all C6-substituted uracil derivatives is the same as the difference for 5-CH_3-U (approximately 11 kJ mol^{-1}). The difference between the O2(N3) and O2(N1) proton affinities for the C6-substituted uracil derivatives (5−10 kJ mol^{-1}) is slightly larger than that for the C5-substituted derivatives (2−5 kJ mol^{-1}).

In summary, although internal hydrogen bonding complicates the trends in the proton affinities of uracil derivatives, the proton affinity generally decreases with an increase in the electronegativity of the substituent. Furthermore, regardless of the C5 or C6 substituent, the O4 position has a larger proton affinity than the O2 site, except when hydrogen bonding occurs between O4 and the C5 substituent. Since a large proton affinity typically reflects a strong proton-acceptor site, our calculated proton affinities suggest that the binding energy will be larger for complexes involving O4 compared with O2 for most uracil derivatives.

Acidity. Acidity is defined as the enthalpy of deprotonation, where a decrease in the deprotonation enthalpy represents an increase in the acidity. The present study focuses on the acidity of the N1 and N3 sites of various uracil derivatives. Our calculated results, which are displayed in Table 2, are in agreement with previously reported experimental and computational data.[34,44]

(i) C5-Substituted Uracil Derivatives. The N1 acidity of thymine is 7.1 kJ mol^{-1} smaller than that of uracil (Table 2), which is due to destabilization of the anion by the electron-donating properties of the methyl substituent. With the addition of an amino group, the N1 acidity further decreases. This arises at least in part because, although neutral 5-NH_2-U has a slightly nonplanar amino group, this substituent has increased puckering and becomes staggered with respect to the C4−C5 bond in the corresponding N1 anion.[59] 5-OH-U is only slightly more acidic than uracil, while 5-F-U has the greatest N1 acidity, which is 22.0 kJ mol^{-1} larger than that of unsubstituted uracil. These trends are clearly displayed in Figure 2.

TABLE 3: Binding Strengths (kJ mol^{-1}) of Complexes between Ammonia, Water or Hydrogen Fluoride, and (Neutral) Uracil Derivatives[a]

XH	binding site	U[b]	5-CH_3-U	5-NH_2-U	5-OH-U	5-F-U	6-CH_3-U	6-NH_2-U	6-OH-U	6-F-U
NH_3	O2(N3)	22.5	22.2	23.6	25.9	25.7	21.8	20.5	22.3	25.1
	O4(N3)	23.4	22.8	22.9	25.1	26.1	23.1	22.6	23.8	26.0
	O4(C5)	10.2	6.6	15.2	27.4	5.2	10.6	12.1	13.2	10.7
H_2O	O2(N3)	22.4	22.9	24.8	25.2	23.7	22.4	21.5	22.1	22.4
	O4(N3)	24.9	24.2	23.7	23.8	24.9	25.4	26.2	25.7	25.7
	O4(C5)	29.5	9.9	19.7	24.3	12.0	20.7	22.9	22.7	18.7
HF	O2(N3)	35.6	37.4	40.1	39.3	35.0	36.9	36.7	35.4	33.9
	O4(N3)	40.1	40.1	38.1	35.6	36.3	42.0	45.2	41.9	38.8
	O4(C5)	37.3	33.4	35.1	35.8	28.0	39.8	43.8	41.2	35.1

[a] See Schemes 1−3 for the structure, chemical numbering, and notation used for the uracil derivative complexes. [b] Reference 42.

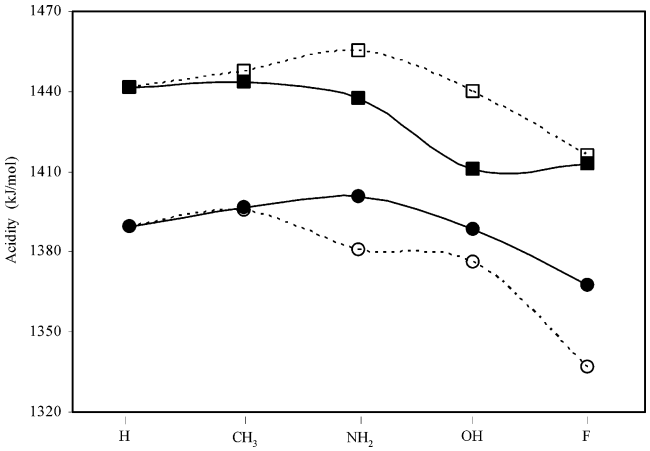


Figure 2. The N1 (●) and N3 (■) acidities for C5 (solid line) and C6 (dashed line) substituted uracil derivatives.

Although the N3 acidities are consistently greater than those at N1, the trend in the acidities with respect to the C5 substituent changes slightly (Figure 2). Specifically, 5-NH_2-U has a slightly greater N3 acidity compared with 5-CH_3-U, while the N3 acidity of 5-OH-U is significantly greater than that of uracil. These differences can be partially explained by geometrical features. For example, the 5-NH_2-U N3 anion has a planar amino group, while the N1 anion adopts a puckered and staggered conformation with respect to the uracil ring. Therefore, additional stabilization is provided by the C5 substituent in the N3 anion.[60] Similarly, hydrogen-bonding interactions between the C5 hydroxyl group and O4 are stronger in the N3 anion, compared with those in the N1 anion and the neutral counterpart.[61]

(ii) C6-Substituted Uracil Derivatives. Although the general trend in C6-substituted (O4) proton affinities is simplified compared with that for the C5-substituted proton affinities, the C6 substituents may influence the trend in N1 acidities since the substituents are in closer proximity to the N1 site. Our calculations show that 6-F-U has the largest (N1) acidity, which is the same as previously discussed for the C5 derivatives (Table 2). The hydroxyl derivative has the next largest acidity. 6-NH_2-U is more acidic than 6-CH_3-U, which is the least acidic C6 derivative and has an acidity slightly less than that of uracil (Figure 2).

The N3 acidities for C6-substituted uracils are less than the N1 counterparts as noted for the C5 derivatives (Figure 2). The order in the N3 acidities with respect to the C6 substituent changes slightly compared with that for the N1 acidities. Differences occur since the C6-amino or hydroxyl hydrogen in the N1 anion provides stabilization of the (N1) anionic site, which is not possible in the N3 anion. This arises due to geometrical changes. For example, the greater (N1) acidity of 6-NH_2-U compared with 6-CH_3-U likely arises due to stabilization from increased planarity of the amino group in the (N1) anion compared with the neutral counterpart.[62] Similar stabilization of the N1 anion is likely provided by the C6-hydroxyl group.

The N1 acidities are significantly larger for the C6-substituted uracils compared with the corresponding C5 derivatives (Figure 2), which occurs due to interactions between the N1 site and the C6 substituents and the closer proximity of the (C6) electronegative substituent to the anionic site. However, the N3 acidities are significant smaller for the C6-substituted uracils compared with the C5 derivatives (Figure 2). Therefore, the difference between the N1 and N3 acidities is larger for the C6 derivatives (average 73 kJ mol^{-1}) compared with the C5-derivatives (average 44 kJ mol^{-1}).

Our calculations indicate that electron-withdrawing groups, such as fluorine, stabilize the N1 and N3 anions, and thereby increase the acidities. Thus, substituents that decrease the proton affinity (basicity) of the uracil carbonyl groups increase the deprotonation enthalpy (acidity) of its NH bonds. Since the substituent trend found for the proton affinity is reversed for the acidity and the complexes studied in the present work involve bidentate hydrogen bonds (Scheme 3), the substituent trend in the binding energies will depend on whether the interaction with the uracil acceptor or donor dominates. Furthermore, different molecules will bind to uracil derivatives with varying affinities based on their intrinsic properties. Thus, the trends in the binding strengths of complexes with uracil derivatives may be difficult to predict.

Binding Strengths of (Neutral) Substituted Uracil Complexes with NH_3, H_2O, or HF. Studying complexes formed between various C5- and C6-substituted uracils and different small molecules will provide additional information about the ability of uracil derivatives to form hydrogen bonds and the stability of these interactions. Furthermore, these complexes will allow us to consider the effects of hydrogen bonds on molecular properties, such as the (N1) acidity.

In the present study, NH_3, H_2O, and HF were added to three positions with respect to the substituted uracils (Scheme 3). Our notation for these complexes indicates the uracil hydrogen bond acceptor and donor sites. For example, O2(N3) involves O2 as the acceptor and the N3 hydrogen as the donor.

(i) 5-Substituted Uracil Derivatives. The binding strengths of the complexes involving (neutral) uracil derivatives, which are defined as the enthalpy required to break the bonds between the small molecules and the uracil derivatives, are provided in Table 3. The binding strengths follow the trend previously discussed for the O2 and O4 proton affinities (Table 1). More specifically, the binding energies of the O4(N3) complexes are marginally larger than those of the O2(N3) complexes (by 2−5 kJ mol^{-1}) for uracil, thymine, and 5-F-U, but smaller than those of the O2(N3) complexes for 5-OH-U and 5-NH_2-U due to

hydrogen bonding between the C5 substituent and the O4 carbonyl. However, the differences between the O2(N3) and O4(N3) binding energies are smaller than the differences between the O2 and O4 proton affinities.

The range in the binding energy with respect to the substituent is approximately 3−5 kJ mol^{-1} at O4(N3) and 1−5 kJ mol^{-1} at O2(N3). Since substituents that decrease the proton affinity of the carbonyl group also increase the acidity of the uracil proton donor, a balance between the proton affinity and acidity of the uracil sites likely leads to the small C5-substituent effect on the binding strengths. For example, 5-F-U has a small proton affinity and a large acidity, while 5-NH_2-U has a large proton affinity and a small acidity. These differences effectively cancel and the binding energies of 5-F-U and 5-NH_2-U complexes are roughly equivalent.

The range in the binding strengths of the O4(C5) complexes with respect to the C5 substituent (22, 20, and 9 kJ mol^{-1} for NH_3, H_2O, and HF complexes, respectively) is larger than that for the O4(N3) or O2(N3) complexes (1−5 kJ mol^{-1}). The greater dependence of the O4(C5) binding energy on the uracil derivative is due to different hydrogen-bonding interactions between the small molecule and the C5 substituent, which will now be discussed in more detail.

Ammonia and water are approximately 46° and 15°, respectively, out of the molecular plane in the thymine O4(C5) complexes. The binding strengths of the O4(C5) complexes between NH_3, H_2O, or HF and thymine or uracil are significantly less than those of the corresponding O4(N3) complexes due to a dramatic decrease in the acidity of the uracil donor. Since NH_3 has a larger proton affinity than H_2O or HF, the binding energy in the ammonia complexes are affected by the change in the proton donating properties of the uracil derivative to the greatest extent.

Due to deviations from the hydrogen-bonding patterns displayed in Scheme 3, the difference between the O4(N3) and O4(C5) binding strengths is greater for 5-F-U compared with uracil. More specifically, only one (F−H···O4) hydrogen bond is present in the O4(C5) complex between hydrogen fluoride and 5-fluorouracil. Furthermore, water and ammonia donate one hydrogen atom to O4 and another to the C5-fluoro group rather than acting as both a proton donor and acceptor as indicated in Scheme 3. All 5-fluorouracil complexes are planar with the exception of the ammonia complex where XH (Scheme 3) is approximately 62° out of the molecular plane.

The 5-OH-U and 5-NH_2-U O4(C5) complexes contain bidentate hydrogen bonds with significant X−H···O and X···H−Y interactions (Scheme 3). These strong hydrogen bonds lead to larger O4(C5) binding energies in these derivatives compared with 5-CH_3-U and 5-F-U (by up to 22 kJ mol^{-1} for NH_3 complexes). Furthermore, 5-OH-U has a consistently larger O4(C5) binding strength compared with 5-NH_2-U, which is at least in part due to the greater acidity of O−H compared with N−H bonds. It should also be noted that ammonia, water, and hydrogen fluoride are approximately 21°, 22°, and 16° out of the molecular plane, respectively, in the 5-NH_2-U complexes. Interestingly, 5-NH_2-U has a slightly smaller, while 5-OH-U has a slightly larger, binding strength at O4(C5) compared with O4(N3). This trend can also be explained by differences in the properties of the uracil hydrogen bond donor and the out-of-plane position of XH.

Complexes with H_2O and NH_3 have similar binding strengths, where the average binding energy is 24 kJ mol^{-1} for both molecules at O2(N3) or O4(N3). Since these complexes contain bidentate hydrogen bonds, the similarity between the results for NH_3 and H_2O could be due to the balance between the proton affinity and acidity of the uracil sites, as well as the relative properties of ammonia and water. Specifically, NH_3 complexes are likely stabilized to the greatest extent by interactions between ammonia and the uracil N3 hydrogens, while H_2O complexes are likely stabilized to the greatest extent by interactions between water and the uracil carbonyl groups.

Hydrogen fluoride binds stronger to the uracil derivatives due to its much greater acidity, and therefore stronger interactions with the carbonyl group, compared with water or ammonia. The binding of HF is stronger at O4(N3) than O2(N3), where the average values are approximately 37 and 41 kJ mol^{-1}, respectively. Exceptions to this trend occur for the 5-amino- and 5-hydroxyl-substituted uracils which exhibit stronger binding at O2(N3). These binding strengths reflect the general trend in the O4 and O2 proton affinities (Table 1).

(ii) C6-Substituted Uracil Derivatives. Irregularities in the general trends in the proton affinities and acidities of C5-substituted uracils (Tables 1 and 2) are also present in the trends in the corresponding binding strengths (Table 3). Since the trends in the proton affinities and acidities of the C6-substituted uracils are more systematic, consideration of the binding strengths of the C6-substituted uracil complexes is important.

In general, the binding energies for the complexes with the C6 derivatives differ by 0−5 kJ mol^{-1} from those for the C5 derivatives. Following the trend in the O2(N3) and O4(N3) proton affinities (Figure 2), the C6 derivatives have smaller O2(N3) and larger O4(N3) binding strengths compared with the C5-substituted uracils. Furthermore, the effects of the C6 substituents on the binding strengths are similar to those discussed for the C5 derivatives. However, the C6-amino and hydroxyl substituents have a reduced effect on the trend in the O4(C5) binding strengths due to the absence of internal hydrogen bonds with O4, which result in unique hydrogen-bonding patterns in C5-substituted uracil complexes.

The trend with respect to the molecule bound to uracil (XH) is the same regardless of the C5 or C6 substituent. Ammonia and water complexes have roughly the same binding strengths, while hydrogen fluoride complexes have larger binding energies. The effect of the binding position is similar for the C6 and C5 derivatives. Specifically, binding of a small molecule at O2(N3) is marginally weaker than binding at O4(N3) (by 2−9 kJ mol^{-1}) for all C6 substituents. The trend at O4(C5) varies with XH, where weaker and stronger binding occurs at O4(C5) compared with O2(N3) in ammonia and hydrogen fluoride complexes, respectively.

Figure 3a displays a summary of the trends in the neutral O2(N3) binding strengths, which provide a representative example of the trends calculated for all binding sites considered in the present study. Figure 3a clearly shows that the binding strengths of hydrogen fluoride complexes are notably larger than the binding strengths of water and ammonia complexes, which are very similar to each other. The graph shows the notable differences between the binding strengths for the C5 and C6 amino- or hydroxyl-substituted uracils. However, with the exception of complications arising due to internal hydrogen bonding in the C5-amino and hydroxyl derivatives, the nature and the position of the substituent have only a marginal effect on the binding strengths of the neutral uracil complexes.

Binding Strengths of Anionic Substituted Uracil Complexes with NH_3, H_2O, or HF. The orientation of the small molecules relative to the ring of the (N1) anionic substituted uracils is similar to that found for the (unsubstituted) anionic uracil complexes.[42] More specifically, with a negative charge

TABLE 4: Binding Strengths (kJ mol^{-1}) of Complexes between Ammonia, Water or Hydrogen Fluoride, and the (N1) Anions of Uracil Derivatives[a]

XH	binding site	U[b]	5-CH_3-U	5-NH_2-U	5-OH-U	5-F-U	6-CH_3-U	6-NH_2-U	6-OH-U	6-F-U
NH_3	O2(N3)	19.3	19.5	18.0	18.2	16.6	19.7	19.7	19.0	17.9
	O4(N3)	18.1	17.8	18.2	16.2	13.4	19.9	19.1	18.2	17.4
	O4(C5)	19.3	18.1	17.2	14.1	19.1	19.6	20.6	19.7	18.1
H_2O	O2(N3)	42.6	43.3	44.1	44.4	41.9	43.1	43.2	41.7	35.9
	O4(N3)	40.6	40.2	39.6	36.3	37.6	41.1	42.5	40.8	39.1
	O4(C5)	42.5	39.7	40.9	37.2	41.0	42.9	44.8	43.3	40.2
HF	O2(N3)	87.1	88.6	90.2	90.9	86.1	88.0	88.0	85.3	81.5
	O4(N3)	82.8	82.3	81.4	76.8	77.3	83.7	86.3	83.3	80.4
	O4(C5)	84.2	78.2	82.1	81.8	73.2	85.0	88.3	85.7	80.2

[a] See Schemes 1−3 for the structure, chemical numbering, and notation used for the uracil derivative complexes. [b] Reference 42.

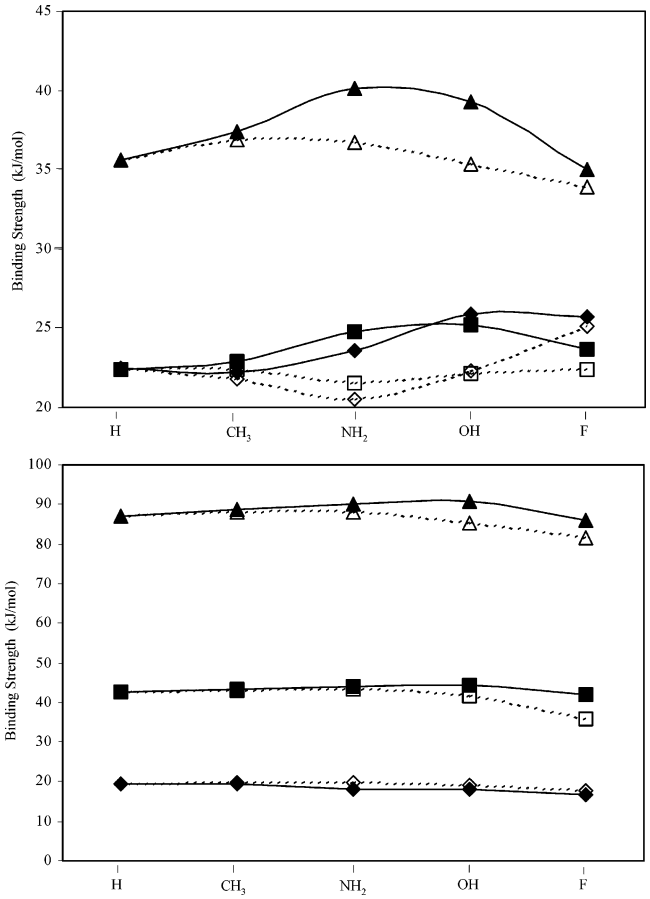


Figure 3. Summary of the (a) neutral and (b) anionic binding strengths (kJ mol^{-1}) for C5 (solid line) and C6 (dashed line) substituted uracil derivatives complexed with NH_3 (◆), H_2O (■), and HF (▲) at O2(N3).

on the uracil derivative, the small molecules (XH) migrate toward the carbonyl group and thus N3−H···X hydrogen bonds in the O2(N3) and O4(N3) complexes are eliminated. Migration of the small molecule in the O4(C5) anionic complexes also occurs, which diminishes interactions with the C5 substituents. It should also be noted that XH is located out of the molecular plane in the 5-NH_2-U complexes by approximately the same degree as in the corresponding neutral complexes, but XH moves into the molecular plane for the anionic 5-CH_3-U complexes.

(i) C5-Substituted Uracil Derivatives. Despite the elimination of bidentate hydrogen bonds, the binding energies are greater for all (N1) anionic complexes with water and hydrogen fluoride (Table 4) compared with the neutral complexes (Table 3). The binding strengths of the ammonia O4(C5) anionic complexes are also larger than those for the corresponding neutral complexes with the exception of the 5-OH-U complex. However, the binding strengths of the ammonia O2(N3) and O4(N3) anionic complexes are less than those for the neutral counterparts.

The relative trends in the binding strengths of the neutral and anionic O2(N3) and O4(N3) complexes likely arise due to an increase in the proton affinity of the carbonyl groups and a decrease in the N3 acidity upon anion formation. More specifically, water and hydrogen fluoride are stronger acids than ammonia and therefore form stronger complexes with the anions through the increased proton affinity. However, ammonia is a strong base and therefore the binding energies of ammonia complexes are affected to a greater extent by the decrease in the N3 acidity upon anion formation, which leads to weaker (anionic) complexes. Similar arguments can be used to explain the smaller binding strength of the 5-OH-U anionic O4(C5) ammonia complex compared with the corresponding neutral complex.

The elimination of bidentate hydrogen bonds simplifies the trend in the binding strengths with respect to the C5 substituent and the small molecule bound to the uracil derivatives. Specifically, the binding strengths of the anionic complexes depend almost exclusively on the acidity of XH (Scheme 3), where the binding strength increases according to $NH_3 < H_2O < HF$. The dominating effect of the molecule bound (XH) diminishes the effect of the substituent and the binding position, where all O2(N3), O4(N3), and O4(C5) binding strengths are similar regardless of the C5 substituent. Interestingly, the minimal effect of the binding position on the binding strength indicates that the proton affinity of the carbonyl group plays a minor role in the trend in the binding strengths.

(ii) C6-Substituted Uracil Derivatives. The trends in the binding strengths of the complexes involving the C5 and C6 uracil derivatives are very similar. As found for the C5 derivatives, the binding strengths of complexes with the C6-substituted uracils depend predominately on the acidity of the molecule bound to the uracil ring (XH, Scheme 3), where binding strengths increase with the acidity of XH. In general, ammonia and water bind more strongly (by 1−5 kJ mol^{-1}), while hydrogen fluoride binds less strongly (by 1−6 kJ mol^{-1}), to the C6 derivatives compared with the C5 derivatives.

The trends in the anionic binding energies are summarized in Figure 3b for the O2(N3) complexes, which provide a representative example of the trends for all binding sites considered in the present study. Clearly, the difference between the binding strengths for the C5- and C6-substituted derivatives is less visible for the anionic complexes (Figure 3b) compared with the neutral complexes (Figure 3a). Furthermore, although the binding strengths are largest for complexes with hydrogen fluoride in both the anionic and neutral complexes, the difference in the binding energy of water and ammonia to the uracil derivatives is notable for the anionic complexes (Figure 3b). Thus, the binding strengths of the uracil anionic complexes

TABLE 5: Calculated (N1) Acidity (kJ mol^{-1}) of Uracil Derivatives in Complexes with Ammonia, Water, and Hydrogen Fluoride[a]

XH	binding site	U[b]	T	5-NH_2-U	5-OH-U	5-F-U	6-CH_3-U	6-NH_2-U	6-OH-U	6-F-U
NH_3	O2(N3)	1392.6	1399.1	1406.3	1396.2	1376.4	1397.7	1381.4	1379.2	1344.2
	O4(N3)	1394.8	1401.4	1405.6	1397.4	1380.0	1398.7	1384.2	1381.5	1345.7
	O4(C5)	1380.3	1384.9	1398.8	1401.8	1353.5	1386.6	1372.1	1369.4	1329.7
H_2O	O2(N3)	1369.2	1376.1	1381.5	1369.2	1349.1	1374.8	1358.9	1356.3	1319.9
	O4(N3)	1373.7	1380.5	1384.9	1375.9	1354.6	1379.8	1367.0	1360.8	1323.6
	O4(C5)	1366.4	1366.6	1379.6	1375.7	1337.1	1373.3	1358.8	1353.0	1316.4
HF	O2(N3)	1338.0	1345.3	1350.6	1335.6	1316.2	1344.4	1329.4	1326.0	1289.4
	O4(N3)	1346.7	1354.2	1357.5	1347.3	1326.3	1353.8	1339.5	1334.5	1295.4
	O4(C5)	1342.5	1351.7	1353.8	1342.4	1322.0	1350.3	1336.2	1331.4	1295.5

[a] See Schemes 1−3 for the structure, chemical numbering, and notation used for the uracil derivative complexes. [b] Reference 42.

TABLE 6: The Effects of Hydrogen Bonding with Ammonia, Water, and Hydrogen Fluoride on the (N1) Acidity of Uracil Derivatives[a,b]

XH	binding site	U[c]	5-CH_3-U	5-NH_2-U	5-OH-U	5-F-U	6-CH_3-U	6-NH_2-U	6-OH-U	6-F-U
NH_3	O2(N3)	−3.2	−2.6	−5.5	−7.8	−9.1	−2.2	−0.8	−3.3	−7.2
	O4(N3)	−5.4	−4.9	−4.8	−9.0	−12.7	−3.2	−3.6	−5.6	−8.7
	O4(C5)	9.1	11.6	2.0	−13.4	13.8	8.9	8.5	6.5	7.3
H_2O	O2(N3)	20.2	20.4	19.3	19.2	18.2	20.9	21.7	19.6	17.1
	O4(N3)	15.7	16.0	15.9	12.5	12.7	15.7	13.6	15.1	13.4
	O4(C5)	23.0	29.9	21.2	12.7	30.2	22.2	21.8	22.9	20.6
HF	O2(N3)	51.4	51.2	50.2	52.8	51.1	51.1	51.2	49.9	47.6
	O4(N3)	42.7	42.3	43.3	41.1	41.0	41.7	41.1	41.4	41.6
	O4(C5)	46.9	44.8	47.0	46.0	45.3	45.2	44.4	44.5	41.5

[a] The reported values are the calculated acidity of isolated uracil derivative (Table 2) minus the calculated acidity of uracil derivative complex (Table 5). A positive value represents an increase in the acidity. [b] See Schemes 1−3 for the structure, chemical numbering, and notation used for the uracil derivative complexes. [c] Reference 42.

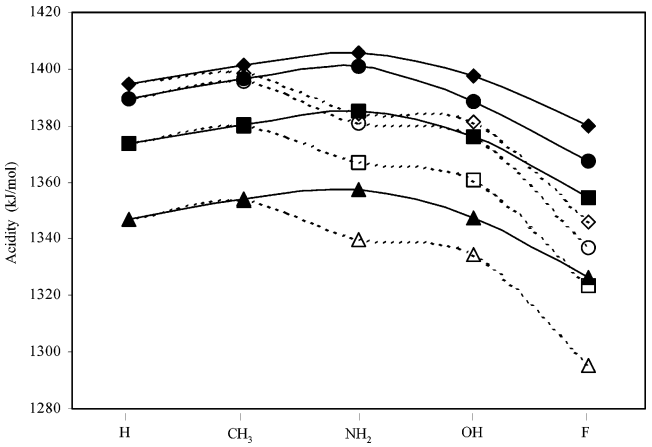


Figure 4. N1 acidity for C5 (solid) and C6 (dashed) substituted uracil derivatives uncomplexed (●) and complexed with NH_3 (◆), H_2O (■), and HF (▲) at O2(N3).

depend on the small molecule bound to the uracil derivative to a greater extent than the nature or position of the substituent.

Acidity of Substituted Uracil Complexes with NH_3, H_2O, or HF. As mentioned in the Introduction, the effects of hydrogen bonds on the properties of biomolecules are important to consider. In the present work, we concentrate on the (N1) acidities of substituted uracil complexes, which are listed in Table 5. The acidities of the complexed uracil derivatives fall within a 117 kJ mol^{-1} range.

The trend in the acidities with respect to the C5 or C6 substituent is the same for the uncomplexed (Table 2) and complexed (Table 5) derivatives. This can clearly be seen in Figure 4, which provides a comparison of the trends in the acidities of the O2(N3) complexes. Specifically, the acidity of the complex increases with an increase in the electronegativity of the uracil substituent. Furthermore, the C6-substituted uracil complexes have greater (N1) acidities than the C5-substituted uracil complexes by 2−35 kJ mol^{-1}, where the largest differences generally occur between 6-F-U and 5-F-U complexes.

The effects of the binding position of the small molecule (XH, Scheme 3) on the acidities are relatively small. In general, the O4(C5) complexes have the largest acidities and the O4(N3) complexes have the smallest acidities for any choice of substituent and XH. However, there are several exceptions to this trend.

More importantly, the acidities of uracil derivatives are greatly affected by the properties of the molecule bound to the uracil ring (XH), where ammonia complexes have the smallest acidities and hydrogen fluoride complexes have the largest acidities (Figure 4). Thus, the most acidic complexes involve HF bound to any position (Scheme 3) in 6-F-U, while the smallest acidity occurs for 5-NH_2-U complexes involving NH_3.

As mentioned, we are primarily interested in the effects of hydrogen bonding on the N1 acidity of uracil derivatives and the dependence of these effects on the C5 or C6 uracil substituent. The effect of hydrogen bonding on the acidity of a uracil derivative (Δ) is calculated as the difference between the acidity of the isolated uracil derivative (Table 2) and the acidity of the complex (Table 5). A positive Δ represents an increase in the acidity upon hydrogen bond formation.

Table 6 displays the effects of hydrogen bonding on the (N1) acidities. The nature of the molecule bound to the uracil ring (XH) affects Δ to a greater extent than the substituent, the substituent position, or the position of XH. Among the molecules bound to the uracil derivatives, hydrogen fluoride increases the acidity to the greatest extent (by 40−53 kJ mol^{-1}). Water also increases the acidity (by approximately 13−30 kJ mol^{-1}). However, the acidity generally decreases upon binding of ammonia to C5- and C6-substituted uracils by roughly 1−13 kJ mol^{-1}.

It should be noted that, due to a simple thermodynamic cycle,[42] Δ can also be calculated as the difference between the binding strengths of the anionic (Table 4) and neutral (Table

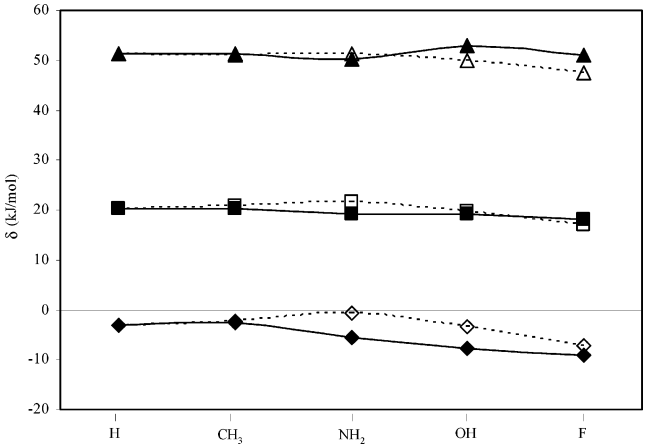


Figure 5. The effects on hydrogen bonding with NH_3 (◆), H_2O (■), and HF (▲) at O2(N3) on the (N1) acidity of C5 (solid line) and C6 (dashed line) substituted uracil derivatives (Δ, kJ mol^{-1}).

3) complexes. Thus, the observed trends in the effects of hydrogen bonds on the acidity of uracil derivatives can be understood by comparing the geometries and binding strengths of the neutral and anionic complexes.

As previously discussed, upon formation of the (N1) anion, XH migrates to coordinate more strongly with the uracil proton acceptor, which leads to a larger binding energy. The magnitude of the anionic binding energies strongly increases with the acidity of XH (NH_3 < H_2O < HF). Since the binding strengths of water and ammonia neutral complexes are similar, while those of hydrogen fluoride complexes are only slightly larger, the effects of XH on the anionic binding strengths are still observed in Δ for the uracil derivatives.

Most importantly, since the binding strengths of the neutral (Table 3) and anionic (Table 4) complexes are not greatly dependent upon the C5 or C6 substituent, Δ is relatively independent of the nature and position of the uracil substituent (Table 6). Figure 5 displays the change in Δ for O2(N3) complexes as a function of the C5 and C6 substituent, which provides a representative example of the general trends observed for O4(N3). However, it should be noted that the effects of hydrogen-bonding interactions at O4(C5) in C5-substituted uracils are more dependent upon the substituent (Table 6) since the binding strength of the O4(C5) neutral complex significantly changes with the C5 substituent and XH (Table 3).

In summary, the effects of hydrogen bonding on the (N1) acidities change most significantly with the small molecule bound to the uracil derivatives (XH) and are not strongly dependent upon the binding position (Table 6). More importantly, the magnitude of the effects of hydrogen bonding on the acidity of the uracil derivatives is not significantly dependent on the C5 or C6 substituent (see, for example, Figure 5). However, it must be stressed that, as shown in Figure 4, the (N1) acidities of the complexed derivatives (Table 5) are very dependent on the C5 or C6 substituent, as was discussed for the uncomplexed acidities (Table 2).

Conclusions

Since hydrogen bonds play fundamental roles in many biological processes, it is important to consider hydrogen-bonding interactions between biomolecules and their derivatives. In the present work, hydrogen-bonding interactions between various C5- and C6-substituted uracils are considered, where the substituents were chosen to cover a range in electronegativities. Computational chemistry was used to study the dependence of the properties of uracil, such as the proton affinity, acidity, and hydrogen-bonding interactions, on the C5 and C6 substituent. Various hydrogen-bonded complexes were considered where ammonia, water, and hydrogen fluoride were bound to different positions with respect to the neutral and (N1) anionic uracil ring.

Our calculations indicate that the proton affinities and acidities of uracil sites decrease and increase, respectively, with an increase in the electronegativity of the C5 or C6 substituent. Since the proton affinities and acidities significantly depend on the nature of the substituent, small molecules may be expected to bind to different uracil derivatives with varying strengths. However, the binding strengths are relatively independent of the substituent. This suggests that the differences in the proton affinities and acidities effectively cancel, which is likely due to the formation of bidentate hydrogen bonds in the complexes. It should also be noted that the binding strengths of water and hydrogen fluoride to the anionic derivatives are larger than those for the neutral complexes, while the reverse is generally true for complexes involving ammonia.

Acidities of substituted uracil complexes display a similar trend as the acidities of the isolated derivatives, where the acidity increases with the electronegativity of the substituent. The acidities of the complexes span a 117 kJ mol^{-1} range and hydrogen-bonding interactions are found to increase the acidity of uracil derivatives by up to 50 kJ mol^{-1}. The nature of the molecule bound to the uracil ring (XH) changes the magnitude of the effect of hydrogen bonding on the uracil acidity to a greater extent than the binding position, the nature of the uracil substituent, or the substituent position. These trends can be explained by considering differences in the geometries and binding strengths of the neutral and (N1) anionic substituted uracil complexes.

The present study was prompted due to our interest in the role of hydrogen bonds in the mechanism of action of uracil DNA glycosylase (UDG), which removes uracil from DNA. UDG is also known to excise 5-fluorouracil, 5-hydroxyuracil, isodialuric acid, and 5,6-dihydroxyuracil.[9−11] However, experiments have shown that the excision rate varies with the substrate. For example, 5-hydroxyuracil is excised 3−10 times less efficiently than uracil,[10] while 5-F-U is excised at a rate similar to that of uracil.[9] Furthermore, 6-aminouracil is a known inhibitor and the enzyme does not excise 5-methyluracil (thymine).[3,12]

Since the proposed mechanism of action of uracil DNA glycosylase involves the formation of the uracil (N1) anion, it would be expected that differences in (N1) acidities of uracil derivatives lead to differences in the excision rate. However, the trends in our data are not in agreement with experimental trends. For example, 5-fluorouracil has a significantly larger calculated acidity than uracil, but the two derivatives are excised at a similar rate.

It is interesting to consider whether hydrogen-bonding interactions are at least partially responsible for the enzyme selectivity. Specifically, it could be proposed that hydrogen-bonding interactions within the active site may have different effects on the acidity of different uracil derivatives. However, our calculations indicate that the effects of hydrogen bonding on the (N1) acidities are the same regardless of the uracil derivative considered. Thus, our calculations suggest that the relative N1 acidities of uracil derivatives play at best a minor role in determining deglycosylation rates by uracil DNA glycosylation, and that other factors, such as steric interactions with active site residues, are extremely important for the

selectivity of the enzyme. More elaborate model systems are currently being investigated to address these issues.

In summary, our study provides a better understanding of hydrogen-bonding interactions involving modified uracil derivatives. Although one of the driving forces of the present study was to better understand interactions within active sites of enzymes that repair damaged nucleobases, such as the DNA glycosylases, our findings will also have more general implications for understanding the use of modified nucleobases in biochemical applications, such as therapeutics, that rely on hydrogen-bonding interactions.

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(58) The only noteworthy geometrical feature that may affect the relative trends in the data is the planar amino groups in the protonated forms of 6-NH_2-U, which contains a slightly puckered amino group in the neutral form.

(59) When the C5-amino group is staggered with respect to the C4−C5 bond in both the neutral and anion forms, the calculated acidity of 5-NH_2-U is only 2.1 kJ mol^{-1} smaller than that of uracil.

(60) It should be noted that minima with staggered amino groups were found for the N3 anion, but these structures are up to 43 kJ mol^{-1} higher in energy than the structure with a planar amino group.

(61) The hydrogen bond distance between the C5-hydroxyl hydrogen and O4 is significantly shorter than the N3 anion (1.894 Å) compared with that in the N1 anion (2.167 Å) and the neutral form (2.176 Å), which likely provides greater stabilization in, and thereby increases the acidity of, the N3 anion.

(62) The amino hydrogen directed toward N1 is 34° out of the molecular plane in neutral 6-NH_2-U, but only 19° out of the plane in the N1 anion.