

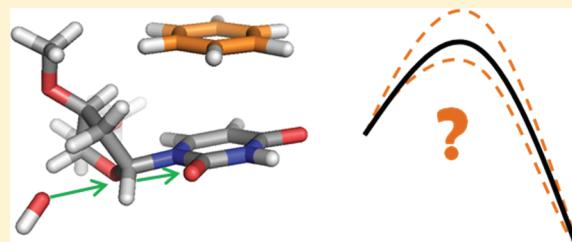
# Combined Effects of $\pi-\pi$ Stacking and Hydrogen Bonding on the (N1) Acidity of Uracil and Hydrolysis of 2'-Deoxyuridine

Jennifer L. Kellie, Lex Navarro-Whyte, Matthew T. Carvey, and Stacey D. Wetmore\*

Department of Chemistry & Biochemistry, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta T1K 3M4, Canada

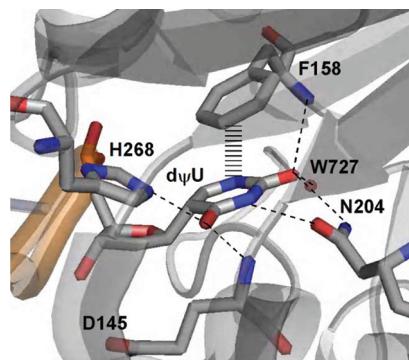
Supporting Information

**ABSTRACT:** M06-2X/6-31+G(d,p) is used to study the simultaneous effects of  $\pi-\pi$  stacking interactions with phenylalanine (modeled as benzene) and hydrogen bonding with small molecules (HF, H<sub>2</sub>O, and NH<sub>3</sub>) on the N1 acidity of uracil and the hydrolytic deglycosylation of 2'-deoxyuridine (dU) (facilitated by fully (OH<sup>-</sup>) or partially (HCOO<sup>-</sup>···H<sub>2</sub>O) activated water). When phenylalanine is complexed with isolated uracil, the proton affinity of all acceptor sites significantly increases (by up to 28 kJ mol<sup>-1</sup>), while the N1 acidity slightly decreases (by ~6 kJ mol<sup>-1</sup>). When small molecules are hydrogen bound to uracil, addition of the phenylalanine ring can increase or decrease the acidity of uracil depending on the number and nature (acidity) of the molecules bound. Furthermore, a strong correlation between the effects of  $\pi-\pi$  stacking on the acidity of U and the dU deglycosylation reaction energetics is found, where the hydrolysis barrier can increase or decrease depending on the nature and number of small molecules bound, the nucleophile considered (which dictates the negative charge on U in the transition state), and the polarity of the (bulk) environment. These findings emphasize that the catalytic (or antecatalytic) role of the active-site aromatic amino acid residues is highly dependent on the situation under consideration. In the case of uracil–DNA glycosylase (UNG), which catalyzes the hydrolytic excision of uracil from DNA, the type of discrete hydrogen-bonding interactions with U, the nature of the nucleophile, and the anticipated weak, nonpolar environment in the active site suggest that phenylalanine will be slightly antecatalytic in the chemical step, and therefore experimentally observed contributions to catalysis may entirely result from associated structural changes that occur prior to deglycosylation.



## INTRODUCTION

The acidity of the uracil nucleobase (U) has been under computational investigation since the late 1990s<sup>1–11</sup> primarily due to interest in the mechanism of action of uracil–DNA glycosylase (UNG). UNG catalyzes the hydrolysis of the 2'-deoxyuridine (dU) glycosidic bond in single- and double-stranded DNA.<sup>12,13</sup> This is an essential process since deamination of cytosine to uracil occurs frequently in DNA<sup>14</sup> and can lead to C:G → T:A transversion mutations if left unrepaired.<sup>15</sup> Since the first X-ray crystal structure of the UNG substrate-bound complex<sup>16</sup> does not reveal a likely proton donor to the uracil nucleobase, it was proposed that the N1 deprotonated uracilate anion (U<sup>-</sup>) is a product of this reaction. Raman–IR evidence later verified that the product is at least a mixture of anionic and neutral uracil.<sup>17</sup> Nevertheless, since the spontaneous depyrimidination of DNA is slow (far slower than depurination),<sup>18,19</sup> it is unlikely that uracil is a good leaving group unless protonated by a general acid. To address this apparent contradiction, hydrogen-bonding interactions in the active site of UNG (Figure 1) have been carefully scrutinized. Indeed, there are several hydrogen-bonding interactions between the enzyme and the uracil nucleobase (Figure 1). For example, H268 interacts with O2 of uracil and has been proposed to provide significant stabilization to the nucleobase anion through a strong (charge neutral) hydrogen bond.<sup>17,20</sup> Furthermore, many computational studies have calculated the N1 acidity of uracil



**Figure 1.** Active site of uracil–DNA glycosylase (UNG) highlighting the interactions between the enzyme and the pseudouridine (dψU) inhibitor (PDB ID: 1EMH).<sup>65</sup>

in the presence of various small molecules, including water,<sup>1,5,6,8,11</sup> cyclen derivatives,<sup>21</sup> ammonia,<sup>6,8</sup> HF,<sup>6,8</sup> and amino acid functional groups.<sup>10</sup> These studies have found that hydrogen-bonding interactions with the nucleobase can significantly influence the acidity, which is a measure of the stability of the glycosidic bond.

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In addition to hydrogen bonding, the active site of UNG contains a  $\pi-\pi$  stacking interaction between uracil and phenylalanine (F158, Figure 1). The structure and binding strength of the U–F interaction has been investigated with various methods,<sup>10,22–24</sup> but these features have not been examined using full optimizations and their effects on the properties of uracil have yet to be determined. This is relevant since it has been shown that stacking interactions can dramatically alter the ionization potential and electron affinity of nucleobases.<sup>25–30</sup> Nevertheless, there have been very few studies on the effects of stacking interactions on the acidity<sup>10,31</sup> or proton affinity<sup>32,33</sup> of biological molecules. Furthermore, the effects have been reported to be catalytic regardless of the property examined. For example, Versee et al. reported that  $\pi-\pi$  stacking between benzene (phenylalanine) and pyridine (purine) increases the basicity (proton affinity) of pyridine by 2.5 pK<sub>a</sub> units,<sup>32</sup> while Olasz et al. found that stacking between benzene and phenol (tyrosine) decreases the acidity of phenol by 0.3 pK<sub>a</sub> units.<sup>31</sup> Although there may be an apparent disparity between these results at first glance, it must be recognized that one study examined  $\pi-\pi$  effects on a property involving a cationic species and the other examined a property involving an anionic species.

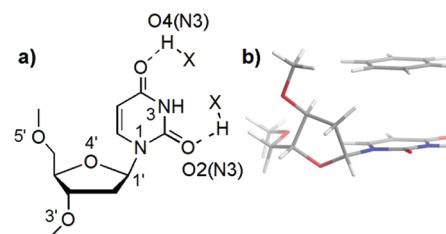
In addition to examining the stability of (N1/N9) nucleobase anions in the presence of noncovalent interactions, our group has considered the hydrolysis of nucleosides<sup>34–36</sup> and nucleotides.<sup>37–39</sup> Most relevant to UNG, we investigated the effects of four different deoxyribose sugar models, nine nucleophiles of varying strength, and hydrogen bonding between uracil and up to three small molecules (H<sub>2</sub>O, HF, and NH<sub>3</sub>).<sup>37</sup> It was found that hydrogen bonding at O2 and O4 of uracil can greatly reduce the barrier to deglycosylation, but not to the same extent that the acidity is increased. Similarly, a follow-up study revealed that hydrogen-bonding interactions with the nucleobase can significantly enhance the hydrolytic deglycosylation of the remaining canonical DNA nucleosides.<sup>38</sup> There have also been numerous theoretical studies on other damaged nucleobases, such as oxidized thymine,<sup>40,41</sup> and the effects of other features on the deglycosylation of natural bases, such as the effects of platination,<sup>42</sup> protonation,<sup>42,43</sup> and oxidation,<sup>39,44,45</sup> on the deglycosylation of guanine. These studies reveal differences in the effects of interactions on nucleobase acidity and nucleotide stability, which indicates that studies on full nucleotide models may be essential.

The present study seeks to consolidate the previous areas of research on the acidities of the nucleobases,<sup>6,8,10,11,46</sup> deglycosylation barriers of 2'-deoxynucleotides,<sup>34–37</sup> and  $\pi-\pi$  (stacking) interactions between the amino acids and nucleobases<sup>22,24,47–50</sup> and thereby fill an important void in the literature. Specifically, the effects of stacking with phenylalanine on the (N1) acidity of uracil, as well as the (concerted, S<sub>N</sub>2) hydrolysis barrier, of 2'-deoxyuridine were systematically studied in the presence and absence of hydrogen-bonding interactions with small molecules. New insights into the preferred structures of nucleobase–amino acid stacking interactions are obtained due to the use of full optimizations for the first time. Interestingly, the (anionic)  $\pi-\pi$  stacking interactions between the uracilate anion and phenylalanine are found to be attractive and stable with respect to optimization. Furthermore, the effects of  $\pi-\pi$  interactions on important molecular properties of biomolecules are revealed and found to be highly dependent on the environment. Finally, the results of this study show that small gas-phase acidity models can be utilized to predict the effect of noncovalent interactions on hydrolysis barriers and therefore can be used to estimate the

binding energies and catalytic contributions of residues included in large-scale models, which are otherwise difficult to calculate and/or measure.

## COMPUTATIONAL DETAILS

The initial geometries for systems that include small molecules were taken from our previous (B3LYP) study of the uracil (N1) acidity<sup>6</sup> and (concerted) hydrolysis of 2'-deoxyuridine (dU).<sup>37</sup> In the case of dU hydrolysis, two nucleophiles were considered, namely the hydroxyl anion (OH<sup>-</sup>) and a formate–water complex (HCOO<sup>-</sup>·H<sub>2</sub>O), which represent fully and partially activated water, respectively. For comparison to our previous study, the 3' and 5' oxygen atoms of the sugar moiety were capped with methyl groups (Figure 2a) when examining dU hydrolysis,<sup>37</sup>



**Figure 2.** Identification of uracil (a) hydrogen-bonding and (b) stacking orientations, as well as atomic numbering, utilized in this study.

which prevents unrealistic interactions between the 3' and 5' substituents and the nucleophile or nucleobase. All combinations of one small molecule (XH = H<sub>2</sub>O, HF, NH<sub>3</sub>) interacting at the O2(N3) or O4(N3) site of uracil (U) were examined (see Figure 2a for definition of hydrogen-bonding sites). These sites were selected to best match the interactions in the uracil–DNA glycosylase (UNG) active site (Figure 1), while the selected small molecules span a range of possible acidities and hydrogen-bond strengths. In the U acidity study, the effects of all combinations of two small molecules simultaneously bound to uracil were also considered. In the case of dU hydrolysis, the effects of occupation of both sites by the same type of small molecule was examined, which captures the possible extremes of the synergistic effects of more than one simultaneous interaction with the nucleobase. For systems including stacking with phenylalanine (F), a benzene ring was first introduced above the uracil ring in an orientation (Figure 2b) consistent with the UNG active site (Figure 1). Subsequently, the heavy atoms of the rings were aligned, the inter-ring separation was set to 3.4 Å, and the system was fully optimized. A previous study by our group found a small increase in binding energy when the phenylalanine model was extended to include the peptide backbone; however, the difference was primarily due to backbone–nucleobase interactions and not due to a change in the magnitude of the  $\pi-\pi$  stacking interaction.<sup>51</sup>

All geometries were optimized at the M06-2X/6-31+G(d,p) level of theory.<sup>52</sup> The M06-2X functional was selected due to its superior treatment of noncovalent interactions (specifically,  $\pi-\pi$  stacking)<sup>50</sup> as well as proton transfer and S<sub>N</sub>2-type reactions.<sup>53,54</sup> Frequency calculations were carried out at the same level of theory to characterize the structures as minima or transition states and to obtain scaled (0.9631)<sup>55</sup> zero-point vibrational energy (ZPVE) corrections. Higher-level single-point energies were determined with M06-2X and the 6-311+G(2d,p) basis set. Gibbs energies were not utilized in the present study to better allow direct comparisons between changes in acidities and

deglycosylation reaction energetics as well as to our previous work.<sup>37,38</sup> Corrections for basis set superposition error (BSSE) were not included in the reported energies since this has been shown to be small for the method implemented,<sup>52,56</sup> and the uncorrected results will allow for easy comparison to large active-site models where counterpoise calculations are not feasible. Solvent-phase single-point energies were determined using the IEF-PCM methodology with the (G09) default settings for water ( $\epsilon = 78$ ) and ether ( $\epsilon = 4$ ), which is commonly used to represent the protein environment. All calculations were carried out with Gaussian 09.<sup>57</sup>

## RESULTS AND DISCUSSION

**Acidity of Uracil.** The gas-phase proton affinities and acidities of various sites in uracil were initially calculated for the bare nucleobase (Table 1). The M06-2X values are in good

**Table 1. Gas-Phase Proton Affinities and Acidities (kJ mol<sup>-1</sup>) of Select Sites in Isolated Uracil and Uracil Complexed with Phenylalanine**

	U		U:F	
	B3LYP <sup>a</sup>	M06-2X <sup>b</sup>	M06-2X <sup>b</sup>	$\Delta(\pi-\pi)^c$
Proton Affinity				
O2(N1)	812.1	809.7	837.9	28.2
O2(N3)	817.3	814.6	842.4	27.9
O4(N3)	844.3	838.1	865.6	27.6
O4(C5)	855.6	849.9	877.6	27.7
Acidity				
N1	1389.4	1385.6	1392.0	-6.4
N3	1441.5	1437.3	1433.5	3.9

<sup>a</sup>B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d,p) values with scaled (0.9806) zero-point corrections from ref 6. <sup>b</sup>M06-2X/6-311+G(2d,p)//M06-2X/6-31+G(d,p) with scaled (0.9631) zero-point energy corrections. <sup>c</sup>A positive value indicates an increase in the proton affinity or acidity upon complexation with F (modeled as benzene) relative to isolated uracil.

agreement with the previously reported B3LYP values.<sup>6</sup> Specifically, the difference between the M06-2X and B3LYP values is less than 6 kJ mol<sup>-1</sup>, where M06-2X predicts a greater (magnitude decrease in) acidity and smaller proton affinity than B3LYP for all uracil sites. Nevertheless, these differences are small and the predicted trends are the same for both methods, which supports the use of M06-2X in the present work.

As previously discussed in the literature,<sup>6</sup> the N1 site of uracil is the most acidic (1385.6 kJ mol<sup>-1</sup>) and the O4(C5) site is the most basic (849.9 kJ mol<sup>-1</sup>). When uracil is complexed with phenylalanine (U:F), there is a large increase in the proton affinity of various uracil sites ( $|\Delta(\pi-\pi)| > 25$  kJ mol<sup>-1</sup>), while there is a small change in uracil acidity ( $|\Delta(\pi-\pi)| < 5$  kJ mol<sup>-1</sup>) (Table 1). This result is in line with previous studies that found a large increase in proton affinity of pyridine<sup>32</sup> and guanine,<sup>33</sup> but a small change in the acidity of phenol<sup>31</sup> upon stacking with benzene. Although the proton affinity consistently increases, the N1 acidity decreases by 4.8 kJ mol<sup>-1</sup>, while the N3 acidity increases by 3.9 kJ mol<sup>-1</sup>, upon addition of the benzene ring.

The significant difference between the effect of stacking on the proton affinity and the acidity of U can be explained by changes in the interaction energy with the respective ions (Table S1). Specifically, the interaction between phenylalanine and the uracil cation are on the order observed for similar  $\pi^+-\pi$  systems<sup>49,58,59</sup> and therefore are exceptionally strong (between -51.8 and

-52.4 kJ mol<sup>-1</sup> depending on the protonation site). This is nearly double the strength of the neutral U:F pair (-24.2 kJ mol<sup>-1</sup>). However, there is a much smaller change in the interaction energy when uracil is deprotonated and a greater variation with respect to the deprotonation site (-19.5 and -28.1 kJ mol<sup>-1</sup> for N1 and N3, respectively). The greater dependence on the deprotonation site arises since that the two rings in the anionic U:F complexes are in a tilted (nonplanar) arrangement (Table S2), which allows for C-H... $\pi$  interactions that become stronger in the case of the N3 anion due to a greater localization of negative charge and larger tilt angle.

The calculated (N1) acidities of U when the base is hydrogen bound to one or two small molecules (Table 2) are generally within 6.5 kJ mol<sup>-1</sup> of the corresponding (previously reported) B3LYP values,<sup>6</sup> where uracil is typically predicted to be more acidic with M06-2X as discussed for the isolated base. In line with the similar energetics, the M06-2X optimized geometries (Figure 3 and Figures S1-1 and S1-2) are very similar to the previously reported B3LYP results, where the average hydrogen-bond distances and angles differ by 0.037 Å and 2.1°, respectively, across all complexes considered. The largest difference in the B3LYP and M06-2X acidities (10.7 kJ mol<sup>-1</sup>) occurs when water is hydrogen bound at both the O2(N3) and O4(N3) sites, which arises due to a shorter O-H...O2 hydrogen bond (by 0.038 Å) and distance between the two water molecules (by 0.166 Å) in the M06-2X optimized anionic complex.

As reported in our previous study,<sup>37</sup> the magnitude of the change in acidity due to hydrogen-bonding interactions between uracil and various small molecules covers a broad range (-10.9 to 87.4 kJ mol<sup>-1</sup>). Additionally, there is a clear trend in the increase in N1 acidity of uracil with the acidity of the small molecule bound ( $\text{NH}_3 < \text{H}_2\text{O} < \text{HF}$ ) as well as the number of small molecules bound. Furthermore, hydrogen-bond interactions at O2 provide more stabilization to the uracil anion, and therefore increase the acidity to a greater extent, than those at O4, which matches the relative hydrogen-bond distances in the anionic complexes (Figure 3 and Figure S2) and corresponds to delocalization of the negative charge over N1 and O2.

As mentioned in the Computational Details, a model phenylalanine (benzene) ring was added to the uracil-XH hydrogen-bonded complexes, and the entire system was relaxed without constraints. The average distance between the center of the benzene ring and the plane of uracil is 3.11 and 3.22 Å in the neutral and anionic complexes, respectively (Table S2). Furthermore, Figure 4 shows that the phenylalanine ring is slightly tilted relative to the U ring, which directs either the  $\pi$  cloud or a proton toward the nucleobase in the neutral and anionic complexes, respectively. The tilt angle (angle between the planes defined using the endocyclic atoms of the two rings) ranges from 4.8–20.2° in the neutral U:F complexes and 2.4–31.8° in the anionic U<sup>-</sup>:F complexes, with average values of 8.6° and 9.5°, respectively (Table S2). Interestingly, with the exception of a few systems containing strong X-H...O4 interactions (e.g., U<sup>-</sup>:F-O4<sub>Wat</sub>, U<sup>-</sup>:F-O4<sub>HF</sub>, and U<sup>-</sup>:F-O2<sub>Am</sub>O4<sub>Wat</sub>), the anionic systems are more parallel than the neutral systems. This implies that  $\pi^--\pi$  stacking interactions between uracil and phenylalanine are slightly more stable, or less prone to transition to T-shaped interactions, than the neutral  $\pi-\pi$  counterparts, despite the anticipated strength of a T-shaped  $\pi^--\pi$  interaction.

It is noteworthy that the fully optimized location of the phenylalanine ring above the uracil  $\pi$ -system does not vary greatly among the systems studied. Specifically, benzene is preferentially centered over N1 or N3 of uracil (Figure 4 and Table S2).

**Table 2. Calculated N1 Acidity ( $\text{kJ mol}^{-1}$ ) of Uracil Complexed with Water, Ammonia, Hydrogen Fluoride, and/or Phenylalanine in Various Environments<sup>a</sup>**

O2(N3)	O4(N3)	gas phase			ether ( $\epsilon = 4$ )			water ( $\epsilon = 78$ )		
		acidity	$\Delta(\text{acidity})^b$	$\Delta(\pi-\pi)^c$	acidity	$\Delta(\text{acidity})^b$	$\Delta(\pi-\pi)^c$	acidity	$\Delta(\text{acidity})^b$	$\Delta(\pi-\pi)^c$
U										
		1385.6			1239.4			1197.2		
H <sub>2</sub> O		1368.3	17.3		1232.9	6.5		1192.8	4.5	
NH <sub>3</sub>		1394.4	-8.8		1253.3	-13.9		1211.2	-13.9	
HF		1333.8	51.8		1203.1	36.3		1166.6	30.6	
	H <sub>2</sub> O	1380.3	5.3		1238.4	1.0		1198.3	-1.0	
	NH <sub>3</sub>	1396.4	-10.9		1254.4	-15.0		1211.6	-14.3	
	HF	1342.2	43.4		1211.1	28.3		1173.9	23.3	
H <sub>2</sub> O	H <sub>2</sub> O	1355.8	29.8		1227.4	12.0		1191.0	6.2	
H <sub>2</sub> O	NH <sub>3</sub>	1374.1	11.5		1242.0	-2.6		1203.5	-6.3	
H <sub>2</sub> O	HF	1336.9	48.7		1215.7	23.7		1181.9	15.4	
NH <sub>3</sub>	H <sub>2</sub> O	1378.9	6.7		1245.4	-6.0		1206.0	-8.8	
NH <sub>3</sub>	NH <sub>3</sub>	1388.8	-3.3		1249.7	-10.3		1208.4	-11.2	
NH <sub>3</sub>	HF	1356.5	29.1		1232.3	7.1		1196.5	0.7	
HF	H <sub>2</sub> O	1327.7	57.8		1207.6	31.8		1174.5	22.7	
HF	NH <sub>3</sub>	1347.6	38.0		1223.9	15.5		1188.7	8.6	
HF	HF	1298.2	87.4		1180.6	58.8		1148.7	48.5	
U:F										
		1392.0	-6.4	-6.4	1248.5	-9.1	-9.1	1204.7	-7.5	-7.5
H <sub>2</sub> O		1370.5	15.1	-2.2	1237.1	2.3	-4.3	1197.6	-0.4	-4.9
NH <sub>3</sub>		1388.6	-3.0	5.8	1253.1	-13.7	0.1	1211.2	-13.9	0.0
HF		1337.0	48.6	-3.2	1207.9	31.5	-4.8	1169.9	27.4	-3.3
	H <sub>2</sub> O	1383.7	1.9	-3.4	1248.4	-9.0	-10.0	1206.0	-8.8	-7.7
	NH <sub>3</sub>	1391.2	-5.7	5.2	1256.5	-17.1	-2.1	1215.9	-18.6	-4.3
	HF	1350.4	35.2	-8.2	1222.4	17.0	-11.3	1185.2	12.1	-11.2
H <sub>2</sub> O	H <sub>2</sub> O	1353.7	31.8	2.0	1230.0	9.4	-2.6	1194.9	2.3	-3.9
H <sub>2</sub> O	NH <sub>3</sub>	1371.7	13.8	2.3	1242.0	-2.6	0.0	1202.2	-4.9	1.3
H <sub>2</sub> O	HF	1331.5	54.1	5.4	1211.8	27.6	3.9	1177.4	19.8	4.5
NH <sub>3</sub>	H <sub>2</sub> O	1376.6	8.9	2.2	1250.7	-11.3	-5.3	1213.4	-16.1	-7.4
NH <sub>3</sub>	NH <sub>3</sub>	1383.3	2.3	5.6	1252.2	-12.8	-2.5	1212.3	-15.1	-3.9
NH <sub>3</sub>	HF	1354.2	31.4	2.3	1233.5	5.9	-1.2	1198.6	-1.4	-2.1
HF	H <sub>2</sub> O	1327.3	58.3	0.5	1210.1	29.3	-2.5	1177.7	19.6	-3.1
HF	NH <sub>3</sub>	1346.0	39.5	1.5	1225.9	13.5	-2.0	1191.7	5.5	-3.1
HF	HF	1305.2	80.4	-7.0	1190.0	49.4	-9.4	1158.7	38.6	-9.9

<sup>a</sup>(PCM-)M06-2X/6-311+G(2d,p)//M06-2X/6-31+G(d,p) values including scaled (0.9631) zero-point energy corrections. <sup>b</sup>A positive  $\Delta(\text{acidity})$  indicates that N1 is more acidic in the complex compared to isolated uracil. <sup>c</sup>The difference in acidity between U and U:F systems with equivalent hydrogen-bonding interactions.

Despite previous reports of a minimum with phenylalanine centered over N3 of neutral uracil,<sup>10,22,23</sup> benzene typically resides over the most acidic (N1) site in complexes with neutral uracil. This discrepancy likely arises since full optimizations were not carried out in the other studies. Indeed, the present work indicates phenylalanine prefers to be centered above N1 rather than N3 by 1.6  $\text{kJ mol}^{-1}$  in the fully optimized (neutral) U:F complex, which is in agreement with previous *ab initio* potential energy surface scans that report isoenergetic minima over N1 and N3.<sup>24</sup> However, when two small molecules are simultaneously bound to uracil, the benzene ring generally centers over N3. This relocation of phenylalanine upon addition of a second hydrogen-bonding interaction permits weak interactions between the benzene ring and the small molecules (see, for example, U:F–O<sub>2</sub><sub>Wat</sub>O<sub>4</sub><sub>Wat</sub> in Figure 3), which do not occur when only one molecule is hydrogen bound to uracil due to a more highly constrained hydrogen-bonding network. Phenylalanine has an even greater preference to reside over N3 in the anionic complexes, which reduces repulsion between the negative charge in the N1–O2 region and the benzene  $\pi$ -system.<sup>60</sup>

The hydrogen-bonding network between the small molecules and uracil is maintained in the U:F systems (Figure 3 and Figures S3 and S4). On average, the hydrogen-bonding interactions at O2 and O4 tighten, and the interactions at N3–H loosen (by up to 0.03 Å) in the presence of benzene, which is anticipated based on the changes to the proton affinity and acidity of uracil sites (Table 1). The largest changes in bond length occur in the anionic systems (~0.09 Å). These results indicate that hydrogen-bond donation to both neutral and anionic uracil is enhanced in the presence of  $\pi-\pi$  interactions. Further support for the synergy of  $\pi-\pi$  and hydrogen-bonding contacts is obtained from the binding strengths of the U:F complexes (Table S1). Specifically, the average binding energy of phenylalanine to the U–XH hydrogen-bonded complexes (−28.4  $\text{kJ mol}^{-1}$  (neutral) and −28.6  $\text{kJ mol}^{-1}$  (anionic)) are much larger than those to isolated (neutral or anionic) uracil (−25.9  $\text{kJ mol}^{-1}$  for U:F and −19.5  $\text{kJ mol}^{-1}$  for U<sup>−</sup>:F). Although some of this increase in binding strength may arise due to interactions between benzene and the small molecule (XH), the stronger binding is at least in part a result of increased

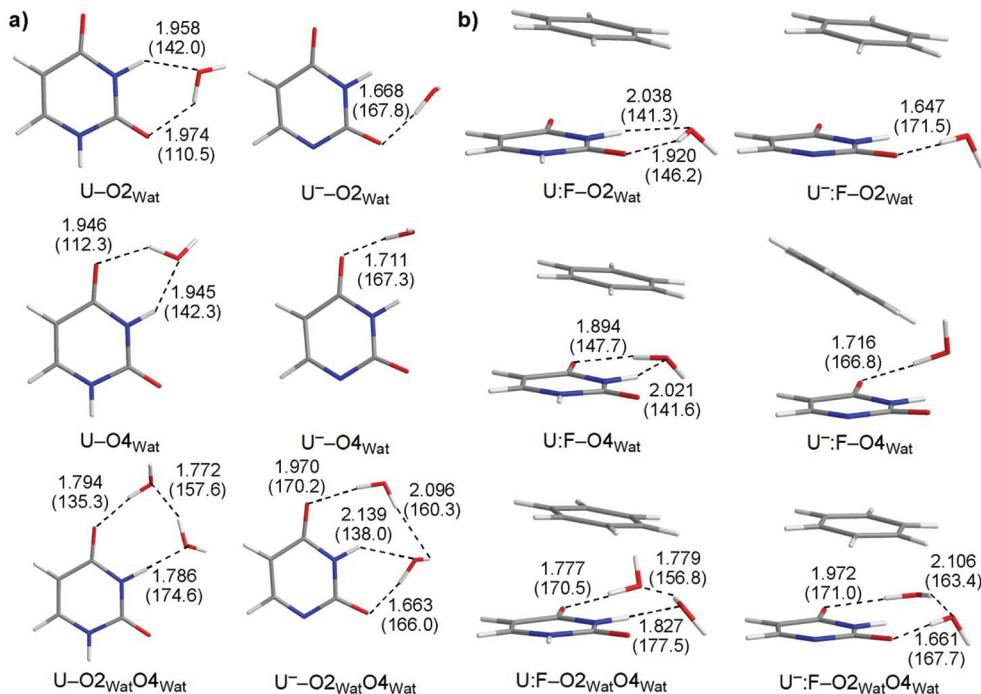


Figure 3. Representative structures with select M06-2X/6-31+G(d,p) hydrogen-bonding distances ( $\text{\AA}$ ) and angles (deg, in parentheses) in the neutral (left) and N1 anionic (right) complexes of (a) U and (b) U:F with one or two water molecules bound.

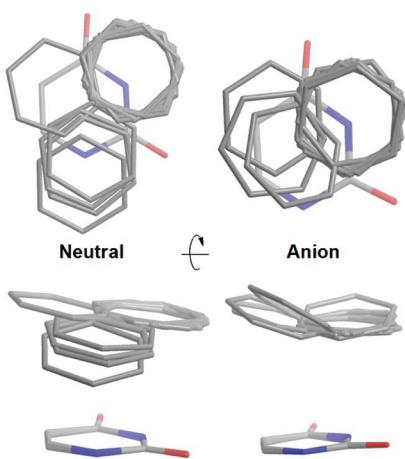


Figure 4. Top (top) and side (bottom) views of the relative position of the phenylalanine and uracil rings in all neutral (left) and anionic (right) complexes examined.

positive charge on uracil and a decreased negative charge on the uracil anion upon binding of XH before stacking with phenylalanine. The exception to this trend is complexes between uracil and a single ammonia molecule, which increases the negative charge on neutral uracil and therefore decreases the (neutral) U:F binding strength. Interestingly, binding of benzene to the uracil anion still increases upon complexation with ammonia, which could be because ammonia provides some stabilization to uracil or the presence of ammonia–phenylalanine interactions.

The N1 acidity of (isolated) U decreases by  $6.4 \text{ kJ mol}^{-1}$  upon complexation with phenylalanine. Although the acidity of complexes involving ammonia increase by  $\sim 5 \text{ kJ mol}^{-1}$ , addition of the benzene ring to complexes with one small molecule hydrogen bound to uracil typically lowers the acidity by  $2.2\text{--}8.2 \text{ kJ mol}^{-1}$  (Table 2). However, when U is stabilized by two hydrogen-bound small molecules, addition of benzene

increases the N1 acidity of the U–XH complex. For example, the complex with water at O<sub>2</sub>(N3) and HF at O<sub>4</sub>(N3) increases the acidity by  $48.7 \text{ kJ mol}^{-1}$  in the absence of stacking, while the U:F system shows an increase in acidity by  $54.1 \text{ kJ mol}^{-1}$ , which leads to a positive  $\Delta(\pi\text{-}\pi)$  of  $5.4 \text{ kJ mol}^{-1}$  (Table 2). This suggests that multiple-hydrogen bonding contacts can overcome the adverse effects of  $\pi\text{-}\pi$  stacking in anionic systems. Additionally, these results are indicative of a synergistic interplay between the  $\pi\text{-}\pi$  and hydrogen-bonding interactions, which for some systems includes a contribution from interactions between the small molecule and the benzene ring.

An exception to the synergy between  $\pi\text{-}\pi$  and hydrogen-bonding interactions on the acidity of U discussed above occurs when two strong acids are hydrogen bound to the nucleobase. For example, when two HF molecules are bound to uracil, addition of a benzene ring decreases the N1 acidity of uracil by  $7 \text{ kJ mol}^{-1}$  due to the greater positive charge on (neutral) uracil, which enhances binding to neutral uracil and makes deprotonation of the complex less favorable. This suggests that the nature of the effects of  $\pi\text{-}\pi$  (stacking) interactions on the properties of the nucleobase are highly dependent on other direct contacts with the surroundings. In addition to specific interactions, the bulk environment may affect the contributions of the  $\pi\text{-}\pi$  contacts. Although it is not clear how accurately implicit solvation methods treat weakly bound systems such as  $\pi\text{-}\pi$  interactions,<sup>61</sup> it is interesting to note that the introduction of bulk solvent through implicit single-point calculations decreases both  $\Delta(\text{acidity})$  and  $\Delta(\pi\text{-}\pi)$ . Even in the presence of the weak dielectric ( $\epsilon = 4$ ) often used to represent a protein environment, the synergy between the hydrogen-bonding and stacking interactions is almost completely absent, where only a few systems possess a positive  $\Delta(\pi\text{-}\pi)$  (Table 2).

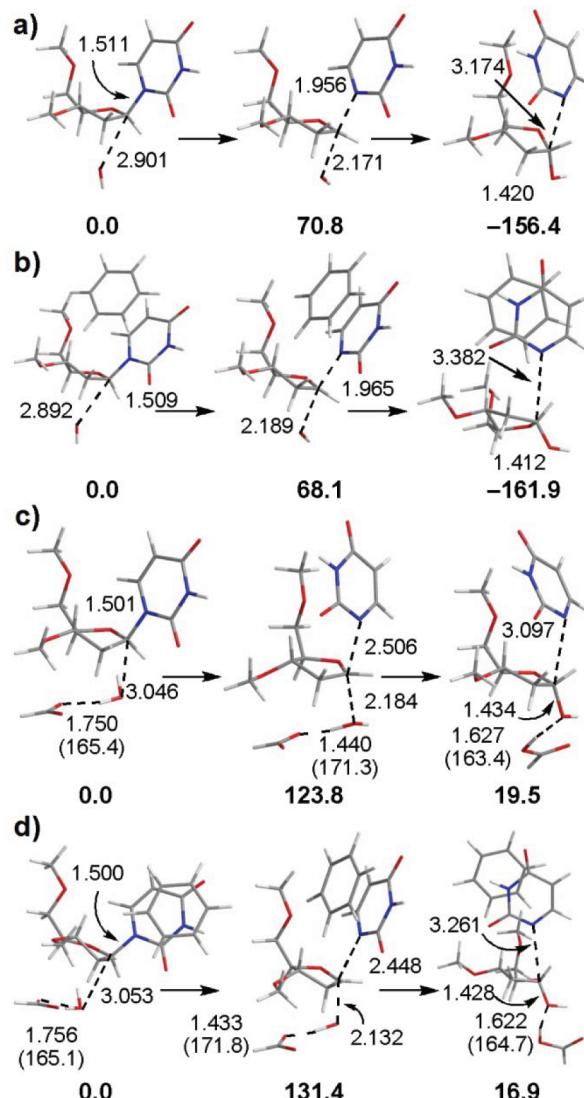
The above results lead to a more complicated view of the possible roles of aromatic residues in the active sites of enzymes such as the DNA glycosylases. Specifically, it has been proposed that aromatic residues may lower the barrier for acid catalysis

by preferentially stabilizing the protonated substrate.<sup>32</sup> In the case of uracil, the calculate N1 acidity decreases by 6.4 kJ mol<sup>-1</sup> upon complexation with a benzene ring, which implies that phenylalanine, as well as likely  $\pi-\pi$  interactions with other conjugated amino acids, is inherently slightly anticatalytic toward deglycosylation reactions. This conclusion holds true when one small molecule is hydrogen bound to uracil. However, when two small molecules are hydrogen bound to uracil, a synergistic interplay between  $\pi-\pi$  (stacking) and hydrogen-bonding interactions can increase the acidity in nonpolar environments. Nevertheless, if more than one highly acidic molecule is hydrogen bound to uracil,  $\pi-\pi$  interactions are slightly anticatalytic since uracil becomes partially protonated and the strength of the at least partial  $\pi^+-\pi$  interaction leads to a preference for the reactant (uracil) over the product (uracil anion) state. Indeed, the active site of AAG, a DNA glycosylase that excises both neutral and cationic nucleotides, is lined with aromatic residues, which likely explains the observed relatively slow reaction rate.<sup>62</sup> Furthermore, when nonpolar (bulk) environments are taken into account,  $\pi-\pi$  interactions are anticatalytic regardless of the hydrogen-bonding interactions present. Therefore, the effects of  $\pi-\pi$  interactions on the acidity of a nucleobase can be catalytic or anticatalytic, where the final situation highly depends on environmental factors. Therefore, the role of  $\pi-\pi$  interactions must be carefully examined on a case-by-case basis and must be carefully considered when addressing enzymatic reactions.

In the case of UNG, the present study suggests that the active-site phenylalanine group may in fact be slightly anticatalytic toward dU deglycosylation. Indeed, when ammonia is hydrogen bound at O2(N3) and water at O4(N3), which most closely resembles the hydrogen-bonding interactions in UNG (Figure 1), addition of a benzene ring decreases the acidity by 5.3 kJ mol<sup>-1</sup> in the presence of a weak dielectric. It is interesting to note that this result is consistent with our recent computational study on the mechanism of UNG action, which reported that F158 increases the deglycosylation barrier by  $\sim 2$  kJ mol<sup>-1</sup>.<sup>63</sup> Although this suggestion disagrees with experiments on UNG which show a slight catalytic effect of F158,<sup>64</sup> neither the acidity nor the large model study accounts for initial conformational changes dictated by this residue.<sup>65</sup> Nevertheless, the close agreement between the predicted effect on the acidity of uracil and the effect on the deglycosylation reaction suggests that the calculation of changes in the acidity may be a useful predictor of the effect of different residues on deglycosylation reactions. This connection will be further explored in the following section.

**Hydrolysis of 2'-Deoxyuridine.** As discussed in the Computational Details, the (concerted) hydrolysis of dU by fully ( $\text{OH}^-$ ) and partially ( $\text{HCOO}^- \cdots \text{H}_2\text{O}$ ) activated water is considered in the present study to determine the combined effects of nucleobase hydrogen-bonding and  $\pi-\pi$  stacking interactions on the reaction energetics. The M06-2X-optimized structures of all reactant, transition state, and product complexes studied can be found in the Supporting Information (Figures S5–S10), while the reactions with no hydrogen-bound molecules are presented in Figure 5.

The deglycosylation reactions mediated by a hydroxyl anion or formate-activated water are shown in Figures 5a and 5c, respectively. The nucleophile is initially complexed to the nucleotide through hydrogen-bonding interactions with the sugar moiety and moves closer to C1' in the transition state. The formation of a partial positive charge on the sugar moiety in the



**Figure 5.** Select M06-2X/6-31+G(d,p) distances (Å) and angles (deg, in parentheses) of representative (concerted) deglycosylation mechanisms for dU (a, c) and dU:F (b, d) initiated by a hydroxyl anion (a, b) or formate-activated water (c, d). Relative energies (kJ mol<sup>-1</sup>) are reported for the gas phase.

transition state is stabilized by negative charges on both the nucleophile and the departing nucleobase. In the product complex, a bond is fully formed between the nucleophile and the sugar moiety, while the base anion is stabilized through hydrogen-bonding interactions with the sugar–nucleophile complex. The highly charged and reactive  $\text{HO}^-$  nucleophile affords a tighter transition state and smaller barrier than the more stable (less powerful)  $\text{HCOO}^- \cdots \text{H}_2\text{O}$  nucleophile, which also has a decreased ability to stabilize the positive charge forming on the sugar moiety in the transition state.

The general structures of the M06-2X optimized stationary points along the hydroxyl mediated reaction (Figure 5a) are very similar to the B3LYP geometries published previously.<sup>37</sup> However, the nucleophile is significantly closer (by 0.361 Å) to the anomeric carbon in the M06-2X reactant, and the M06-2X transition state occurs slightly later. The largest differences occur in the product, where B3LYP characterizes a complex with a C2'-H...O2 interaction, while the M06-2X structure contains a O5' lone pair– $\pi$  interaction. Similarly, when the reaction is

**Table 3. Comparison of Barrier Heights ( $\text{kJ mol}^{-1}$ ) for the Hydrolytic Deglycosylation of dU Complexed with Water, Ammonia, Hydrogen Fluoride, and/or Phenylalanine by an  $\text{OH}^-$  Nucleophile<sup>a</sup>**

O2(N3)	O4(N3)	dU				dU:F					
		$\Delta E^\ddagger$	$\Delta\Delta E^\ddagger$	$\Delta E_R$	$\Delta\Delta E_R$	$\Delta E^\ddagger$	$\Delta\Delta E^\ddagger$	$\Delta(\pi-\pi)^\ddagger$ <sup>b</sup>	$\Delta E_R$	$\Delta\Delta E_R$	$\Delta(\pi-\pi)_R^c$
Gas Phase											
		70.8		-156.4		68.1	-2.7	-2.7	-161.9	-5.6	-5.5
H <sub>2</sub> O		65.7	-5.1	-170.1	-13.7	72.6	1.8	6.9	-169.4	-13.0	0.7
NH <sub>3</sub>		72.2	1.4	-158.3	-1.9	74.3	3.5	2.1	-157.9	-1.5	0.4
HF		56.2	-14.6	-189.1	-32.7	56.3	-14.5	0.1	-186.1	-29.7	3.0
	H <sub>2</sub> O	66.0	-4.8	-166.6	-10.2	67.5	-3.3	1.5	-165.8	-9.4	0.8
	NH <sub>3</sub>	71.4	0.6	-167.9	-11.5	73.3	2.5	1.9	-157.0	-0.6	10.9
	HF	59.3	-11.5	-179.3	-22.9	62.5	-8.4	3.1	-172.6	-16.2	6.7
H <sub>2</sub> O	H <sub>2</sub> O	60.0	-10.8	-183.6	-27.2	57.6	-13.2	-2.4	-180.0	-23.6	3.6
NH <sub>3</sub>	NH <sub>3</sub>	67.2	-3.7	-174.6	-18.2	65.3	-5.5	-1.9	-165.9	-9.5	8.7
HF	HF	48.5	-22.3	-205.6	-49.2	48.8	-22.0	0.3	-201.2	-44.8	4.4
	Ether ( $\epsilon = 4$ )										
		88.1		-127.2		88.3	0.1	0.1	-123.3	4.0	3.9
H <sub>2</sub> O		83.4	-4.7	-138.3	-11.1	91.3	3.2	7.9	-130.7	-3.5	7.6
NH <sub>3</sub>		91.5	3.4	-127.8	-0.6	96.0	7.8	4.5	-118.3	9.0	9.5
HF		71.1	-17.0	-161.9	-34.7	73.0	-15.2	1.9	-151.7	-24.5	10.2
	H <sub>2</sub> O	84.6	-3.5	-135.0	-7.8	87.1	-1.1	2.5	-127.9	-0.7	7.1
	NH <sub>3</sub>	90.7	2.6	-135.5	-8.3	94.0	5.9	3.3	-117.3	10.0	18.2
	HF	75.1	-13.0	-150.7	-23.5	80.7	-7.5	5.5	-135.2	-7.9	15.5
H <sub>2</sub> O	H <sub>2</sub> O	78.5	-9.6	-151.5	-24.3	76.4	-11.7	-2.1	-141.6	-14.4	9.9
NH <sub>3</sub>	NH <sub>3</sub>	86.8	-1.3	-141.4	-14.2	85.9	-2.3	-0.9	-126.2	1.0	15.2
HF	HF	63.4	-24.7	-176.0	-48.8	63.0	-25.1	-0.4	-168.8	-41.5	7.2
	Water ( $\epsilon = 78$ )										
		98.5		-110.8		99.4	0.9	0.9	-103.2	7.6	7.6
H <sub>2</sub> O		93.7	-4.7	-121.3	-10.5	100.7	2.2	6.9	-111.2	-0.4	10.1
NH <sub>3</sub>		101.9	3.5	-112.8	-2.1	106.4	7.9	4.4	-98.6	12.1	14.2
HF		80.6	-17.9	-145.7	-34.9	82.3	-16.2	1.7	-133.5	-22.8	12.2
	H <sub>2</sub> O	94.9	-3.5	-117.7	-7.0	98.1	-0.4	3.1	-107.8	3.0	9.9
	NH <sub>3</sub>	101.2	2.7	-119.5	-8.7	105.3	6.9	4.2	-97.2	13.6	22.3
	HF	85.3	-13.2	-133.4	-22.6	90.5	-8.0	5.2	-114.9	-4.1	18.5
H <sub>2</sub> O	H <sub>2</sub> O	88.9	-9.6	-133.4	-22.6	87.1	-11.4	-1.8	-120.6	-9.8	12.8
NH <sub>3</sub>	NH <sub>3</sub>	97.6	-0.9	-124.0	-13.2	96.7	-1.8	-0.9	-106.4	4.3	17.6
HF	HF	72.6	-25.9	-158.0	-47.2	72.1	-26.4	-0.5	-149.9	-39.2	8.1

<sup>a</sup>(PCM-)M06-2X/6-311+G(2d,p)//M06-2X/6-31+G(d,p) values including scaled (0.9631) zero-point energy corrections. <sup>b</sup>The difference between dU and dU:F barriers for systems with equivalent hydrogen-bonding interactions. <sup>c</sup>The difference between dU and dU:F reaction energies for systems with equivalent hydrogen-bonding interactions.

mediated by formate-activated water, there are few differences in the B3LYP and M06-2X geometries. Although the  $\text{HCOO}^- \cdots \text{H}_2\text{O}$  M06-2X transition state is slightly earlier than previously reported and contains a lone pair- $\pi$  interaction that was not previously reported, it is still far later than the hydroxyl-mediated reaction.

Since it is well-known that B3LYP underestimates barrier heights,<sup>66</sup> it is not surprising that the M06-2X barrier heights (Tables 3 and 4) are  $\sim 20 \text{ kJ mol}^{-1}$  larger than the previously reported B3LYP values.<sup>37</sup> Perhaps more importantly, the calculated change in the deglycosylation barrier height ( $\Delta\Delta E^\ddagger$ ) due to the various nucleobase hydrogen-bonding interactions predicted by the two methods are generally within  $2 \text{ kJ mol}^{-1}$  for the  $\text{OH}^-$  nucleophile and  $4 \text{ kJ mol}^{-1}$  for the  $\text{HCOO}^- \cdots \text{H}_2\text{O}$  nucleophile. In addition, the B3LYP and M06-2X changes in reaction energies ( $\Delta\Delta E_R$ ) are generally within  $5 \text{ kJ mol}^{-1}$ . This difference between the two methods comes from slight changes in the product complex discussed above. The greatest differences in barrier height and reaction energy involve the ammonia molecule, where the M06-2X method finds interactions with ammonia to be more beneficial than B3LYP.<sup>37</sup>

At the M06-2X level of theory, the barrier to deglycosylation (Tables 3 and 4) is decreased in the presence of interactions with HF ( $11\text{--}40 \text{ kJ mol}^{-1}$ ) significantly more than with water ( $5\text{--}20 \text{ kJ mol}^{-1}$ ) or ammonia (which is only beneficial when two NH<sub>3</sub> molecules are present). This is similar to the trend discussed for the effects of XH on the N1 acidity of uracil (Table 2). However, in contrast to the acidity, the effects of hydrogen-bonding interactions are not always larger at O2 over O4 when hydrolysis is facilitated by the OH<sup>-</sup> nucleophile. For example, ammonia and water have nearly equivalent effects at both sites, while HF lowers the hydrolysis barrier by  $\sim 3 \text{ kJ mol}^{-1}$  more when located at O2(N3) than O4(N3). This likely occurs due to the early transition state, which does not exhibit a large negative charge on uracil and therefore permits a balance between the larger proton affinity of O4 (Table 1) and the negative charge building on O2. In contrast, the later transition states associated with the formate-activated water nucleophile have approximately 2–4  $\text{kJ mol}^{-1}$  lower barriers when a small molecule interacts with O2 rather than O4 due to a greater (negative) charge at O2. The increased charge on the base in the formate-activated water transition states also allows

**Table 4. Comparison of Barrier Heights ( $\text{kJ mol}^{-1}$ ) for the Hydrolytic Deglycosylation of dU Complexed with Water, Ammonia, Hydrogen Fluoride, and/or Phenylalanine by an  $\text{HCOO}^- \cdots \text{H}_2\text{O}$  Nucleophile<sup>a</sup>**

O2(N3)	O4(N3)	dU				dU:F					
		$\Delta E^\ddagger$	$\Delta\Delta E^\ddagger$	$\Delta E_R$	$\Delta\Delta E_R$	$\Delta E^\ddagger$	$\Delta\Delta E^\ddagger$	$\Delta(\pi-\pi)^\ddagger$ <sup>b</sup>	$\Delta E_R$	$\Delta\Delta E_R$	$\Delta(\pi-\pi)_R$ <sup>c</sup>
Gas Phase											
		123.8		19.5		131.4	7.6	7.6	16.9	-2.5	-2.5
H <sub>2</sub> O		115.2	-8.6	3.9	-15.6	124.7	0.9	9.5	7.5	-12.0	3.6
NH <sub>3</sub>		124.2	0.4	18.5	-1.0	134.4	10.6	10.2	3.2	-16.3	-15.3
HF		100.0	-23.8	-9.2	-28.7	109.4	-14.4	9.4	-11.1	-30.6	-1.9
	H <sub>2</sub> O	117.7	-6.1	9.0	-10.5	123.1	-0.7	5.4	6.0	-13.5	-3.0
	NH <sub>3</sub>	127.9	4.1	10.6	-8.9	134.8	11.1	7.0	18.8	-0.7	8.2
	HF	106.2	-17.6	-2.4	-21.8	114.5	-9.3	8.3	1.7	-17.7	4.1
H <sub>2</sub> O	H <sub>2</sub> O	104.4	-19.4	5.1	-14.4	113.6	-10.2	9.2	3.9	-15.6	-1.2
NH <sub>3</sub>	NH <sub>3</sub>	110.8	-13.0	22.6	3.1	128.6	4.8	17.8	-3.7	-23.1	-26.3
HF	HF	87.0	-36.8	-28.1	-47.5	96.3	-27.5	9.3	-27.0	-46.5	1.1
Ether ( $\epsilon = 4$ )											
		132.1		32.3		140.0	7.9	7.9	37.6	5.3	5.3
H <sub>2</sub> O		124.8	-7.4	18.6	-13.7	134.9	2.8	10.2	28.9	-3.4	10.3
NH <sub>3</sub>		134.9	2.8	29.4	-2.9	146.1	14.0	11.2	26.7	-5.6	-2.7
HF		105.7	-26.5	-1.6	-33.9	115.7	-16.5	10.0	6.7	-25.7	8.3
	H <sub>2</sub> O	127.6	-4.6	25.4	-7.0	134.0	1.8	6.4	29.0	-3.4	3.6
	NH <sub>3</sub>	138.5	6.3	27.0	-5.3	146.0	13.9	7.5	42.9	10.6	15.8
	HF	113.2	-18.9	6.3	-26.0	123.1	-9.0	9.9	22.1	-10.2	15.7
H <sub>2</sub> O	H <sub>2</sub> O	113.4	-18.7	12.0	-20.3	124.2	-8.0	10.8	20.4	-12.0	8.4
NH <sub>3</sub>	NH <sub>3</sub>	123.2	-9.0	31.8	-0.5	140.8	8.7	17.7	21.7	-10.6	-10.1
HF	HF	95.1	-37.1	-17.0	-49.3	102.8	-29.4	7.7	-8.4	-40.7	8.6
Water ( $\epsilon = 78$ )											
		136.3		38.6		143.2	6.9	6.9	44.8	6.3	6.3
H <sub>2</sub> O		128.6	-7.8	24.4	-14.2	138.4	2.1	9.9	36.6	-1.9	12.2
NH <sub>3</sub>		138.1	1.8	33.3	-5.3	149.3	13.0	11.2	36.6	-2.0	3.3
HF		109.0	-27.3	2.9	-35.6	118.1	-18.2	9.1	13.7	-24.9	10.8
	H <sub>2</sub> O	131.7	-4.7	32.7	-5.8	138.2	1.8	6.5	37.0	-1.6	4.3
	NH <sub>3</sub>	141.7	5.3	32.9	-5.7	149.6	13.2	7.9	50.8	12.3	18.0
	HF	117.3	-19.1	11.3	-27.3	126.9	-9.4	9.7	31.6	-7.0	20.3
H <sub>2</sub> O	H <sub>2</sub> O	117.7	-18.6	15.7	-22.8	128.6	-7.7	10.9	25.7	-12.9	10.0
NH <sub>3</sub>	NH <sub>3</sub>	127.9	-8.4	35.3	-3.2	145.0	8.7	17.1	32.1	-6.4	-3.2
HF	HF	100.0	-36.3	-9.8	-48.3	105.8	-30.5	5.8	-0.4	-39.0	9.4

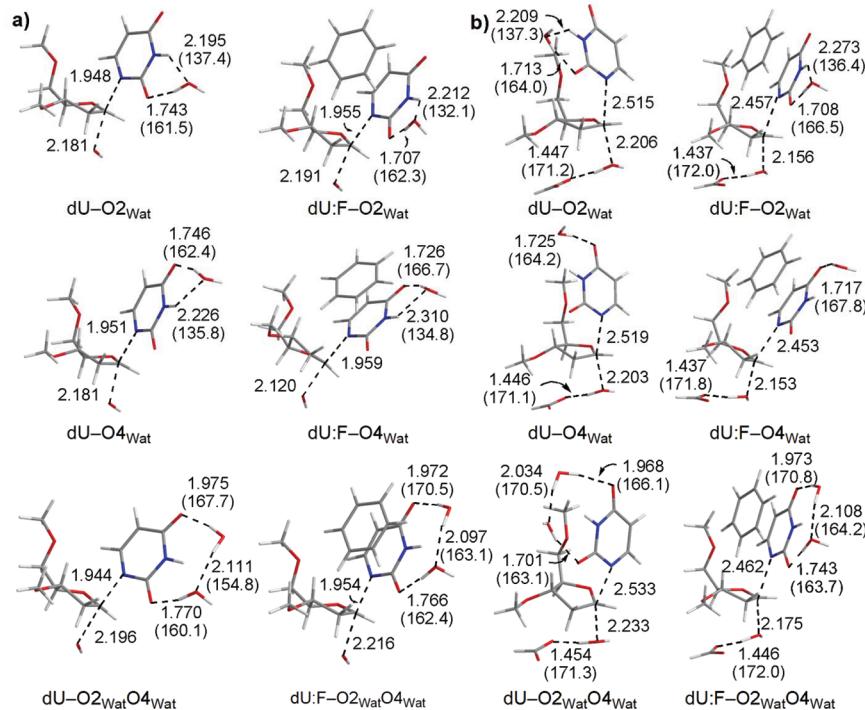
<sup>a</sup>(PCM-)M06-2X/6-311+G(2d,p)//M06-2X/6-31+G(d,p) values including scaled (0.9631) zero-point energy corrections. <sup>b</sup>The difference between dU and dU:F barriers for systems with equivalent hydrogen-bonding interactions. <sup>c</sup>The difference between dU and dU:F reaction energies for systems with equivalent hydrogen-bonding interactions.

the hydrogen-bound small molecule(s) to have a greater effect on the reaction than hydrolysis facilitated by fully activated water. While bulk solvent increases the calculated  $\Delta E^\ddagger$  and decreases the  $\Delta E_R$ , the effects are consistent for all reactions regardless of the molecules interacting with uracil, which results in the effects of solvent on  $\Delta E^\ddagger$  and  $\Delta\Delta E_R$  being only  $\sim 2 \text{ kJ mol}^{-1}$ .

When phenylalanine is complexed with dU along the hydrolysis pathways, the transition states become looser for the OH<sup>-</sup> nucleophile and tighter for the HCOO<sup>-</sup>...H<sub>2</sub>O nucleophile than in the corresponding parent complex (Figures 5 and 6 and Figures S5–S10). Nevertheless, the presence of a benzene ring does not significantly alter the reactant and transition state complexes, where the glycosidic and nucleophilic distances differ by less than 0.02 Å. In dU-XH complexes, the hydrogen bonds involving O2 and O4 tighten and those at N3 slightly loosen upon complexation with the benzene ring, which was observed in the acidity portion of this study. Otherwise, there is very little structural change in the transition states and the reactant complexes (see, for example, Figure 5) upon introducing  $\pi-\pi$  interactions with the nucleobase. However, there are considerable differences between the dU and dU:F product complexes.

Specifically, the lone-pair- $\pi$  interactions between the uracil moiety and the 5'-oxygen in the deoxyribose moiety present in the dU products (and transition states involving formate-activated water) are absent in the dU:F systems since the benzene ring sterically blocks the  $\pi$ -face of uracil.

As observed for the U:F systems, the tilt angle between the phenylalanine and U rings is small in the reactant ( $<10^\circ$  on average) and generally decreases as the reaction proceeds (Table S4). The exception is the products with ammonia hydrogen bound to uracil, which distort (tilt angle  $\sim 50^\circ$ ) to stabilize the uracil anion through weak C–H... $\pi$  interactions between phenylalanine and the nucleobase. In addition to the tilt angle, the distance between the center of benzene and the uracil plane is only slightly further than observed in the U:F complexes (Table S4). However, in the case of the dU:F systems, phenylalanine has a greater preference to be centered over N3 (or the N3-H bond) due to steric interactions between the sugar and phenylalanine ring (Table S4). It is particularly interesting to note that the benzene ring remains in a stacked orientation with respect to uracil in the transition states and products along the deglycosylation mechanisms,



**Figure 6.** Transition states, including select M06-2X/6-31+G(d,p) hydrogen-bond distances ( $\text{\AA}$ ) and angles (deg, in parentheses), for the deglycosylation of dU (left) and dU:F (right) facilitated by the (a) hydroxyl and (b) formate-activated water nucleophile, where one (top and middle) or two (bottom) water molecules are bound.

which once again emphasizes the stability of these interactions upon full optimization.

Despite the small differences in the location of phenylalanine in the U:F and dU:F systems, benzene generally only binds 2–7  $\text{kJ mol}^{-1}$  stronger to the reactant and product complexes compared to isolated neutral and anionic uracil, respectively (Tables S2 and S3). The binding strengths of the transition state complexes are weaker than the reactant and product complexes when one small molecule is hydrogen bound to U, but stronger when two small molecules are bound, which correlates with the relative effect of one versus two small molecules on the acidity. The small changes in the structures and binding strengths for the hydrolysis reactions compared with the acidity study are consistent with the small predicted effects of the sugar ring on the stacking between nucleobases and amino acids.<sup>51,67</sup>

The  $\pi-\pi$  interactions with benzene slightly lower the barrier to dU hydrolysis by the hydroxyl anion (by 2.7  $\text{kJ mol}^{-1}$ , Table 3) in the absence of small molecules bound to uracil. However, the corresponding formate-activated water hydrolysis barrier increases by 7.6  $\text{kJ mol}^{-1}$  upon addition of the phenylalanine ring (Table 4). The trend in the effects of the small molecule bound and the binding site discussed for the dU reactions hold true upon complexation with phenylalanine. More importantly, when one small molecule hydrogen bonds to uracil, the  $\Delta(\pi-\pi)^{\ddagger}$  due to hydrolysis by  $\text{OH}^-$  is generally less than 2  $\text{kJ mol}^{-1}$  (Table 3) and indicates an increase in the reaction barrier. However, when two small molecules are hydrogen bound to uracil,  $\Delta(\pi-\pi)^{\ddagger}$  is negative, where the barrier lowering likely arises due to a net tightening of the O2 and O4 hydrogen bonds in these complexes. On the other hand, when the formate-activated water nucleophile is considered,  $\Delta(\pi-\pi)^{\ddagger}$  is positive by up to 10  $\text{kJ mol}^{-1}$  if one small molecule is bound to uracil and 20  $\text{kJ mol}^{-1}$  if two small molecules are involved. The differences

in the effect of phenylalanine on the dU hydrolysis barrier outlined here for the two nucleophiles can be rationalized based on the nature of the transition state (which is later for  $\text{HCOO}^- \cdots \text{H}_2\text{O}$ ) and once again emphasizes the sensitivity of the role of  $\pi-\pi$  interactions to the situation under investigation, including the nature of the nucleophile and other interactions occurring with the substrate.

Despite the emphasis that the effect of  $\pi-\pi$  interactions on the N1 acidity of uracil is dependent on the polarity of the surrounding environment, the effect of introducing stacking with the nucleobase on dU deglycosylation ( $\Delta(\pi-\pi)^{\ddagger}$ ) is relatively unchanged in the presence of solvent (Tables 3 and 4). In general, the effect of introducing more polar environments is <2  $\text{kJ mol}^{-1}$ , where phenylalanine tends to be more anticatalytic in more polar surroundings. However, the effect of bulk solvent on  $\Delta(\pi-\pi)_R^{\ddagger}$  is more significant, sometimes being over 10  $\text{kJ mol}^{-1}$  and leading to more endothermic products. The less powerful nucleophile (formate-activated water) tends to be more greatly affected by the environment than the stronger nucleophile ( $\text{OH}^-$ ), which supports our proposal that both the environment and the nucleophile must be carefully considered when predicting the roles of active-site aromatic amino acid residues.

As mentioned previously, our calculated effects of hydrogen-bonding and  $\pi-\pi$  stacking on the acidity of uracil correlate with the effects predicted using a much larger computational model of the UNG active site. Since it can be difficult to fully characterize deglycosylation barriers in the presence of weak noncovalent interactions such as stacking using currently available computational methodologies, it is interesting to further consider whether smaller acidity studies can be used to anticipate the changes in reaction barriers and the current work presents a large database for direct comparison. There is a linear relationship between the calculated change in acidity and the change in barrier heights (Figure S11). The constant relating  $\Delta E^{\ddagger}$  to  $\Delta(\text{acidity})$  varies

from 0.23 ( $\text{OH}^-$ ) to 0.36 ( $\text{HCOO}^- \cdots \text{H}_2\text{O}$ ), which is in accord with the later transition states (charge build up on uracil) for the formate-activated water nucleophile. When  $\pi-\pi$  interactions with benzene are introduced into the systems, the ratio slightly increases (to 0.28 and 0.43 for  $\text{OH}^-$  and  $\text{HCOO}^- \cdots \text{H}_2\text{O}$ , respectively). Thus, regardless of whether  $\pi-\pi$  interactions are present, changes in acidity of uracil are predictive of changes in the dU deglycosylation barrier in the presence of the same discrete nucleobase interactions.

In summary, the results presented in this section once again demonstrate that  $\pi-\pi$  stacking interactions can be slightly catalytic or antecatalytic depending on the other molecules interacting with the nucleobase, the power of the nucleophile and the (bulk) environment. Regardless, these effects can be accurately estimated using small model studies of the effects on the nucleobase acidity. In terms of UNG, the  $\pi-\pi$  stacking between phenylalanine and uracil may be slightly antecatalytic for the chemical step due to the residues hydrogen bound to uracil (Figure 1), the estimated (weakly polar) environment, and the water nucleophile that is activated by an active-site amino acid residue. Therefore, the observed small catalytic contribution of this residue<sup>64</sup> may arise due to reactant destabilization through structural changes due to sterics (as discussed by Parikh et al.<sup>65</sup>) rather than an intrinsic lowering of the deglycosylation barrier. We note that these steric considerations are not accounted for in the current models and occur prior to the reaction considered in our previous large-scale study.<sup>63</sup> Thus, although  $\pi-\pi$  interactions play a catalytic role in some environments, it is possible that these contacts with the substrate serve a structural rather than catalytic purpose in some active sites.

## CONCLUSIONS

In this study, the effect of  $\pi-\pi$  stacking, as well as the simultaneous effects of hydrogen bonding, with uracil on the nucleobase acidity was investigated. To the best of our knowledge, this work represents one of the first contributions to the literature that uses fully optimized systems to carefully examine both the effects of  $\pi-\pi$  stacking interactions on the acidity (and basicity) of different sites in a biomolecule and the potential catalytic contribution of the same interactions to a chemical reaction. The proton affinity of uracil is greatly enhanced in the presence of stacking with phenylalanine, which indicates that aromatic residues in active sites may lower the barrier for acid catalysis. However, stacking slightly decreases the N1 acidity of uracil. Nevertheless, when two small molecules are simultaneously hydrogen bound to the nucleobase, the negative effect of the  $\pi-\pi$  interactions on the nucleobase acidity is reduced or even reversed such that  $\pi-\pi$  interactions increase the acidity of uracil. The effect of  $\pi-\pi$  stacking on the glycosidic bond (N1–C1') stability was further explored by studying the barrier to hydrolytic deglycosylation of dU. Once again, the  $\pi-\pi$  interactions were found to be slightly catalytic or antecatalytic, where the final situation arising depends on the number and type of small molecules bound to uracil, the power of the nucleophile (or the degree of dissociation in the transition state), and the polarity of the surrounding (bulk) environment. Thus, stacking interactions in the active sites of enzymes that catalyze DNA glycosidic bond cleavage reactions should not be ignored when designing large-scale active site models. Interestingly, a linear relationship between the change in acidity and barrier height was found, which suggests that the effects of multiple noncovalent interactions on the deglycosylation of the nucleobases can be reasonably approximated by small model, acidity calculations. Therefore, future

studies interested in the effects of  $\pi-\pi$  interactions on nucleobase deglycosylation reactions must carefully consider the conditions under which the reaction proceeds and can use acidity calculations to quickly estimate the individual and synergistic role of the various components.

## ASSOCIATED CONTENT

### Supporting Information

Structures of all U complexes (Figures S1–S4 and Table 2) and reactants, transition states, and products for the hydrolysis of dU (Figures S5–S10 and Table 4); binding energies in U:F (Table S1) and dU:F (Table S2) complexes; the relation between change in acidity and change in barrier height (Figure S11); and the full citation for ref 57. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: stacey.wetmore@uleth.ca.

### Notes

The authors declare no competing financial interest.

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