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Uracil and Thymine Reactivity in the Gas Phase: The S_N2 Reaction and Implications for Electron Delocalization in Leaving Groups

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Abstract: The gas-phase substitution reactions of methyl chloride and 1,3-dimethyluracil (at the N1−CH_3) are examined computationally and experimentally. It is found that, although hydrochloric acid and 3-methyluracil are similar in acidity, the leaving group abilities of chloride and N1-deprotonated 3-methyluracil are not: chloride is a slightly better leaving group. The reason for this difference is most likely related to the electron delocalization in the N1-deprotonated 3-methyluracil anion, which we explore further herein. The leaving group ability of the N1-deprotonated 3-methyluracil anion relative to the N1-deprotonated 3-methylthymine anion is also examined in the context of an enzymatic reaction that cleaves uracil but not thymine from DNA.

Introduction

Uracil and thymine are pyrimidine nucleobases that differ in structure by only a methyl group at C5 (**1a** and **3a**). Uracil naturally occurs in RNA, while thymine is its DNA counterpart.

Although uracil and thymine are very similar in structure, the presence of uracil in DNA is problematic.[1−4] Uracil can arise in DNA from cytosine deamination, which is mutagenic; uracil can also be misincorporated into DNA, leading to cytotoxic uracil·adenine base pairs.[1,2,4−6] Uracil is removed from the genome by the enzyme uracil DNA glycosylase (UDG).[5,7−13]

The mechanism of UDG has been shown to involve N1-deprotonated uracil as the leaving group (LG).[5,9,14−30] Deprotonated uracil as a leaving group seems somewhat surprising and begs the question: How good of a leaving group is deprotonated uracil? The N1−H pK_a in water is 9.8, which would indicate a fair or mediocre leaving group ability. In the enzyme, uracil has a depressed pK_a of 6.4.[19,20]

Examining properties in the gas phase is useful for elucidating inherent reactivity in the absence of solvent.[3,17,31−40] In previous studies, we calculated and measured the gas-phase acidity of uracil and found it to be as acidic as hydrochloric acid, indicating that in the gas phase, deprotonated uracil might be, relatively speaking, a good LG.[3] Furthermore, because enzyme environments are sometimes quite nonpolar, reactivity in the gas phase,

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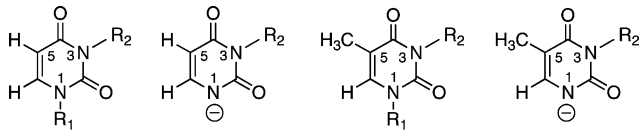
a kind of "ultimate" nonpolar medium, can yield insight into biological reactivity.[3,17,35−43] Uracil is particularly intriguing, we found, because the acidities at the N1 and N3 sites are very different in the gas phase, but coalesce in aqueous solution.[3,17]

Having established that uracil (**1a**) is quite acidic in the gas phase, we now examine the leaving group ability of the conjugate base, N1-deprotonated uracil (**2a**), in a substitution reaction. Because uracil is as acidic as HCl, is deprotonated uracil as good of a leaving group as chloride in the gas phase?



1 **2** **3** **4**

a $R_1 = R_2 = H$
b $R_1 = H, R_2 = CH_3$
c $R_1 = CH_3, R_2 = H$
d $R_1 = R_2 = CH_3$

We are also interested in comparing the leaving group abilities of deprotonated uracil versus deprotonated thymine. The occurrence of uracil in the human genome is typically one uracil per $>10^7$ normal DNA base pairs. The ability of the UDG enzyme to find and excise the few uracils present while leaving the structurally similar thymine untouched is of interest.[44] A recent study indicates that the uracil versus thymine discrimination could be due in part to base-pair dynamics.[4] We wanted to probe the possibility that another contribution to the favorable excision of uracil over thymine could be due to the relative leaving group abilities of the corresponding conjugate bases (deprotonated at N1). If N1-deprotonated thymine (**4a**) is a poorer leaving group than N1-deprotonated uracil (**2a**), this could presumably contribute to the favorable excision of uracil over thymine.[3,17,37,40] Leaving group ability has been implicated in the discrimination of substrates with other glycosylases.[35,38,39,43,45]

Results

S_N2 reactions in the gas phase have been studied for more than two decades. They follow the "classic" gas-phase double-well potential energy surface, where an initial ion−molecule complex is formed that can either dissociate back to reactants or react to products.[34,46−60] Previously measured second-order reaction rate constants and efficiencies for S_N2 reactions of methyl chloride with different anionic nucleophiles are shown in Table 1 (where reaction efficiency is defined as the ratio of the observed rate constant to the estimated collision rate constant calculated by parametrized trajectory calculations).[34,61,62]

Table 1. Second-Order Reaction Rate Constants and Efficiencies for Reactions of Nucleophiles of Varying Proton Affinity with Methyl Chloride[34]

nucleophile	PA, kcal mol^{-1}	rate, $\times 10^{-10}$ cm^3/molecule/s	efficiency, %
NH_2^-	403.4	15	46
CH_3NH^-	403.2	17	64
Ph^-	401.7	8.7	42
H^-	400.4	30	26
HO^-	390.3	20	62
CH_3O^-	382.0	13	50
$PhCH_2^-$	379.2	0.15	0.7
HCC^-	379.0	1.3	4.6
$(CH_3)_3CO^-$	374.7	1.6	7.6
F^-	371.3	13	42
$CF_3CH_2O^-$	361.7	2.2	11
CH_3S^-	357.6	1.1	4.7
HS^-	350.8	0.12	0.5
Cl^-	333.4	0.00035	0.001

Model Systems for Gas-Phase Study. The excision of uracil from DNA involves nucleophilic attack of the C1′ of ribose (Figure 1). Kinetic isotope effects point to a "dissociative S_N2" reaction mechanism ($D_N{*}A_N$).[5,16,25] Our interest is in testing the leaving group ability of deprotonated uracil in a substitution reaction. The simplest model would be to examine reactivity at the N1−CH_3 group of 1-methyluracil (**1c**). However, experiments with 1-methyluracil are limited by the acidity of the N3−H, which has been measured to be 348 ± 3 kcal mol^{-1} in the gas phase.[3,17] Therefore, any nucleophile with a proton affinity (PA) of 348 or greater will likely deprotonate the N3−H. Proton transfers are enthalpically generally barrierless ($\Delta H^{\ddagger} = 0$) and will, if exothermic, compete with the S_N2 reaction.[33] Using nucleophiles with PAs less than 348 kcal mol^{-1} would be too limiting; for substitution reactions with CH_3Cl, anions with a PA at or below 348 kcal mol^{-1} yield very low efficiencies (Table 1). Therefore, to allow the use of more basic nucleophiles, we chose to examine the 1,3-dimethyl substrates (**1d**, **3d**) wherein the methyl group at N3 acts as a sort of "protecting group".

Acidity Studies. With the chosen model systems, our leaving groups are no longer the deprotonated uracil and deprotonated thymine, but rather the 3-methyl derivatives (Figure 2).

As a starting point toward ascertaining whether N1-deprotonated-3-methyluracil is a better leaving group than N1-

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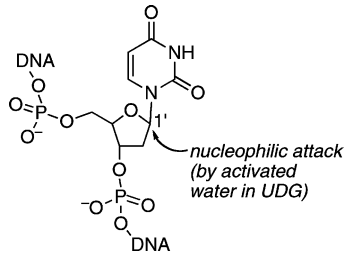

Figure 1. Nucleophilic attack at C1′ to excise uracil.[5]

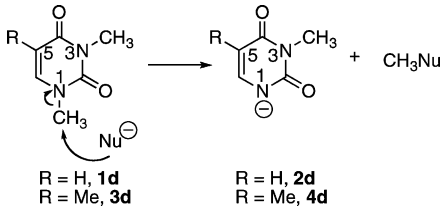

Figure 2. S_N2 reactions studied.

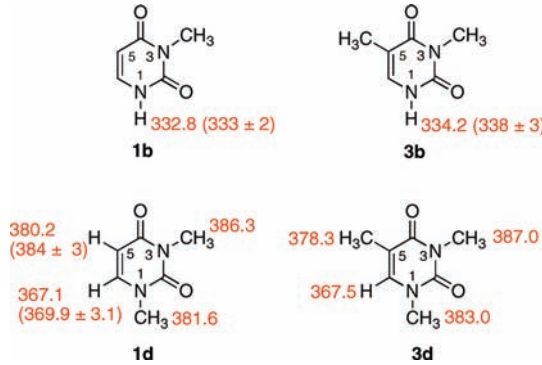

Figure 3. Calculated N1−H acidities of 3-methyluracil[17] (3-MeU, **1b**) and 3-methylthymine (3-MeT, **3b**) and all of the sites of 1,3-dimethyluracil (**1d**) and 1,3-dimethylthymine (**3d**) (B3LYP/6-31+G*, ΔH_{298K}, kcal mol^{-1}). Experimental values where known are in parentheses.[17,63]

deprotonated 3-methylthymine, we assessed the acidity of the N1−H proton in 3-methyluracil (3-MeU, **1b**) and compared it to the acidity of the N1−H proton in 3-methylthymine (3-MeT, **3b**). The acidity of 3-MeU has been calculated previously by us (332.8 kcal mol^{-1}, Figure 3); herein, we calculate the ΔH_{acid} of 3-MeT to be 1.4 kcal mol^{-1} less than the ΔH_{acid} of 3-MeU (332.8 vs 334.2 kcal mol^{-1}, Figure 3).[17] On the basis of these acidities, we would expect deprotonated 3-methyluracil to be a better leaving group than deprotonated 3-methylthymine.

We have also previously measured the gas-phase ΔH_{acid} of 3-MeU (**1b**) to be 333 ± 2 kcal mol^{-1}.[17] We bracket the acidity of 3-MeT herein (**3b**) (Table 2). We find that while the conjugate base of 3-MeT deprotonates 2-chloropropanoic acid (ΔH_{acid} = 337.0 ± 2.1 kcal mol^{-1}) and acids with lower ΔH_{acid} values, it cannot deprotonate trifluoro-*m*-cresol (ΔH_{acid} = 339.3 ± 2.1 kcal mol^{-1}) or reference acids with higher ΔH_{acid} values. Consistent with this, 2-chloropropanoate cannot deprotonate 3-MeT, but trifluoro-*m*-cresolate can. We therefore bracket the ΔH_{acid} of 3-MeT to be 338 ± 3 kcal mol^{-1} (ΔG_{acid} (3-MeT) = 331 ± 3 kcal mol^{-1}).

We also conducted Cooks kinetic method experiments to measure the relative acidity of 3-MeT (**3b**) and 3-MeU (**1b**).[65−69] We accomplished this by dissociating the [(3-MeU)$^-$·H$^+$·(3-MeT)$^-$] dimer. These experiments indicate that 3-MeU (**1b**) is 2−3 kcal mol^{-1} more acidic than 3-MeT (**3b**).

The experiments therefore indicate a difference in acidity between 3-MeU and 3-MeT that is on the order of 2−5 kcal mol^{-1}. Although this is a rather large range, what is consistent is that 3-MeU is more acidic than 3-MeT. This could make the N1$^-$ conjugate base of 3-MeU a potentially better leaving group than that of 3-MeT.

Another important reason to probe acidities is to establish the upper limit of proton affinity for the nucleophiles to be studied experimentally. As we noted earlier, we are using the N3−methyl substrates because the N3−H of 1-methyluracil has a ΔH_{acid} of 348 kcal mol^{-1}, and to avoid competition between S_N2 reaction at the N1−CH_3 and proton transfer at the N3−H, we would be limited to using nucleophiles with proton affinities (PA) under 348 kcal mol^{-1}.[3,17] Now that we have established that we will be using the 1,3-dimethyl derivatives **1d** and **3d**, we need to assess the acidities of all of the sites of those substrates (Figure 3). The C6 protons of both of these derivatives are quite acidic, with values just below that of acetone (the C6 and C5 of 1,3-dMU have also been previously measured by the Gronert lab and our lab).[17,53] Therefore, to avoid competition from deprotonation, nucleophiles with proton affinities below 367 kcal mol^{-1} will be utilized.

S_N2 Reaction Studies: Calculations. a. 1,3-Dimethyluracil (1,3-dMU). We next calculated the energetics associated with S_N2 reactions of 1,3-dMU. We chose formate and methyl thiolate as the nucleophiles. Formate was chosen as a probable slow reaction example, based on the acidity of formic acid (ΔH_{acid} = 346.2 ± 1.2 kcal mol^{-1}) and the known methyl chloride data for nucleophiles in that acidity range (Table 1). Methyl thiolate (ΔH_{acid} = 357.6 ± 2.0 kcal mol^{-1}) was chosen as a faster (although still moderate) reaction example.

The reaction of formate with 1,3-dMU first forms the expected reactant ion−molecule complex, a process that is 23.1 kcal mol^{-1} exothermic (Figure 4). The $\Delta H^{\ddagger}$ barrier to the S_N2 reaction is 32.3 kcal mol^{-1} from this complex (and 9.2 kcal mol^{-1} from the separated reactants (Figure 4)). The product ion−molecule complex is 9.3 kcal mol^{-1} more stable than the separated reactants, but the separated products are 2.7 kcal mol^{-1} higher in energy than the separated reactants. Given that both the transition state (TS) and the separated products are higher in energy than the separated reactants, this reaction is not likely to proceed significantly under our gas-phase conditions.

For the reaction of methyl thiolate with 1,3-dMU (figure in Supporting Information), the transition state is calculated to be just 4.3 kcal mol^{-1} higher than the energy of the separated reactants. The energy of the transition structure must be below the energy of the separated reactants for reaction to be observed, so we could see reaction depending on how accurate the calculations are, and how entropically unfavorable the process is.[34]

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Table 2. Summary of Results of Acidity Bracketing of 3-Methylthymine (3-MeT, **3b**)

reference compound	ΔH_{acid}[a] (kcal mol^{-1})	ΔG_{acid}[a] (kcal mol^{-1})	proton transfer[b] ref. acid	proton transfer[b] conj. base
2,4-pentanedione	343.8 ± 2.1	336.7 ± 2.0	−	+
methyl cyanoacetate	340.80 ± 0.60	334.5	−	+
trifluoro-*m*-cresol	339.3 ± 2.1	332.4 ± 2.0	−	+
2-chloropropanoic acid	337.0 ± 2.1	330.4 ± 2.0	+	−
malononitrile	335.8 ± 2.1	328.1 ± 2.0	+	−
pyruvic acid	333.5 ± 2.9	326.5 ± 2.8	+	−
difluoroacetic acid	331.0 ± 2.2	323.8 ± 2.0	+	−
1,1,1-trifluoro-2,4-pentadione	328.3 ± 2.9	322.0 ± 2.0	+	−

[a] Acidities are in kcal mol^{-1}.[64] [b] A "+" indicates the occurrence, and a "−" indicates the absence of proton transfer.

b. Methyl Chloride. We also calculated the energetics for the reaction of formate (Figure 5) and methyl thiolate (figure in Supporting Information) with methyl chloride, to provide a benchmark for comparison. Interestingly, the methyl chloride reactions are consistently more exothermic and have lower barriers than the uracil reactions. With formate, the $\Delta H^{\ddagger}$ is 1.9 kcal mol^{-1} below the separated reactants (Figure 5). For methyl thiolate, the $\Delta H^{\ddagger}$ is 7.5 kcal mol^{-1} below the separated reactants.

Our calculations therefore indicate that, although 3-MeU and HCl have similar acidities, the 1,3-dMU S_N2 reactions are expected to be slower than those of CH_3Cl.

c. 1,3-Dimethylthymine (1,3-dMT). Our calculations indicate that in keeping with the fact that thymine is less acidic than uracil, the S_N2 reactions wherein deprotonated thymine is a leaving group do have slightly higher barriers than the corresponding reactions with deprotonated uracil. Using 1,3-dMT as the model system, the energy surfaces for reaction with formate and methyl thiolate were calculated (figures in Supporting Information). For the formate reaction, the barrier is 1.2 kcal mol^{-1} higher with 1,3-dMT than with 1,3-dMU (relative to separated reactants) and is also more endothermic. For the methyl thiolate reaction, the barrier for 1,3-dMT is 1 kcal mol^{-1} higher than that with 1,3-dMU. We also find computationally that for a wide range of nucleophiles (formate, acetate, *n*-pentylthiolate, methyl thiolate, and anilide), the exothermicities of the 1,3-dMU versus the 1,3-dMT reactions are always roughly 1.4 kcal mol^{-1} apart (with 1,3-dMU being more exothermic; data in Supporting Information).

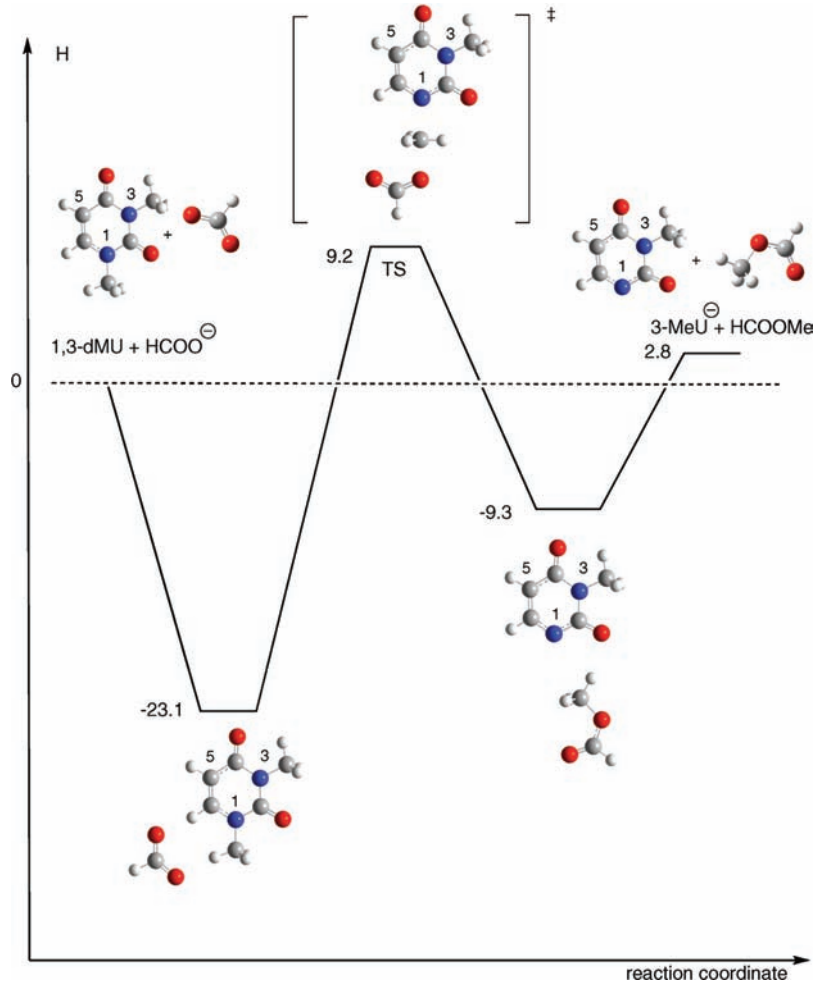

Figure 4. Calculated (B3LYP/6-31+G*) potential energy diagram of the reaction of 1,3-dMU (**1d**) with formate (ΔH, kcal mol^{-1}, 298 K).

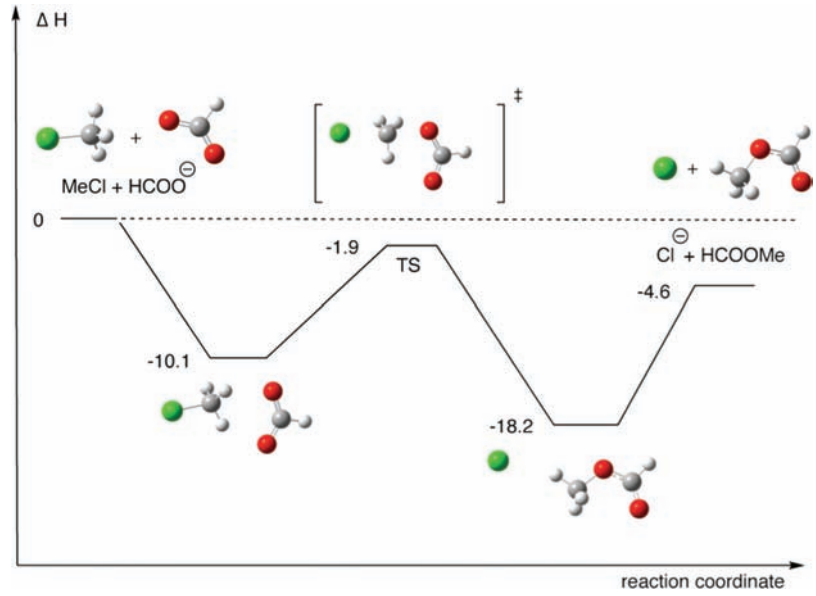

Figure 5. Calculated (B3LYP/6-31+G*) potential energy diagram of the reaction of methyl chloride with formate (ΔH, kcal mol^{-1}, 298 K).

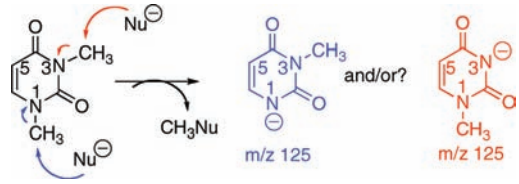

Figure 6. N1 versus N3 attack of 1,3-dimethyluracil.

d. Methyl Chloride versus Pyrimidine Derivatives Acidity Calculations Revisited. Experimentally, HCl and uracil have similar acidities. However, our calculations indicate that, although the N1-deprotonated uracil derivatives are still fairly good leaving groups (for example, the reaction of methyl thiolate, which is not that basic, with 1,3-dMU is exothermic by 23.4 kcal mol^{-1} and has a barrier of only 4.3 kcal mol^{-1} above the separated reactants), chloride is still better.

To assess whether this calculated difference in reactivity might be a computational artifact, we compared the computed acidities of 3-MeU and HCl. We know by experiment that the two have comparable acidities.[3,17] However, we find that the ΔH_{acid} of HCl calculates to 325.1 kcal mol^{-1} at B3LYP/6-31+G*, 7.7 kcal mol^{-1} more acidic than the calculated value for 3-methyluracil, and 8.3 kcal mol^{-1} more acidic than the known measured ΔH_{acid} of HCl (333.4 ± 0.1 kcal mol^{-1}). Essentially, although 3-methyluracil and HCl have the same experimental acidity, the B3LYP/6-31+G* calculations are not accurate: HCl appears to be more acidic. To assess whether the calculated differences in S_N2 reaction barriers for the methyl chloride and pyrimidine derivatives are due to a related computational artifact or truly reflect the reactivity, we conducted experiments, which are described later in this Article.

e. N1 versus N3 Attack. One possible complication for examining these reactions experimentally is a potential competition between N1−CH_3 attack (which we wish to see) and N3−CH_3 attack (which is not of biological interest; Figure 6).

We computationally examined nucleophilic attack at the N1−CH_3 versus N3−CH_3 for a series of nucleophiles (formate, acetate, *n*-pentyl thiolate, *n*-propyl thiolate, methyl thiolate, and anilide) and find that the N1−CH_3 S_N2 reaction is consistently on the order of 12 kcal mol^{-1} more exothermic than the N3−CH_3 path (Table 3).

Table 3. Calculated Enthalpies of S_N2 Reactions of 1,3-dMU (**1d**) with a Series of Nucleophiles: N1−Methyl versus N3−Methyl Group Attack

		ΔH_{rxn}, kcal mol^{-1}	
nucleophile	PA,[64] kcal mol^{-1}	N1 attack	N3 attack
$HCOO^-$	346.2	2.7	15.1
AcO^-	348.1	−6.3	6.0
n-$C_5H_{11}S^-$	352.5	−20.3	−7.9
n-PrS^-	354.2	−20.5	−8.2
CH_3S^-	357.6	−23.4	−11.1
$PhNH^-$	366.4	−30.5	−18.1

We also calculated the activation enthalpies ($\Delta H^{\ddagger}$) for the reactions with formate and methyl thiolate; for both reactions, the barrier for attack at the N1−methyl group is 10 kcal mol^{-1} lower than that at the N3−methyl. These differences in barrier are significant enough that, experimentally, S_N2 reaction at N1−CH_3 should be considerably favored over S_N2 reaction at N3−CH_3. These results are as expected, given that the N1−H is more than 10 kcal mol^{-1} acidic than the N3−H.[3,17]

S_N2 Reaction Studies: Experiments. The calculated barriers for the reaction of formate with 1,3-dMU versus methyl chloride indicate that the methyl chloride reaction should be faster (Figures 4 and 5). Furthermore, Table 1 indicates that a nucleophile with PA of 361.7 ($CF_3CH_2O^-$) reacts with methyl chloride with an efficiency of just 11%. We would therefore expect that the S_N2 reactions of nucleophiles whose PAs are less than the ΔH_{acid} of C6−H ($\Delta H_{acid} \approx 370$ kcal mol^{-1}) with 1,3-dMU and 1,3-dMT will all be relatively slow.

To establish that we can see S_N2 reactivity under our conditions, we repeated the known reactions of 2,2,2-trifluoroethoxide and methyl thiolate with methyl chloride. Our efficiency values for these S_N2 reactions are comparable to those obtained previously (for 2,2,2-trifluoroethoxide, 13.3% (current work) vs 11% (literature); for methyl thiolate, 8.0% (current work) vs 4.7% (literature)).[34,49,57]

The reactions of a series of nucleophiles with 1,3-dMU and 1,3-dMT were studied (Table 4). The S_N2 reaction product was

Table 4. S_N2 Reactions of 1,3-dMU (**1d**) and 1,3-dMT (**3d**)

PA[64] (nucleophile), kcal mol^{-1}	nucleophile, A^-	substrate (efficiency of S_N2 reaction, %)	
		1,3-dMU (**1d**)	1,3-dMT (**3d**)
364.1	$PhNEt^-$	0.38 ± 0.07	0.006 ± 0.003
361.7	$CF_3CH_2O^-$	0.84 ± 0.38	0.004 ± 0.001
359.5	$C_4H_4N^-$	0.35 ± 0.21	0.033 ± 0.019
357.6	CH_3S^-	0.23 ± 0.14	0.009 ± 0.003
354.2	*n*-PrS^-	0.22 ± 0.07	0.014 ± 0.009
353.4	*i*-PrS^-	0.23 ± 0.21	0.004 ± 0.001
352.5	*n*-$C_5H_{11}S^-$	0.28 ± 0.04	0.008 ± 0.006
348.1	AcO^-	0.11 ± 0.09	no S_N2 reaction
346.2	$HCOO^-$	0.08 ± 0.04	no S_N2 reaction
339.3	*m*-CF_3-PhO^-	0.04 ± 0.02	no S_N2 reaction

observed for the reaction of 1,3-dMU (**1d**) with nucleophiles ranging from *m*-CF_3PhO^- (PA = 339 kcal mol^{-1}) to HO^- (PA = 390 kcal mol^{-1}). However, when using nucleophiles with PA greater than about 365 kcal mol^{-1}, both proton transfer and S_N2 reactions were observed. Proton transfer is presumably the result of deprotonation of the most acidic (C6−H) site of 1,3-dMU (calculated ΔH_{acid} = 367.1 kcal mol^{-1}; experimental ΔH_{acid} = 369.9 ± 3.1 kcal mol^{-1}).[17,63] The S_N2 reaction efficiencies of the reactions of nucleophiles with PA less than 365 kcal mol^{-1} with 1,3-dMU are all fairly low (less than 1%) (Table 4). For nucleophiles with PA lower than 339 kcal mol^{-1}, the efficiencies were less than 0.01%.

We also examined the reactions of the same series of nucleophiles with 1,3-dMT (**3d**). For nucleophiles with PA higher than 365 kcal mol^{-1}, both S_N2 reactions and proton transfer were observed (as we saw with 1,3-dMU). S_N2 reaction products were observed for nucleophiles with PAs as low as ∼352 kcal mol^{-1}. Generally, the S_N2 reaction efficiencies for 1,3-dMT (**3d**) are lower than those efficiencies for 1,3-dMU (**1d**). Such small efficiencies are quite challenging to measure, and therefore the precision for the 1,3-dMT (**3d**) measurements varies from 0.001% to 0.02%. For the reaction of nucleophiles with PA smaller than 352.5 kcal mol^{-1} with 1,3-dMT (**3d**), no S_N2 reaction was observed (Table 4).

It therefore appears that the S_N2 reaction proceeds for both 1,3-dMU (**1d**) and 1,3-dMT (**3d**) and that the efficiencies observed for 1,3-dMT (**3d**) are lower than those for 1,3-dMU (**1d**).

Discussion

Acidity. The experimental measurements of acidity indicate that while 3-MeU and HCl have comparable acidities (around 333 kcal mol^{-1}), 3-MeT is slightly less acidic, by 2−5 kcal mol^{-1}. The B3LYP/6-31+G* calculations involving HCl are not quite in agreement with experiment. The computed (B3LYP/6-31+G*) acidity for HCl is 325.1, while that for 3-MeU is 332.8 kcal mol^{-1}. Thus, by calculation, the acidity difference is more than 7 kcal mol^{-1}, while by experiment that difference is much less. The calculations are likely to be in error, because the measured acidity of HCl is very well-known to be ΔH_{acid} = 333.4 ± 0.1 kcal mol^{-1}, and our previous bracketing studies show that reaction of HCl and 3-MeU (i.e., the conjugate base of one with the acid of the other and vice versa) proceeds in both directions.[17] To establish that the discrepancy is due to a computational artifact, we calculated the acidity of HCl and 3-MeU using the CBS-QB3 (complete basis set) model chemistry, which has been shown to accurately calculate thermochemical values.[70−74] Using this method, we find that the calculations and experimental data are in much better agreement: for HCl, ΔH_{acid} = 332.2 kcal mol^{-1} (calc) and 333.4 ± 0.1 kcal mol^{-1} (expt); for 3-MeU, ΔH_{acid} = 335.5 kcal mol^{-1} (calc) and 333 ± 2 kcal mol^{-1} (expt). Therefore, it does appear that the discrepancy between calculations and experiments is a computational issue. We therefore rely on our experimental data; the experimental acidity studies indicate that Cl^- and the conjugate base of 3-MeU might be comparable leaving groups, based on their acidities; 3-MeT would be a slightly worse leaving group.

S_N2 Reactions. Methyl Chloride versus Pyrimidines. The calculated energy diagrams for the reactions of formate and methyl thiolate with CH_3Cl and 1,3-dMU are superimposed in Figures 7 and 8. We are not indicating that the methyl chloride and dimethyluracil systems start with the exact same total energy; we plot them in such a way that the differences in reactivity are easier to see. We leave off 1,3-dMT to keep the diagrams uncluttered. In both the formate and the methyl thiolate reactions, the methyl chloride energetics are more favorable than the 1,3-dMU energetics (lower transition state energy and more exothermic). The relevant values for discussion are the differences in the transition state energies for reaction of a given nucleophile with 1,3-dMU versus methyl chloride (11.1 kcal mol^{-1} for formate (Figure 7); 11.8 kcal mol^{-1} for methyl thiolate (Figure 8)) and the differences in product energies for reaction of a given nucleophile with 1,3-dMU versus methyl chloride (7.3 kcal mol^{-1} for formate (Figure 7); 7.4 kcal mol^{-1} for methyl thiolate (Figure 8)). We know from our acidity calculations that the differences in the product energies are probably due to a computational artifact: HCl calculates to be 7.7 kcal mol^{-1} less acidic than 3-MeU even though by experiment they have comparable acidities (vide supra). This energy difference between the acidity values of HCl and 3-MeU corresponds to the ∼7 kcal mol^{-1} difference in product energies for the reactions in which chloride and deprotonated 3-MeU are the leaving groups (Figures 7 and 8). The transition state energies, however, show a larger difference for the 1,3-dMU versus methyl chloride reactions (11.1 kcal mol^{-1} for formate and 11.8 kcal mol^{-1} for methyl thiolate, favoring the CH_3Cl reaction). The accuracy of these S_N2 reaction transition state energies is certainly called into question based on the failure of B3LYP/6-31+G* to correctly predict the acidity of HCl. However, DFT methods have been shown to be reasonable for S_N2 reaction energetics in degenerate reactions involving chloride as the nucleophile and leaving group.[71,75−78] The consistent qualitative conclusion from Figures 7 and 8 is that the methyl chloride S_N2 reaction appears to have a lower barrier than that of 1,3-

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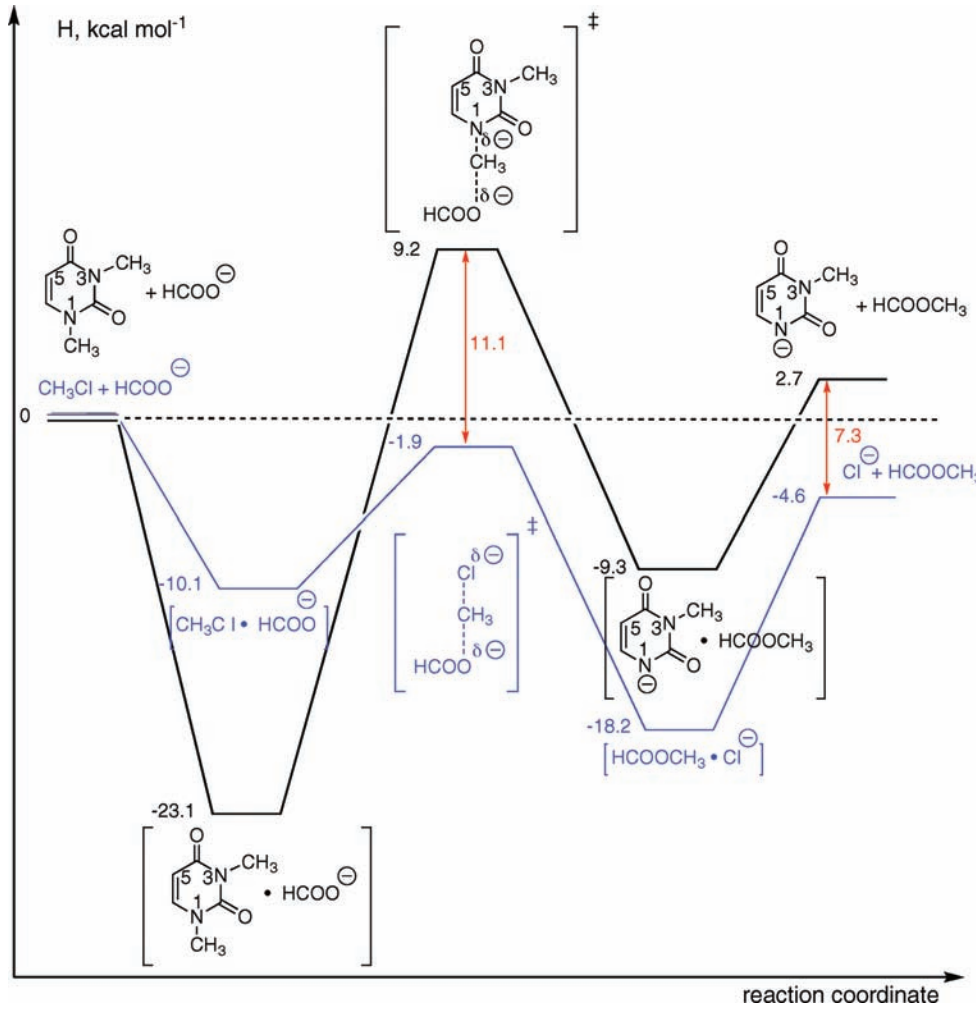

Figure 7. Superimposed energy diagrams for reactions of 1,3-dMU and methyl chloride with formate (B3LYP/6-31+G*, 298 K).

dMU. That is, although chloride and deprotonated 3-MeU have similar basicities, chloride is the better leaving group.

The experimental S_N2 reaction data qualitatively support the calculations (Tables 1 and 4). For example, for the reaction of 1,1,1-trifluoroethoxide with methyl chloride, the efficiency is 11% (literature) to 13% (our lab). With 1,3-dMU, the efficiency with the same nucleophile is less than 1% (a rate difference of about 15×, corresponding to about a 1.5 kcal mol^{-1} difference in barrier). Thus, both calculations and experiments do indicate that, although HCl and 3-MeU have comparable acidities in the gas phase, the conjugate bases do not appear to be equivalently good leaving groups. It appears that the calculations may overestimate that difference (11 kcal mol^{-1} by calculation versus about 2 kcal mol^{-1} by experiment). We understand that our efficiency values for the pyrimidine reactions are incredibly low, and it is in fact almost impossible to fully discount the possibility that the 1,3-dMU sample (and the 1,3-dMT sample) are not contaminated with some monomethyl substrate. Should, for example, 3-methyluracil be present in the 1,3-dMU sample, then deprotonation at N1−H of that contaminant 3-MeU would result in the same product ion expected from the S_N2 reaction. The 1,3-dMU purchased from Sigma Aldrich has a purity of 99%. We synthesized the 1,3-dMT sample from thymine. The compounds appear pure by NMR and also by our mass spectrometric studies of reaction with H_3O^+, where we see only the mass-to-charge ratio corresponding to protonated 1,3-dMU and 1,3-dMT, and do not see the *m*/*z* ratio corresponding to protonated monomethylated compound. We are therefore quite confident that what we see are very slow S_N2 reactions but cannot discount the possibility that deprotonation of a trace amount of monomethylated compound contributes to the observed product ions. However, if there is such a contaminant, that would mean that the contribution of the S_N2 reaction to the total reaction efficiency would be even lower than the reported values. Therefore, the reported efficiency values for the pyrimidine reactions are an upper limit. Our data indicate that the methyl chloride reactions are on the order of 15 times faster than the 1,3-dMU reactions; this is a lower limit in the sense that our S_N2 reaction efficiencies might be lower than reported.

The reason for this is probably related to the nature of the respective leaving groups: deprotonated uracil is a delocalized ion while chloride is not. In the neutral uracil, the N1 electrons can delocalize into the π system, but such delocalization separates charge, and therefore those resonance structures probably contribute less to the actual structure. Deprotonation results in a negative charge that can be stabilized by pushing that charge into the carbonyl oxygens (Figure 9). This is the same argument that, for example, could be used to explain why *N*-bromosuccinimide is an effective brominating agent.

Acidity is, of course, a thermodynamic property, while leaving group ability is essentially a kinetic property: how fast is the S_N2 reaction? In the S_N2 reaction transition state, because the N1-deprotonated uracil anion is not yet fully formed, the stability

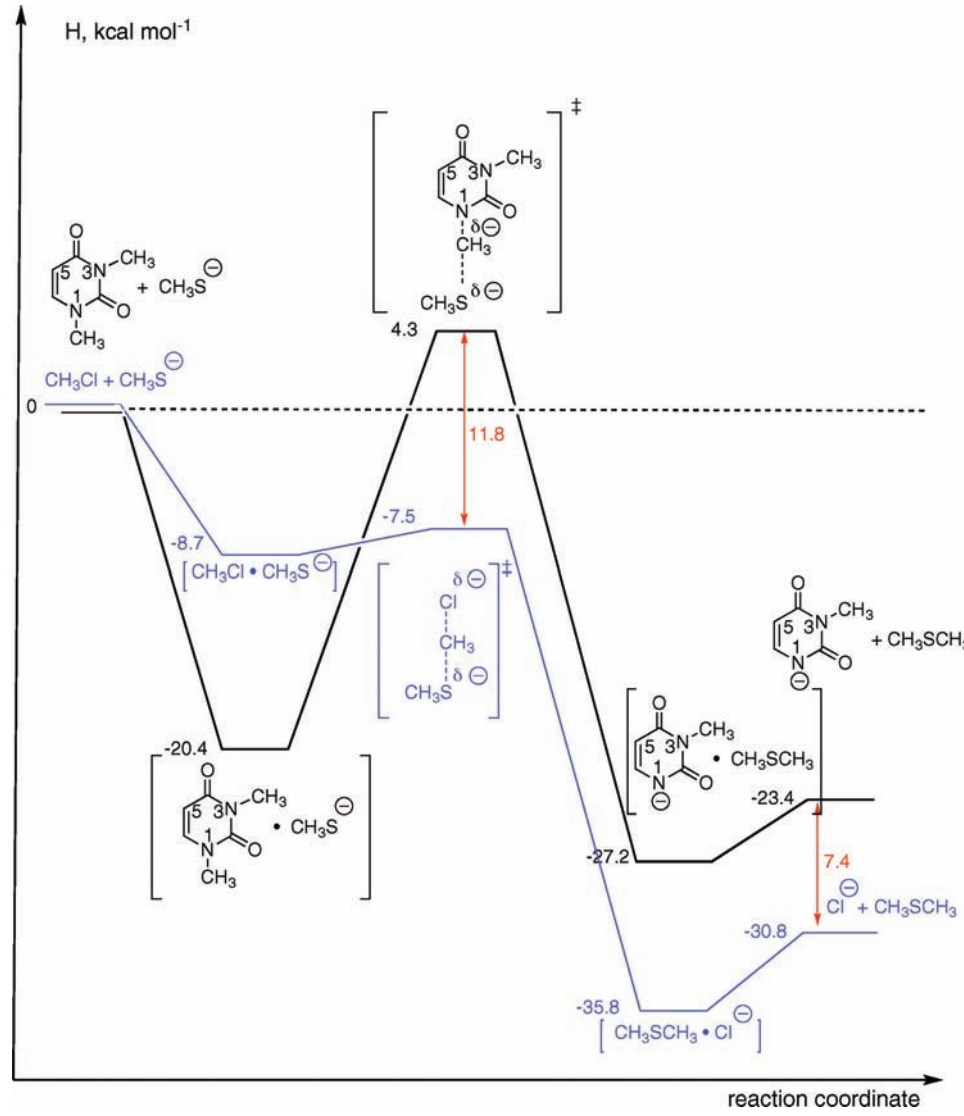

Figure 8. Superimposed PES for reactions of 1,3-dMU and methyl chloride with methyl thiolate (B3LYP/6-31+G*, 298 K).

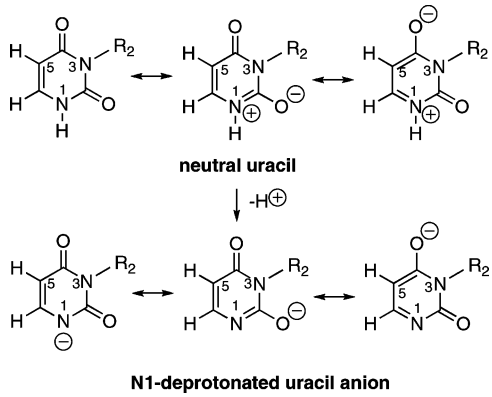

Figure 9. Resonance structures of neutral and N1-deprotonated uracil.

provided by electron delocalization in the product is not yet completely in place. Chloride, on the other hand, is a polarizable entity whose good leaving group properties are likely present in the transition state; little electron reorganization is required. In other words, the special stability enjoyed by the deprotonated uracil $N1^-$ anion, while making uracil as thermodynamically acidic as HCl, helps only partially in the leaving group ability: in the S_N2 reaction transition state, the N1 anion is not fully formed and therefore not fully delocalized.[79−81] (Presumably the carbonyl groups also stabilize the anion by induction, but one would expect that effect to be seen in both the acidity and the S_N2 reactivity.) In summary, delocalization in an anion may make its conjugate acid acidic, but may not affect leaving group ability as much as expected.[79−82]

We examined the bond lengths and charge distribution (CHELPG) for the participants in the S_N2 reaction of formate and methyl thiolate with 1,3-dMU to probe these ideas further. In Table 5, we list the distance for the breaking $N1-CH_3$ bond ($r1$), the distance for the forming $Nu-CH_3$ bond ($r2$), and the charges on the O2 and O4. For the reactant, negative charges of −0.560 and −0.579 reside on the O2 and O4, respectively. Those values increase (negatively) to −0.767 and −0.735 in the product. For the reaction with formate, the transition state is predictably a little late (based on the $r1$ length, relative to

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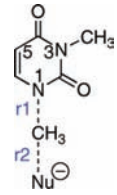
Table 5. Calculated (B3LYP/6-31+G*) Distances and Charges (CHELPG) for S_N2 Reactions of 1,3-dMU



substrate	*r*1 (N1−CH_3 distance) (Å)	*r*2 (CH_3−Nu distance) (Å)	charge, O2	charge, O4
1,3-dMU (reactant)	1.47	N/A	−0.560	−0.579
N1-deprotonated 3-MeU (product)	N/A	N/A	−0.767	−0.735
TS with Nu^- = formate	2.03	1.94	−0.670	−0.686
TS with Nu^- = methyl thiolate	1.94	2.52	−0.641	−0.683
$HCOOCH_3$ (product)	N/A	1.44	N/A	N/A
CH_3SCH_3 (product)	N/A	1.83	N/A	N/A

the methyl thiolate reaction); the Nu−CH_3 bond is more formed (1.94 Å), and the N1−CH_3 bond is quite elongated (2.03 Å). The charge distributions are more negative than in the reactant, but not nearly as negative as in the product (−0.670 and −0.686 for the O2 and O4, respectively). For the reaction with methyl thiolate, the transition state is earlier than that for formate, which would be expected because the reaction with methyl thiolate is more exothermic (methyl thiolate is a more basic nucleophile). The breaking N1−CH_3 bond is shorter (1.94 Å) than the forming Nu−CH_3 bond (2.52 Å). Consistent with the earlier TS, the oxygens are not as negative as they are in the formate transition state (−0.641 and −0.683 for methyl thiolate, versus −0.670 and −0.686 for the O2 and O4, respectively, for formate). There does seem to be an interesting paradox for reactions where the leaving group gains stability from delocalization: as the nucleophile becomes more basic, the reaction becomes more exothermic, which is consistent with a slightly lowered barrier (as we see with the formate reaction, which has a $\Delta H^{\ddagger}$ of 9.2 kcal mol^{-1} above the separated reactants versus that of methyl thiolate, which is only 4.3 kcal mol^{-1}).[83,84] However, as the reaction becomes more exothermic, the transition state may move earlier, where charge might be less delocalized, which would have an opposing effect on rate.

In summary, although uracil is as acidic as HCl, deprotonated uracil is not as good of a leaving group as chloride due to electron delocalization-related issues. This is not to say that deprotonated uracil is necessarily a terrible leaving group; it is clearly the species that leaves in the UDG reaction.[5,18,20,25]

In this study, we are also interested in comparing 1,3-dMU to 1,3-dMT. Because UDG cleaves uracil but not thymine, we wanted to probe whether there was an intrinsic reactivity component that would favor uracil cleavage. The calculations for the formate and methyl thiolate reactions with 1,3-dMU versus 1,3-dMT indicate that the barriers for the 1,3-dMU reactions are usually about 1 kcal mol^{-1} lower (relative to the separated reactants) than the reactions with 1,3-dMT. The measured reaction efficiencies for the uracil reactions are consistently higher than for the analogous thymine reactions (Table 4). The efficiency values are very small, but the 1,3-dMU reactions are always at least 10 times higher in efficiency than the corresponding 1,3-dMT reactions. Assuming that the efficiency results are not due in part to deprotonation reactions of a monomethylated contaminant, it does appear that the uracil reactions are faster than the thymine reactions, which is consistent with the calculations and the measured acidities (wherein 3-MeU is more acidic than 3-MeT, vide supra). Thus, intrinsically, deprotonated uracil is more easily cleavable than deprotonated thymine, which could be one factor (of many) aiding in the discrimination between the two by UDG in DNA.

Last, although we cannot be sure that nucleophilic attack occurs at N1 (rather than N3) of 1,3-dMU and 1,3-dMT, our calculations indicate that the difference in reactivity of those sites is so great (more than 10 kcal mol^{-1}, vide supra) that attack at N1 is likely. Future studies with appropriately deuterated substrates will be conducted.

Conclusions

We find that although the acidities of HCl and 3-methyluracil are comparable in the gas phase, the leaving group abilities of chloride and deprotonated 3-methyluracil are different, with chloride being slightly better. The basis of this difference lies in the fact that deprotonated 3-methyluracil is thermodynamically very acidic due to delocalization, which does not yield as large of a beneficial effect in the S_N2 reaction transition state.

Comparison of calculations to experiments indicates that the B3LYP/6-31+G* estimates the acidity of the pyrimidine derivatives well, but not that of HCl (the error is on the order of 7 kcal mol^{-1}, with the calculations yielding too low of a value). In terms of the S_N2 reactions, the calculations predict that those with methyl chloride would be favored over those of 3-methyluracil. The experiments also indicate that the methyl chloride reactions are faster than those of 1,3-dimethyluracil, with rates of at least 15−20 times more.

We also compared the leaving group ability of 1,3-dimethyluracil versus 1,3-dimethylthymine. Our calculations and experiments indicate that the N1-deprotonated uracil derivative is a slightly better leaving group than the N1-deprotonated thymine derivative, which is consistent with the cleavage of the former but not the latter from DNA.

Experimental Section

1,3-dMU (**1d**) and 3-MeT (**3b**) are commercially available and were used as received. 1,3-dMT (**3d**) was synthesized from thymine (**3a**). The procedure used is similar to that reported in the literature for 1,3-dMU synthesis.[85,86] The product was purified, and the identity was confirmed by ^1H and ^{13}C NMR.

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All nucleophiles except CH_3S^- were generated from commercially available neutral reference acids by deprotonation with hydroxide ions. CH_3S^- was generated via the elimination reaction of hydroxide plus dimethyldisulfide, which is a well-known source of the methyl thiolate anion:[87−89]

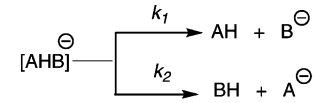
All S_N2 reaction experiments and 3-MeT acidity bracketing were conducted using a dual cell Fourier transform ion cyclotron resonance mass spectrometer (FTMS), which has been described previously.[3,35,39] The magnetic field is produced by a 3.3 T superconducting magnet. Gas-phase reactions occur in a 1 in. cubic cell located inside the instrument, which is pumped down to a baseline pressure 1×10^{-9} Torr. The solid nucleobases were introduced to the cell via the solids probe. Nucleophile anions were generated by deprotonation of commercially available reference acids with hydroxide ions. To introduce the reference acids into the cell, a leak valve or batch inlet was used. Hydroxide ions are produced via electron impact of water (typically 8 eV, 6 μA, 0.5 s).

For our acidity bracketing experiments, we utilized a protocol described previously.[3,17,35,38−40] Proton transfer reactions were conducted in both directions (deprotonated 3-MeT with neutral reference acids and the conjugate bases of neutral reference acids plus 3-MeT). The occurrence or nonoccurrence of proton transfer is denoted as a "+" or "−" in Table 2.

The reaction efficiency is the percentage ratio of collisions that lead to product. We report the ratio of observed rate constant to the estimated collisional rate constant calculated by parametrized trajectory theory.[61,62] Dipole moments were calculated at B3LYP/6-31+G*; polarizability was estimated using the method of Miller and Savchik.[90] Each kinetics experiment was run at least three times; reported values are the average and standard deviation.

The Cooks kinetics method[39,40,65−69] was used to conduct a relative acidity study of 3-MeT (**3b**) and 3-MeU (**1b**). We use a quadrupole ion trap (LCQ) mass spectrometer to conduct the experiments. The detailed protocol for Cooks kinetic experiments conducted in our lab has been described previously.[38,39] Briefly, the "relative" Cooks kinetic method we use herein involves formation of a proton-bound dimer of the two species of interest. The dimer is isolated and dissociated via CID. The ratio of intensities of the two deprotonated substrates yields the ratio of rate constants of the two possible dissociation pathways, which yields the relative acidities of the two substrates.

Proton-bound dimers were generated by electrospray (ESI) from the 1:1 mixture of 250 μM 3-MeT (**3b**) and 250 μM 3-MeU (**1b**)



where AH = 3-MeU, BH = 3-MeT

$$K \quad k_1/k_2$$

$$H_{acid1} - H_{acid2} \quad RT_{eff} \ln K$$

$$\ln (k_1/k_2) = (1/RT_{eff})(H_{acid1} - H_{acid2})$$

solutions in 20% methanol−water. A needle voltage of 4 kV, capillary temperature 150 °C, and a flow rate of about 25 μL/min were typically used. The proton-bound complex $[(3\text{-MeU})^- \cdot H^+ \cdot (3\text{-MeT})^-]$ was isolated and activated for about 30 ms. 40 scans were averaged for the product ions, and the experiment was repeated three times. A T_{eff} of 420 K, obtained from a calibration experiment with 3-MeT (**3b**), was used.

Calculations were conducted at B3LYP/6-31+G* using Gaussian 03;[91−94] the geometries were fully optimized and frequencies were calculated. This method has been shown to be reasonable for calculating S_N2 reaction potential energy surfaces.[71,75,77,78] All of the values reported are at 298 K. No scaling factor was applied. All calculated TS structures have one negative frequency. Partial charges were calculated using CHELPG as implemented in Gaussian 03.[95] As described in this Article, we also for some of the substrates utilized the CBS-QB3 model chemistry.[73,74]

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Supporting Information Available: Cartesian coordinates for all calculated species, additional ΔH and ΔG diagrams, other additional data as noted in the manuscript, and full citations for references with greater than 16 authors. This material is available free of charge via the Internet at http://pubs.acs.org.

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