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Gas-Phase Studies of Purine 3-Methyladenine DNA Glycosylase II (AlkA) Substrates

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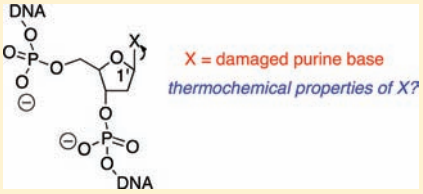
Supporting Information

ABSTRACT: 3-Methyladenine DNA glycosylase II (AlkA) is an enzyme that cleaves a wide range of damaged bases from DNA. The gas-phase thermochemical properties (tautomerism, acidity, and proton affinity) have been measured and calculated for a series of AlkA purine substrates (7-methyladenine, 7-methylguanine, 3-methyladenine, 3-methylguanine, purine, 6-chloropurine, xanthine) that have not been heretofore examined. The damaged nucleobases are found to be more acidic than the normal nucleobases adenine and guanine. Because of this increased acidity, the damaged bases would be expected to be more easily cleaved from DNA by AlkA (their conjugate bases should be better leaving groups). We find that the gas-phase acidity correlates to the AlkA excision rates, which lends support to an AlkA mechanism wherein the enzyme provides a nonspecific active site, and nucleobase cleavage is dependent on the intrinsic *N*-glycosidic bond stability.



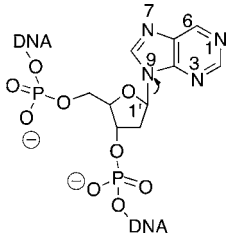
INTRODUCTION

Maintaining the integrity of DNA is essential for the health of living organisms. Unfortunately, DNA is constantly under assault; one of the most common modifications is alkylation, both by cellular metabolites as well as exogenous alkylating agents. Alkylation damage threatens proper cell function and compromises the correct propagation of the genetic code.[1,2] The base excision repair (BER) pathway is the primary means for excising damaged bases. In *Escherichia coli*, 3-methyladenine DNA glycosylase II (called AlkA, after the gene that encodes for it) is an enzyme that is up-regulated following exposure to DNA alkylating agents.[3−5] Because of its ability to cleave a wide range of substrates, AlkA is considered a particularly intriguing enzyme.[1,2] The other alkylation-specific enzyme found in *E. coli*, 3-methyladenine DNA glycosylase I (TAG), is quite specific, catalyzing the excision of only 3-alkyl-substituted adenine and guanine (but not other alkylated nucleobases).[6,7] In contrast, AlkA has a very broad substrate range, catalyzing the excision of various N3- and N7-alkyl purines, O2-alkyl pyrimidines, and other lesions that are not the product of alkylation, such as hypoxanthine, xanthine, and 1,N^6-ethenoadenine.[2,8−11]

Because AlkA cleaves such a diverse set of damaged bases, the active site is thought to be indiscriminate, with the reactivity of the *N*-glycosidic bond of a given substrate dictating the rate of AlkA-catalyzed excision.[12] Excision is believed to occur via an S_N1-type mechanism, where the nucleobase leaves first (Scheme 1).[2]

In prior work, we hypothesized that a related enzyme, alkyl adenine glycosylase (AAG), which catalyzes the excision of a wide range of damaged bases in mammalian cells, may provide a hydrophobic active site that aids in the discrimination of damaged from normal bases by enhancing the differences in their leaving group ability.[13−17] We hypothesize that AlkA may do the same.

Scheme 1



The examination of properties in the gas phase, which provides the "ultimate" nonpolar environment, reveals intrinsic reactivity that can be correlated to activity in other media, such as hydrophobic active sites.[13−16,18−20] In this paper, we calculate and measure the gas-phase acidities and proton affinities of a series of purine substrates (most of which have not been heretofore studied in vacuo) and discuss the results in the context of the AlkA mechanism.

EXPERIMENTAL SECTION

All the purine substrates and reference compounds are commercially available and were used as received.

Bracketing Method. Acidity and proton affinity (PA) bracketing measurements were conducted using a Fourier transform ion cyclotron resonance mass spectrometer (FTMS) with a dual cell setup, which has been described previously.[13,14,16,18,20−22] In our FTMS, two adjoining 2-in. cubic cells are positioned collinearly with the magnetic field produced by a 3.3 T superconducting magnet. The pressure of

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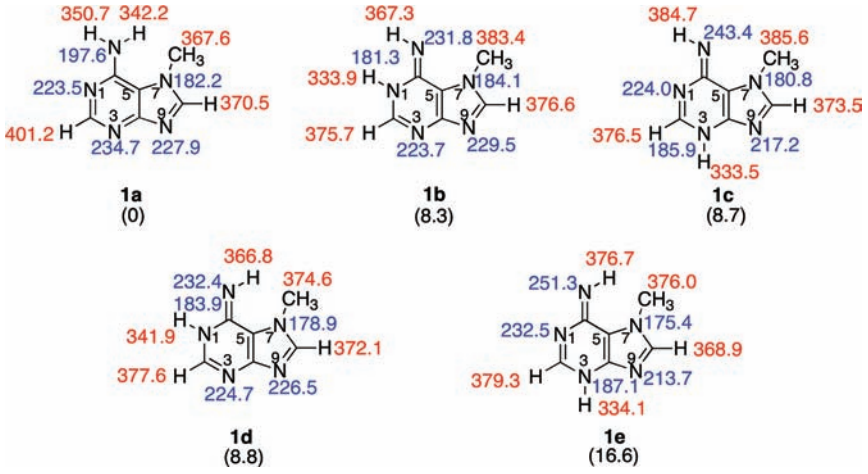

Figure 1. The five possible tautomeric structures of 7-methyladenine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

the dual cell is pumped down to less than 1×10^{-9} Torr. Solid purines are introduced into the cell via a heatable solids probe. Hydroxide or hydronium ions are generated from water pulsed into the cell and ionized by an electron beam [typically 8 eV (for OH^-) or 20 eV (for H_3O^+) and 6 μA, ionization time 0.5 s]. Liquid reference acids or bases are introduced via a batch inlet system or a leak valve and allowed to react with either hydroxide (for acidity measurement) or hydronium ions (for PA measurement).

The typical protocol for bracketing experiments has been described previously by us.[13,14,16,18,20−22] Briefly, ions are generated from a reference compound (acid or base) or the substrate whose acidity or PA is unknown (in our case, a nucleobase), selected, transferred to another adjoining cell via a 2-mm hole in the center of the central trapping plate, cooled by a pulse of argon (that raises the cell pressure to 10^{-5} Torr), and allowed to react with a neutral (either a reference compound or nucleobase). Proton transfer reactions are conducted in both directions. The occurrence of proton transfer is regarded as evidence that the reaction is exothermic ("+" in the tables).

We run bracketing reactions under pseudo-first-order conditions, where the amount of the neutral substrate is in excess relative to the reactant ions. Reading the pressure from an ion gauge is often unreliable, both because of the gauge's remote location as well as varying sensitivity for different substrates.[23,24] We therefore "back out" the neutral pressure from a control reaction (described previously).[13,14,20,22,25,26]

Cooks Kinetic Method. We also used the Cooks kinetic method in a quadrupole ion trap (LCQ) mass spectrometer[27−30] to measure the acidities and proton affinities of substituted purines.

The Cooks kinetic method involves the formation of a proton-bound complex, or dimer, of the unknown AH and a reference acid B_iH of known acidity (eq 1).

$$[AHB_i]^- \xrightarrow{k_1} [B_iH] + A^- \qquad [AHB_i]^- \xrightarrow{k_2} [AH] + B_i^- \qquad \text{(eq 1)}$$

The proton-bound dimer $[AHB_i]^-$ is dissociated via collision-induced dissociation (CID). The rate constants k_1 and k_2 are for the two different dissociation pathways. The relationship of these rate constants to ΔH_{acid} is shown in eq 2:

$$\ln(k_1/k_2) = (1/RT_{eff})(\Delta H_{BiH} - \Delta H_{AH}) \qquad \text{(eq 2)}$$

R is the gas constant and T_{eff} is the effective temperature[31] of the activated dimer.[27−30] The ratio of the intensities of the signals corresponding to the two deprotonated products yields the relative acidity of the two compounds of interest (eq 2), assuming the dissociation has no reverse activation energy barrier and that the dissociation transition state structure is late and therefore indicative of the stability of the two deprotonated products. These assumptions are generally true for proton-bound systems.[30,32,33] In order to obtain the acidity of compound AH, the natural logarithm of the relative intensity ratios is plotted versus the acidities for a series of reference acids, where the slope is $1/RT_{eff}$ and the y-intercept is $-\Delta H_{AH}/RT_{eff}$. The T_{eff} is obtained from the slope. The acidity of compound AH (ΔH_{AH}) is calculated from either eq 2 or the y-intercept.

The same procedure can be applied for PA measurements (via positively charged proton-bound dimers). The Cooks kinetic method data can be found in the Supporting Information.

The proton-bound complex ions are generated by electrospray (ESI) of 100−500 μM solutions of purine and reference acid (or base, for PA measurement). Methanol or water−methanol (20%) solutions are used as solvents.[34] Addition of formic acid is necessary to dissolve xanthine. An electrospray needle voltage of ~4 kV and the flow rate of 25 μL/min is applied. The proton-bound complex ions are isolated and then dissociated using collision-induced dissociation (CID); the complexes are activated for about 30 ms. Finally, the dissociation product ions are detected to give the ratio of the deprotonated (or protonated) analyte and deprotonated (or protonated) reference acid. A total of 40 scans are averaged for the product ions.

Calculations. Calculations are conducted at B3LYP/6-31+G(d),[35−37] M06-2X/6-311+G(2df,2p),[38,39] MP2(full)/6-31+G(d,p),[40−45] and CBS-QB3[46,47] levels using Gaussian 03[48] and Gaussian 09;[49] the geometries are fully optimized and frequencies are calculated. No scaling factor is applied. All the values reported are ΔH at 298 K. Dielectric medium calculations were done using the conductor-like polarizable continuum solvent model (CPCM, full optimization; UAKS cavity) at B3LYP/6-31+G(d) as implemented in Gaussian 03.[50−52] The "total free energy in solution" values are reported, and the solvation free energy of a proton in water or DMSO (−265.9 or −273.3 kcal mol^{-1} respectively) is accounted for.[53]

RESULTS

7-Methyladenine (7meA, 1). *Calculations: Tautomers, Acidity, and Proton Affinity.* In our experience DFT methods generally yield accurate values for thermochemical properties of nucleobases, so we utilized B3LYP/6-31+G(d) to calculate the relative tautomeric stabilities, acidities (ΔH_{acid}), and proton affinities (PA) of 7-methyladenine.[14,18,20−22] 7MeA has five possible tautomeric structures (Figure 1). The most stable tautomer (amino 7MeA **1a**) is over 8 kcal mol^{-1} more stable than the next most stable species. The most acidic site of **1a** is

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predicted to be the exocyclic NH_2 (ΔH_{acid} = 342.2 kcal mol^{-1}). The most basic site of tautomer **1a** is the N3 (PA = 234.7 kcal mol^{-1}).

Experiments: Acidity. We measured the acidity of 7-methyladenine using acidity bracketing (details in the Experimental Section). The conjugate base of 7-methyladenine deprotonates 2,4-pentanedione; the reaction in the opposite direction (the conjugate base of 2,4-pentanedione with 7-methyladenine) also occurs (Table 1). We therefore bracket the ΔH_{acid} of 7meA as 344 ± 3 kcal mol^{-1}.

Table 1. Acidity Bracketing of 7-Methyladenine (1)

		proton transfer[b]	
ref compd	ΔH_{acid}[a]	ref acid	conj base
4-(trifluoromethyl)aniline	353.3 ± 2.1	−	+
m-cresol	349.6 ± 2.1	−	+
acetic acid	347.4 ± 0.5	−	+
butyric acid	346.8 ± 2.0	−	+
2,4-pentanedione	343.8 ± 2.1	+	+
methyl cyanoacetate	340.8 ± 0.6	+	−
α,α,α-trifluoro-*m*-cresol	339.2 ± 2.1	+	−
2-chloropropanoic acid	337.0 ± 2.1	+	−

[a] ΔH_{acid} is in kcal mol^{-1}.[54,55] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

We also measured the acidity of 7meA using the Cooks kinetic method. Seven reference acids were used: butyric acid (ΔH_{acid} = 346.8 ± 2.0 kcal mol^{-1}), valeric acid (ΔH_{acid} = 346.2 ± 2.1 kcal mol^{-1}), isovaleric acid (ΔH_{acid} = 345.5 ± 2.1 kcal mol^{-1}), pivalic acid (ΔH_{acid} = 344.6 ± 2.1 kcal mol^{-1}), methacrylic acid (ΔH_{acid} = 344.1 ± 2.9 kcal mol^{-1}), 4-aminobenzoic acid (ΔH_{acid} = 343.4 ± 2.1 kcal mol^{-1}), and methoxyacetic acid (ΔH_{acid} = 341.9 ± 2.1 kcal mol^{-1}). A ΔH_{acid} of 344 ± 3 kcal mol^{-1} was yielded.

Experiments: Proton Affinity. In bracketing the PA of 7meA, we find that di-*sec*-butylamine (PA = 234.4 ± 2.0 kcal mol^{-1}) deprotonates protonated 7-methyladenine; the opposite reaction (7-methyladenine deprotonating protonated di-*sec*-butylamine) also occurs (Table 2). We therefore bracket the PA of 7meA to be 234 ± 3 kcal mol^{-1}.

Table 2. Proton Affinity Bracketing of 7-Methyladenine (1)

		proton transfer[b]	
ref compd	PA[a]	ref base	conj acid
2,2,6,6-tetramethylpiperidine	235.9 ± 2.0	+	−
N,N-dimethylcyclohexylamine	235.1 ± 2.0	+	−
triethylamine	234.7 ± 2.0	+	−
di-*sec*-butylamine	234.4 ± 2.0	+	+
1-methylpiperidine	232.1 ± 2.0	−	+
1-methylpyrrolidine	230.8 ± 2.0	−	+
pyrrolidine	226.6 ± 2.0	−	+

[a] PA is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

Using the Cooks kinetic method with five reference bases [2,2,6,6-tetramethylpiperidine (PA = 235.9 ± 2.0 kcal mol^{-1}), *N,N*-dimethylcyclohexylamine (PA = 235.1 ± 2.0 kcal mol^{-1}), triethylamine (PA = 234.7 ± 2.0 kcal mol^{-1}), 1-methylpiperidine (PA = 232.1 ± 2.0 kcal mol^{-1}), and *N,N*-dimethylbenzylamine (PA = 231.5 ± 2.0 kcal mol^{-1})], we measure a PA of 234 ± 3 kcal mol^{-1}.

7-Methylguanine (7meG, 2). *Calculations: Tautomers, Acidity, and Proton Affinity.* The B3LYP/6-31+G(d)-calculated properties of 7-methylguanine (**2**) are shown in Figure 2. There are 10 possible tautomers for 7-methylguanine; the six lowest (all below 15 kcal mol^{-1} relative to the most stable tautomer) are shown (the remaining tautomers are in the Supporting Information). The most stable form is the keto-amino **2a**; for this tautomer, the most acidic site is the N1−H (ΔH_{acid} = 335.6 kcal mol^{-1}) and the most basic site is the N9 (PA = 231.4 kcal mol^{-1}).

Experiments: Acidity. We measured the acidity of 7-methylguanine using the bracketing method. The reaction of 2-chloropropionic acid (ΔH_{acid} = 337.0 ± 2.1 kcal mol^{-1}) and deprotonated 7meG proceeds, as does the reaction in the opposite direction (2-chloropropionate with 7meG), allowing us to bracket the ΔH_{acid} to be 337 ± 3 kcal mol^{-1} (Table 3).

Table 3. Acidity Bracketing of 7-Methylguanine (2)

		proton transfer[b]	
ref compd	ΔH_{acid}[a]	ref acid	conj base
2,4-pentanedione	343.8 ± 2.1	−	+
methyl cyanoacetate	340.8 ± 0.6	−	+
α,α,α-trifluoro-*m*-cresol	339.2 ± 2.1	−	+
2-chloropropionic acid	337.0 ± 2.1	+	+
malononitrile	335.8 ± 2.1	+	−
pyruvic acid	335.5 ± 2.9	+	−
difluoroacetic acid	331.0 ± 2.2	+	−

[a] ΔH_{acid} is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

Five reference acids were used in the Cooks kinetic method measurement of 7meG acidity: 3-chloropropionic acid (ΔH_{acid} = 340.8 ± 2.7 kcal mol^{-1}), 2-chloropropionic acid (ΔH_{acid} = 337.0 ± 2.1 kcal mol^{-1}), 4-hydroxybenzoic acid (ΔH_{acid} = 335.9 ± 2.1 kcal mol^{-1}), 2-chlorobenzoic acid (ΔH_{acid} = 335.1 ± 2.1 kcal mol^{-1}), and pyruvic acid (ΔH_{acid} = 333.5 ± 2.9 kcal mol^{-1}). The ΔH_{acid} was found to be 337 ± 3 kcal mol^{-1}.

Experiments: Proton Affinity. We also bracketed the PA of 7meG (Table 4). Protonated 1-methylpyrrolidine reacts with 7meG; likewise, protonated 7meG reacts with 1-methylpyrrolidine, placing the PA at 231 ± 3 kcal mol^{-1}.

For the Cooks PA measurement, six reference bases were used: triethylamine (PA = 234.7 ± 2.0 kcal mol^{-1}), di-*sec*-butylamine (PA = 234.4 ± 2.0 kcal mol^{-1}), 1-methylpiperidine (PA = 232.1 ± 2.0 kcal mol^{-1}), *N,N*-dimethylisopropylamine

Table 4. Proton Affinity Bracketing of 7-Methylguanine (2)

		proton transfer[b]	
ref compd	PA[a]	ref base	conj acid
2,2,6,6-tetramethylpiperidine	235.9 ± 2.0	+	−
di-*sec*-butylamine	234.4 ± 2.0	+	−
1-methylpiperidine	232.1 ± 2.0	+	−
1-methylpyrrolidine	230.8 ± 2.0	+	+
piperidine	228.0 ± 2.0	−	+
4-picoline	226.4 ± 2.0	−	+
3-picoline	225.5 ± 2.0	−	+

[a] PA is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

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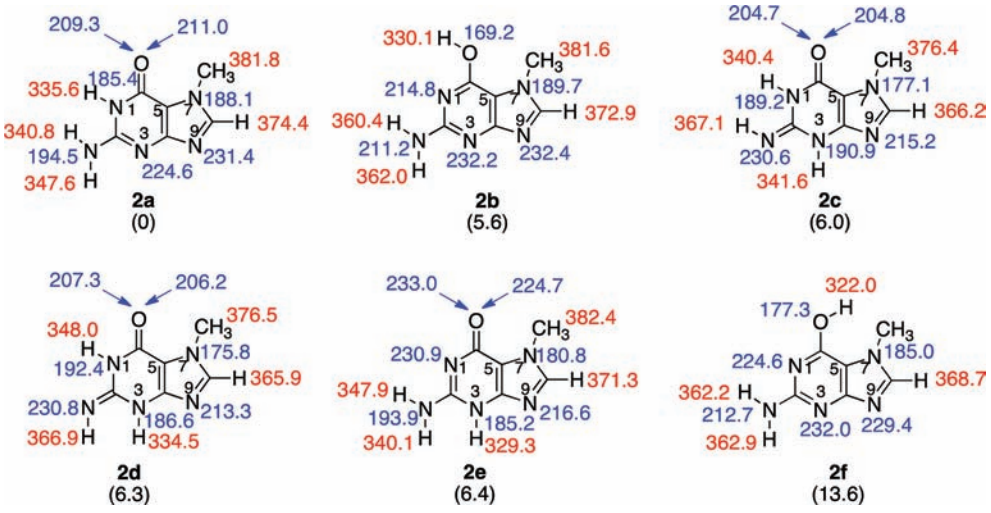

Figure 2. Six of the 10 possible tautomeric structures of 7-methylguanine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

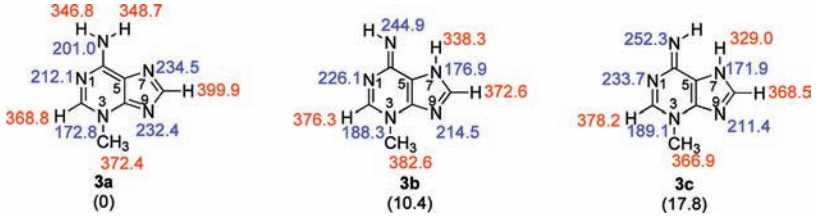

Figure 3. Tautomeric structures of 3-methyladenine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

(PA = 232.0 ± 2.0 kcal mol^{-1}), *N,N*-dimethylbenzylamine (PA = 231.5 ± 2.0 kcal mol^{-1}), and *N*-methylpiperidine (PA = 230.8 ± 2.0 kcal mol^{-1}). A PA of 232 ± 3 kcal mol^{-1} was yielded.

3-Methyladenine (3meA, 3). *Calculations: Tautomers, Acidity, and Proton Affinity.* We previously calculated the acidity and the relative stabilities of the possible tautomers of 3-methyladenine; these data plus new calculations of PA are shown in Figure 3.[16] 3-Methyladenine has five possible tautomers; the three lowest are shown. The most stable is the one with the exocyclic amino group (**3a**), for which the calculated acidity is 346.8 kcal mol^{-1} (for the proton on the amino group). The most basic site has a PA of 234.5 kcal mol^{-1}, at the N7.

Experiments: Acidity and Proton Affinity. The acidity of 3-methyladenine was previously measured by us to be 347 ± 4 kcal mol^{-1}.[16] We bracket the PA of 3meA herein (Table 5). Di-*sec*-butylamine (PA = 234.4 ± 2.0 kcal mol^{-1}) can deprotonate protonated 3-methyladenine, but 1-methylpiperidine (232.1 ± 2.0 kcal mol^{-1}) cannot. In the reverse direction, 3-methyladenine deprotonates protonated 1-methylpiperidine but not protonated di-*sec*-butylamine. We therefore bracket the PA of 3-methyladenine to be 233 ± 3 kcal mol^{-1}.

Table 5. Proton Affinity Bracketing of 3-Methyladenine (3)

ref compd	PA[a]	proton transfer[b] ref base	proton transfer[b] conj acid
2,2,6,6-tetramethylpiperidine	235.9 ± 2.0	+	–
triethylamine	234.7 ± 2.0	+	–
di-*sec*-butylamine	234.4 ± 2.0	+	–
1-methylpiperidine	232.1 ± 2.0	–	+
2,4-lutidine	230.1 ± 2.0	–	+
3-picoline	225.5 ± 2.0	–	+

[a]PA is in kcal mol^{-1}.[54] [b]A "+" indicates the occurrence of proton transfer and a "–" indicates the absence of it.

3-Methylguanine (3MeG, 4). *Calculations: Tautomers, Acidity, and Proton Affinity.* 3-Methylguanine has 14 possible tautomers; the seven structures within 15 kcal mol^{-1} of the most stable form are shown, along with calculated acidities and proton affinities, in Figure 4. The most acidic site of the most stable tautomer **4a** is the N7–H, with a calculated ΔH_{acid} of 328.6 kcal mol^{-1}. The most basic site is on the imino NH (PA = 231.8 kcal mol^{-1}).[56]

Purine. *Calculations: Tautomers, Acidity, and Proton Affinity.* Purine is a known substrate for AlkA and is also of interest as it is the most fundamental structure for the species studied herein (thus its name!). There are four possible purine tautomers, of which the most stable is the canonical structure **5a** (Figure 5). The acidity of this tautomer is calculated to be 329.8 kcal mol^{-1} (at the N9–H); the PA is 219.2 kcal mol^{-1} (at N1).

Experiments: Acidity. We measured the acidity of purine using the bracketing method (Table 6). Deprotonated purine can deprotonate perfluoro-*tert*-butanol (ΔH_{acid} = 331.6 ± 2.2 kcal mol^{-1}) but not pyruvic acid (ΔH_{acid} = 333.5 ± 2.9 kcal mol^{-1}). Pyruvate deprotonates purine but perfluoro-*tert*-butanoxide does not. We therefore bracket the acidity of the more acidic site of purine to be ΔH_{acid} = 333 ± 4 kcal mol^{-1}.

The acidity was also measured using the Cooks kinetic method. Five reference acids were used: 3-(trifluoromethyl)-benzoic acid (ΔH_{acid} = 332.2 ± 2.1 kcal mol^{-1}), 2-nitrobenzoic

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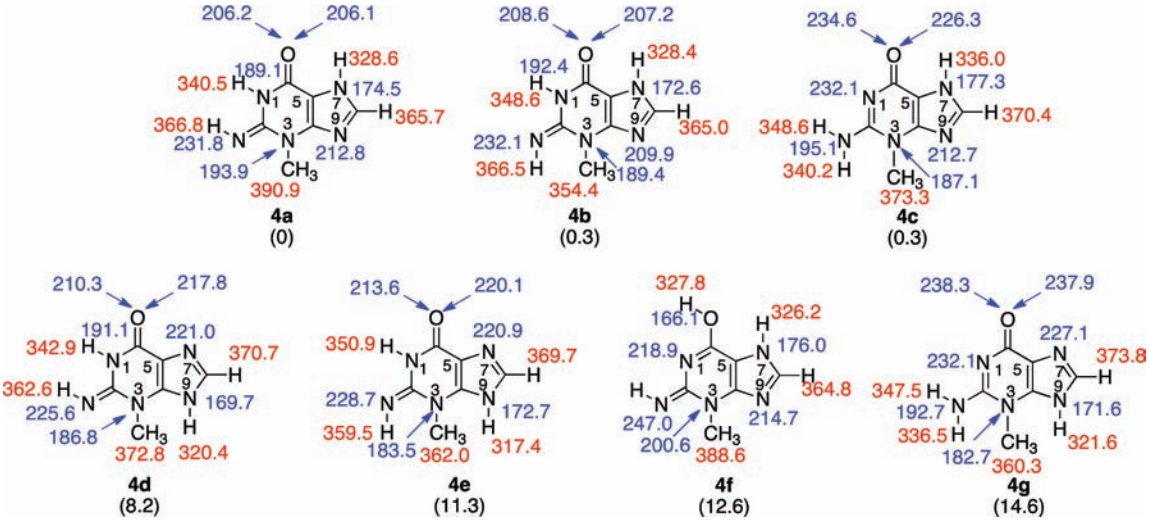

Figure 4. Tautomeric structures of 3-methylguanine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

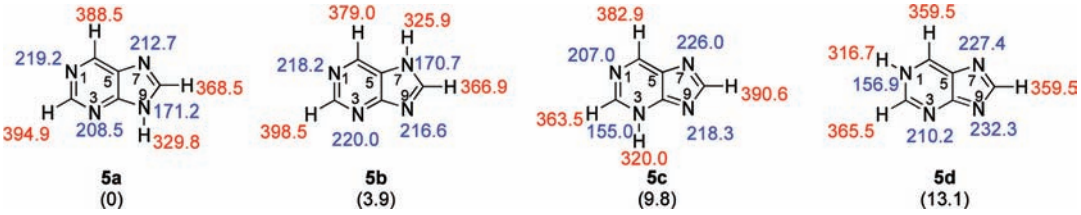

Figure 5. Tautomeric structures of purine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

Table 6. Acidity Bracketing of Purine (5)

ref compd	ΔH_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conj base
2,4-pentanedione	343.8 ± 2.1	−	+
α,α,α-trifluoro-*m*-cresol	339.2 ± 2.1	−	+
pyruvic acid	333.5 ± 2.9	−	+
perfluoro-*tert*-butanol	331.6 ± 2.2	+	−
difluoroacetic acid	331.0 ± 2.2	+	−
3,5-bis(trifluoromethyl) phenol	329.7 ± 2.1	+	−
1,1,1-trifluoro-2,4-pentanedione	328.3 ± 2.9	+	−

[a] ΔH_{acid} is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

Table 7. Proton Affinity Bracketing of Purine (5)

ref compd	PA[a]	proton transfer[b] ref base	proton transfer[b] conj acid
3-picoline	225.5 ± 2.0	+	−
pyridine	222.0 ± 2.0	+	−
N-ethylaniline	221.0 ± 2.0	+	−
n-butylamine	220.2 ± 2.0	+	+
N-methylpropionamide	220.0 ± 2.0	−	+
propylamine	219.4 ± 2.0	−	+
benzylamine	218.3 ± 2.0	−	+
dimethylacetamide	217.0 ± 2.0	−	+
m-toluidine	214.1 ± 2.0	−	+

[a] PA is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

acid (ΔH_{acid} = 331.7 ± 2.2 kcal mol^{-1}), perfluoro-*tert*-butanol (ΔH_{acid} = 331.6 ± 2.2 kcal mol^{-1}), difluoroacetic acid (ΔH_{acid} = 331.0 ± 2.2 kcal mol^{-1}), and 3,5-bis(trifluoromethyl)phenol (ΔH_{acid} = 329.7 ± 2.1 kcal mol^{-1}). An acidity of 332 ± 3 kcal mol^{-1} was yielded.

Experiments: Proton Affinity. The PA of purine was bracketed as shown in Table 7. For the reaction of protonated purine and *n*-butylamine (PA = 220.2 ± 2.0 kcal mol^{-1}) and that of protonated *n*-butylamine and purine, proton transfer is observed in both directions, yielding a bracketed PA of 220 ± 3 kcal mol^{-1}. This value is consistent with a previous equilibrium measurement.[57]

For the Cooks PA measurement of purine, nine reference bases were used: cyclohexylamine (PA = 223.3 ± 2.0 kcal mol^{-1}), ethanolamine (PA = 222.3 ± 2.0 kcal mol^{-1}), octylamine (PA = 222.0 ± 2.0 kcal mol^{-1}), isobutylamine (PA = 221.0 ± 2.0 kcal mol^{-1}), L-phenylalanine (PA = 220.6 ± 2.0 kcal mol^{-1}), *n*-butylamine (PA = 220.2 ± 2.0 kcal mol^{-1}), propylamine (PA = 219.4 ± 2.0 kcal mol^{-1}), benzylamine (PA = 218.3 ± 2.0 kcal mol^{-1}), and dimethylacetamide (PA = 217.0 ± 2.0 kcal mol^{-1}). A PA of 221 ± 3 kcal mol^{-1} was obtained.

6-Chloropurine. *Calculations: Tautomers, Acidity, and Proton Affinity.* 6-Chloropurine has not been experimentally tested as an AlkA substrate. We chose to study this as a potential substrate, based on the assumption that the chloride group would render the compound quite acidic at N9 (more in the Discussion). 6-Chloropurine has four possible tautomeric structures, of which the N9−H canonical is calculated to be most stable (Figure 6). The most acidic site of the canonical tautomer has a calculated ΔH_{acid} of 322.8 kcal mol^{-1} (N9−H); the most basic site, at N1, has a PA of 212.5 kcal mol^{-1}.

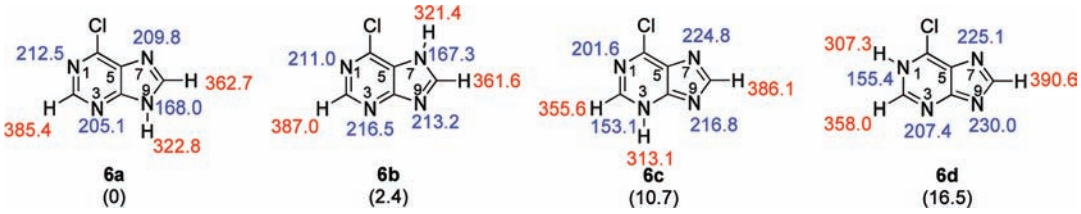

Figure 6. Tautomeric structures of 6-chloropurine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

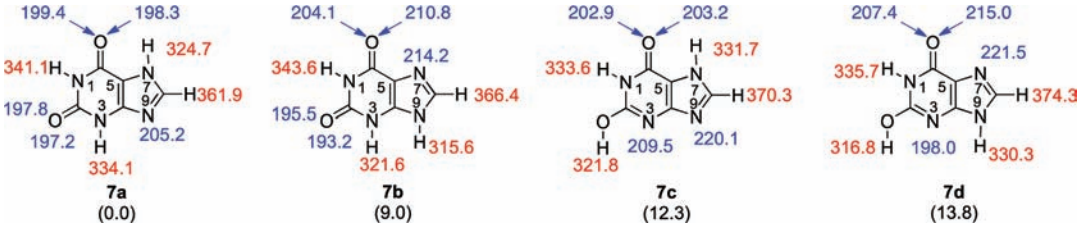

Figure 7. Tautomeric structures of xanthine. Gas-phase acidities are in red; gas-phase proton affinities are in blue. Relative stabilities are in parentheses. Calculations were conducted at B3LYP/6-31+G(d); reported values are ΔH at 298 K.

Experiments: Acidity. 6-Chloropurine is predicted to be quite acidic (calculated ΔH_{acid} of 322.8 kcal mol^{-1}). Because reference acids in this range are limited, we bracketed an upper limit for the ΔH_{acid} of 6-chloropurine (Table 8). The most acidic reference acid we used was 1,1,1-trifluoro-2,4-pentanedione (ΔH_{acid} = 328.3 ± 2.9 kcal mol^{-1}). Deprotonated 6-chloropurine is unable to deprotonate 1,1,1-trifluoro-2,4-pentanedione and acids with higher ΔH_{acid} values (Table 8). Likewise, deprotonated 1,1,1-trifluoro-2,4-pentanedione and stronger bases can deprotonate 6-chloropurine, so we bracket an upper limit of the ΔH_{acid} of 328.3 kcal mol^{-1}.[58]

Table 8. Acidity Bracketing of 6-Chloropurine (6)

ref compd	ΔH_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conj base
2,4-pentanedione	343.8 ± 2.1	−	+
perfluoro-*tert*-butanol	331.6 ± 2.2	−	+
3,5-bis(trifluoromethyl) phenol	329.8 ± 2.1	−	+
1,1,1-trifluoro-2,4-pentanedione	328.3 ± 2.9	−	+

[a] ΔH_{acid} is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

Experiments: Proton Affinity. The bracketing of the PA of 6-chloropurine is shown in Table 9. For the reaction of protonated 6-chloropurine with both methylamine and *m*-toluidine, proton transfer is observed; the reaction occurs in the opposite direction as well, yielding a 6-chloropurine bracketed PA of 214 ± 3 kcal mol^{-1}.[58]

Table 9. Proton Affinity Bracketing of 6-Chloropurine (6)

ref compd	PA[a]	proton transfer[b] ref base	proton transfer[b] conj acid
pyridine	222.0 ± 2.0	+	−
propylamine	219.4 ± 2.0	+	−
dimethylacetamide	217.0 ± 2.0	+	−
3-chloropyridine	215.9 ± 2.0	+	−
methylamine	214.9 ± 2.0	+	+
m-toluidine	214.1 ± 2.0	+	+
N-methylacetamide	212.4 ± 2.0	−	+
aniline	210.9 ± 2.0	−	+
2,4-pentanedione	208.8 ± 2.0	−	+

[a] PA is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

Xanthine. *Calculations: Tautomers, Acidity, and Proton Affinity.* Of the 33 possible tautomers of xanthine, there are four below 15 kcal mol^{-1} (Figure 7; all tautomers are shown in Supporting Information). The N7H structure **7a** is the most stable, with an acidity of 324.7 kcal mol^{-1} (N7−H) and a PA of 205.2 kcal mol^{-1} (at N9).

Experiments: Acidity. As with 6-chloropurine, xanthine has a very low predicted acidity value (324.7 kcal mol^{-1}). We ascertain an upper limit of 328.3 kcal mol^{-1} for xanthine acidity (Table 10). We also observe that bromide [ΔH_{acid}(HBr) = 323.5 ± 0.1 kcal mol^{-1}] is unable to deprotonate xanthine, which establishes a lower limit for xanthine acidity.

Table 10. Acidity Bracketing of Xanthine (7)

ref compd	ΔH_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conj base
perfluoro-*tert*-butanol	331.6 ± 2.2	−	+
difluoroacetic acid	331.0 ± 2.2	−	+
3,5-bis(trifluoromethyl) phenol	329.8 ± 2.1	−	+
1,1,1-trifluoro-2,4-pentanedione	328.3 ± 2.9	−	+
hydrobromic acid	323.5 ± 0.1	N/A	−

[a] ΔH_{acid} is in kcal mol^{-1}.[54] [b] A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.[59]

For the Cooks acidity measurement, 10 reference acids were used [L-asparagine (ΔH_{acid} = 331.6 ± 3.1 kcal mol^{-1}), L-histidine (ΔH_{acid} = 328.6 ± 1.9 kcal/mol), difluoroacetic acid (ΔH_{acid} = 331.0 ± 2.2 kcal mol^{-1}), dichloroacetic acid (ΔH_{acid} = 328.4 ± 2.1 kcal mol^{-1}), *o*-hydroxybenzoic acid (ΔH_{acid} = 325.5 ± 2.2 kcal mol^{-1}), 3,5-bis(trifluoromethyl)pyrazole (ΔH_{acid} = 324.6 ± 2.1 kcal mol^{-1}), 3,5-bis(trifluoromethyl)-benzoic acid (ΔH_{acid} = 324.4 ± 2.1 kcal mol^{-1}), trifluoroacetic acid (ΔH_{acid} = 323.8 ± 2.9 kcal mol^{-1}), hydrobromic acid (ΔH_{acid} = 323.5 ± 0.1 kcal mol^{-1}), and heptafluorobutyric acid (ΔH_{acid} = 321.9 ± 2.2 kcal mol^{-1})], yielding an acidity (ΔH_{acid}) of 327 ± 3 kcal mol^{-1}.

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Experiments: Proton Affinity. In bracketing the PA of xanthine, we find that reaction with 4′-*tert*-butylacetophenone (PA = 210.9 ± 2.0 kcal mol^{-1}) proceeds in both directions, placing the PA at 211 ± 3 kcal mol^{-1} (Table 11).

Table 11. Proton Affinity Bracketing of Xanthine (7)

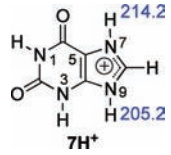
		proton transfer[b]	
ref compd	PA[a]	ref base	conj acid
m-toluidine	214.1 ± 2.0	+	−
o-toluidine	212.9 ± 2.0	+	−
DMSO	211.4 ± 2.0	+	−
4′-*tert*-butylacetophenone	210.9 ± 2.0	+	+
2,4-pentanedione	208.8 ± 2.0	−	+
m-chloroaniline	207.5 ± 2.0	−	+
ethyl sulfide	204.8 ± 2.0	−	+
4-methylcyclohexanone	201.9 ± 2.0	−	+
3-pentanone	200.0 ± 2.0	−	+
acetone	194.0 ± 2.0	−	+

[a]PA is in kcal mol^{-1}.[54] [b]A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

For the Cooks PA measurement, seven reference bases were used: pyrazole (PA = 213.7 ± 2.0 kcal mol^{-1}), benzamide (PA = 213.2 kcal mol^{-1}), *o*-toluidine (PA = 212.9 ± 2.0 kcal mol^{-1}), glycine (PA = 211.9 ± 2.0 kcal mol^{-1}), DMSO (PA = 211.4 ± 2.0 kcal mol^{-1}), aniline (PA = 210.9 ± 2.0 kcal mol^{-1}), and thymine (PA = 210.5 ± 2.0 kcal mol^{-1}). A PA of 212 ± 3 kcal mol^{-1} was obtained.

DISCUSSION

Calculated versus Experimental Values. The calculated acidity and proton affinity values for all the substrates studied herein are summarized in Table 12. Generally, B3LYP/6-31+G(d) appears to provide fairly accurate predictions for the thermochemical values.[60] The one instance where the calculated and experimental data are quite disparate is for the PA of xanthine: the calculated value is 205.2 kcal mol^{-1}, yet we measure a PA of 211 kcal mol^{-1} (by bracketing). This is a fairly significant discrepancy. One possibility is that we have a mixture of the two most stable xanthine tautomers (**7a** and **7b**), and the more basic **7b** (calculated PA of 214.2 kcal mol^{-1}) influences the experimentally observed value. However, it seems unlikely that the neutral xanthine would be a mixture, as the predicted difference in stability of **7a** versus **7b** is 9 kcal mol^{-1} (Figure 7). Because DFT methods and non-DFT methods have been known to yield different relative enthalpies for nucleobase tautomers, we also calculated the relative enthalpies of **7a** and **7b** using CBS-QB3 and MP2(full)/6-31+G(d,p).[61−63] We find that the enthalpy differences are comparable to the B3LYP value (8.7 kcal mol^{-1} for CBS-QB3; 8.6 kcal mol^{-1} for the MP2 calculation).[20,22,61−68] IR−UV double resonance spectroscopy experiments in the gas phase also indicate the dominance of **7a**.[69] Furthermore, when examining the reaction of protonated xanthine with neutral reference bases, the protonated xanthine will always have the structure **7H**$^+$, regardless of the starting neutral structure. That is, both **7a** and **7b** when protonated will have the structure **7H**$^+$. If the calculations are correct, one would expect bases with PAs greater than 205 kcal mol^{-1} to deprotonate **7H**$^+$, but we only see proton transfer with reference bases whose PAs are greater than 211 kcal mol^{-1} (Table 11).



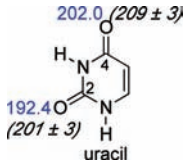
7H$^+$

In terms of the thermochemical properties of nucleobases, a discrepancy of this magnitude between predicted [B3LYP/6-31+G(d)] and measured values was also observed by us nearly a decade ago, when we examined the pyrimidine nucleobase uracil (Figure 8).[70] The B3LYP/6-31+G(d)-calculated value for the PA of the most basic site O4 is 202.0 kcal mol^{-1}. However, the measured value is 209 ± 3 kcal mol^{-1}. We were also able to measure the less basic site; the calculated value is 192.4 kcal mol^{-1}, but the measured value is 201 ± 3 kcal mol^{-1}. Thus, as with xanthine, the measured proton affinities are significantly higher than the calculated values. The difference between the O2 and O4 proton affinities are comparable between experiment (8 kcal mol^{-1}) and calculation (10 kcal mol^{-1}), but the absolute values differ (Figure 8). These data, coupled with the present xanthine results, lead us to believe that for these substrates, the PA calculations may not be accurate.[71]

Table 12. Calculated (B3LYP/6-31+G(d); 298 K) and Experimental Data for Damaged Bases

substrate	calcd value	exptl value[b]
	ΔH_{acid}[a]	
7-methyladenine (**1**)	342.2	344 (344)
7-methylguanine (**2**)	335.6	337 (337)
3-methyladenine (**3**)	346.8	347
3-methylguanine (**4**)	328.6	N/A
purine (**5**)	329.8	333 (332)
6-chloropurine (**6**)	322.8	<328
xanthine (**7**)	324.7	323−328 (327)
	PA[a]	
7-methyladenine (**1**)	234.7	234 (234)
7-methylguanine (**2**)	231.4	231 (232)
3-methyladenine (**3**)	234.5	233
3-methylguanine (**4**)	231.8	N/A
purine (**5**)	219.2	220 (221)
6-chloropurine (**6**)	212.5	214
xanthine (**7**)	205.2	211 (212)

[a]ΔH_{acid} and PA values are in kcal mol^{-1}. [b]The first listed experimental value is bracketed; Cooks kinetic method value, if available, is in parentheses. Error is ±3−4 kcal mol^{-1}.



uracil

Figure 8. B3LYP/6-31+G(d)-calculated proton affinity values for uracil (in blue); values in parentheses are experimental (bracketing). All values are ΔH in kcal mol^{-1} at 298 K.

In an attempt to find a method/level that would accurately calculate the PA of xanthine (and uracil), we tried M06-2X/6-311+G(2df,2p), CBS-QB3, and MP2(full)/6-31+G(d,p) (Table 13).[38,39,46,47,72−74] As can be seen from Table 13, neither the DFT nor non-DFT methods correctly predict the

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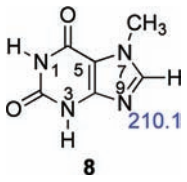
Table 13. Calculated and Experimental Values for the N9 Proton Affinity of Xanthine Tautomer 7a and O2 and O4 Proton Affinities of Uracil

	PA[a]		
method/level	xanthine **7a** N9	uracil O4	uracil O2
B3LYP/6-31+G(d)	205.2	202.0	192.4
M06-2X/6-311+G(2df,2p)	205.6	204.7	196.2
CBS-QB3	206.8	204.4	196.8
MP2(full)/6-31+G(d,p)	206.4	203.2	195.3
experimental value	211−212	209	201

[a]All PA values are ΔH in kcal mol^{-1} at 298 K.

PAs of xanthine and uracil. We are unsure why the PAs of all the other nucleobases are calculated with accuracy but xanthine and uracil are not. The only similarity we see when comparing the N9 site of xanthine **7a** with the O2 and O4 sites of uracil is that all the protonation sites are β to an N−H. This is not true for any of the other measured substrates. What is not clear is why this would cause the calculated PA to be lower than the measured PA. These results, however, do point to the need to be cautious in trusting calculated values, even for series (as in this case, nucleobases) that appear to be similar in structure. In fact, we also calculated the PA for several of the other damaged nucleobases at CBS-QB3 and found that the values are comparable to those calculated at B3LYP/6-31+G(d) (data in Supporting Information). Therefore, neither DFT nor non-DFT methods are entirely reliable for the computation of PAs for these substrates, thus highlighting the importance of experiments.

As a final test of our hypothesis that the PA calculations are poor for xanthine, we studied the methylated derivative 7-methylxanthine (**8**). With the parent xanthine, one might always have the concern that the discrepancy between the calculated and measured PA could result from the presence of more than one tautomer. 7-Methyxanthine is "locked" into a structure analogous to **7a**; the structure analogous to **7b** cannot be accessed. The PA value of 7-methylxanthine calculated at B3LYP/6-31+G(d) is 210.1 kcal mol^{-1} (Figure 9). If our



8

Figure 9. B3LYP/6-31+G(d)-calculated proton affinity value for 7-methylxanthine (ΔH in kcal mol^{-1} at 298 K).

hypothesis that xanthine and uracil have a structural component that renders their PAs difficult to predict computationally is correct, the measured PA of 7-methylxanthine (**8**) will be higher than the computational value.

Our results for the bracketing of 7-methylxanthine are shown in Table 14. Proton transfer occurs in both directions with 3-fluoropyridine (PA = 215.6 ± 2.0 kcal mol^{-1}) and 2-chloropyridine (PA = 215.3 ± 2.0 kcal mol^{-1}), yielding a PA for 7-methylxanthine of 215 ± 3 kcal mol^{-1}. As with the unsubstituted xanthine, the measured value is higher than the calculated value: for 7-methylxanthine, the measured PA is 5 kcal mol^{-1} higher than the B3LYP/6-31+G(d) value (for the parent xanthine the difference is 6 kcal mol^{-1}). Therefore, we

Table 14. Proton Affinity Bracketing of 7-Methylxanthine (8)

		proton transfer[b]	
ref compd	PA[a]	ref base	conj acid
n-butylamine	220.2 ± 2.0	+	−
N-methylaniline	219.1 ± 2.0	+	−
3-bromopyridine	217.5 ± 2.0	+	−
3-fluoropyridine	215.6 ± 2.0	+	+
2-chloropyridine	215.3 ± 2.0	+	+
m-toluidine	214.1 ± 2.0	−	+
DMSO	211.4 ± 2.0	−	+
aniline	210.9 ± 2.0	−	+
pyrrole	209.2 ± 2.0	−	+

[a]PA is in kcal mol^{-1}.[54] [b]A "+" indicates the occurrence of proton transfer and a "−" indicates the absence of it.

conclude that, for xanthine PA, the measured value of 211−212 kcal mol^{-1} is correct. It simply appears that the PA of xanthine cannot be easily calculated with accuracy.

Biological Implications. AlkA is a glycosylase with a particularly broad substrate range, cleaving a wide variety of damaged bases from double-stranded DNA.[10−12,75−85] The exact mechanism by which AlkA cleaves damaged bases with greater efficiency than the normal bases adenine and guanine is unknown. The main hypothesis is that cleavage is related to the intrinsic stability of the *N*-glycosidic bond and that the enzyme merely provides a non-base-specific active site.[1,2,12,81] Therefore, the better a leaving group of the nucleobase is, the more easily it is cleaved. Since acidity and leaving group ability are generally correlated, we would expect the damaged bases to be more acidic than the normal bases.

We further postulate, based on our previous studies of other glycosylases, that AlkA may provide a nonpolar active site that serves to *enhance* the differences in acidity between damaged and normal nucleobases and, in doing so, aids in the discrimination of normal from damaged bases.[13,14,16−18] Thus, not only do we expect the damaged bases to be more acidic than the normal bases, but those differences in acidity should be significantly greater in the gas phase (and in a nonpolar active site of an enzyme) than in aqueous solution.

We first sought to compare the acidities of damaged and normal substrates of AlkA to ascertain whether the damaged bases are more acidic. If rate of excision is based on the intrinsic stability of the *N*-glycosidic bond, then the acidity of the N9 position is relevant (Figure 10). (Note that the biologically relevant structure is not always the most stable structure in the gas phase.) The substrates studied herein, as well as other known substrates of AlkA that we have previously studied (hypoxanthine, 1,N^6-ethenoadenine, adenine, guanine), are shown.[13,14,16,20]

One interesting feature of AlkA is that it cleaves both positively charged and neutral nucleobases. For example, when adenine and guanine are alkylated at N7 to form 7meA and 7meG, the result is a positively charged nucleobase (**1aH**$^+$ and **2aH**$^+$ in Figure 10). Cleavage of that positively charged nucleobase results in a neutral nucleobase leaving group (path A in Scheme 2, where Scheme 2 shows possible cleavage paths for 3meA). Therefore, the relevant acidity to correlate to leaving group ability is the N9−H acidity value for the positively charged substrates, as shown for **1aH**$^+$, **2aH**$^+$, **3aH**$^+$, and positively charged 3MeG in Figure 10. Those values are in blue because they are equivalent to the PA values at those

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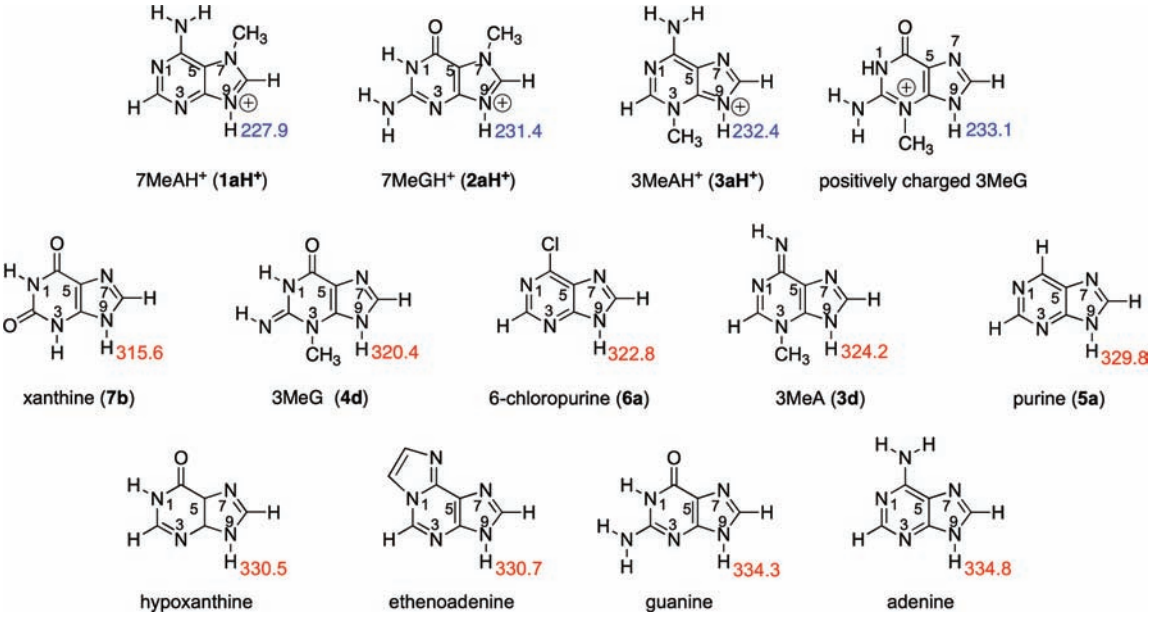
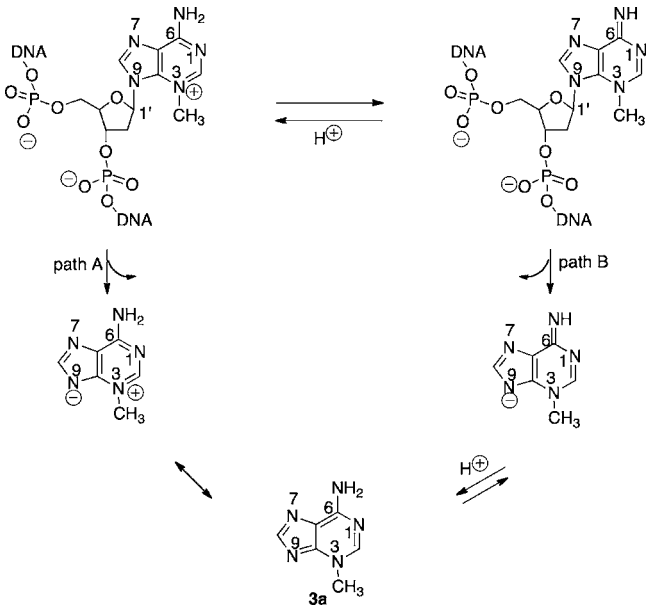


Figure 10. Gas-phase acidity [ΔH_{298K}, calculated, B3LYP/6-31+G(d), in kcal mol^{-1}] of biologically relevant structures.[13,14,16,20]

Scheme 2



positions for the corresponding neutral substrates (**1a**, **2a**, **3a**, and 3MeG).

Other nucleobases, such as xanthine (**7**), 6-chloropurine (**6**), purine (**5**), hypoxanthine, ethenoadenine (eA), guanine, and adenine (Figure 10) are neutral substrates for AlkA. In these cases, the leaving group ability would be related to the acidity of the neutral nucleobase at N9−H (values shown in red, Figure 10).[17,86,87]

3MeA (**3**) and 3meG (**4**) are intriguing because cleavage could either occur from the positively charged form (path A, Scheme 2) or from the neutral form (path B, Scheme 2). That is, cleavage of the nucleobase could occur via path A to yield the neutral nucleobase, or a proton could be lost prior to cleavage, such that the deprotonated nucleobase is the leaving group (path B). Therefore, these two substrates appear twice in Figure 10, in both positively charged and neutral forms.

The substrates in Figure 10 are arranged in order of *decreasing* acidity. The substrate with the lowest acidity value [amino 7meAH$^+$ (**1aH**$^+$)] is expected to be the best leaving group. The question is, do these relative acidities correlate to known AlkA experimental excision rates? Known data for the rate constants by which AlkA cleaves various nucleobases are compiled in Table 15.[88] The nucleobases are listed in the order

Table 15. Rate Constants for Excision of Various Nucleobases by AlkA Compared to Gas-Phase Acidity

substrate	k_{st} (min^{-1})^{a,b}	ΔH_{acid} (kcal mol^{-1})^c
7meGH$^+$ (**2H**$^+$)	300	231.4
3meAH$^+$(**3H**$^+$)/3meA (**3**)	0.5	232.4/324.2
ethenoadenine	7.5×10^{-2}	330.7^d
purine (**5**)	5.4×10^{-2}	329.8
hypoxanthine	2.9×10^{-2}	330.5^e
guanine	6.9×10^{-3}	334.3^f
adenine	5.2×10^{-3}	334.8^g

^{a}Ref 12. $^b k_{st}$ is single turnover rate constant with saturating AlkA. ^{c}Calculated ΔH_{acid} values at 298 K [B3LYP/6-31+G(d)]. ^{d}Ref 13. ^{e}Ref 14. ^{f}Ref 20. ^{g}Refs 16, 20.

of *decreasing* rate constants. The data show a rough correlation between the rate constant for excision and the calculated ΔH_{acid} value. 7MeGH$^+$ is cleaved the most quickly, and the acidity value is the lowest (most acidic substrate). The next most easily cleaved nucleobase is 3meAH+ and then 3meA. Thus, whether 3-methyladenine is excised as a neutral (path A, Scheme 2) or in the anionic deprotonated form (path B, Scheme 2), the acidity value correlates to the leaving group ability in a qualitative sense; that is, both acidity values are higher than that for 7meG but less than that for eA (the next most easily cleaved nucleobase). For the next three nucleobases (ethenoadenine, purine, hypoxanthine), the gas-phase acidities are similar (around 329−330 kcal mol^{-1}) and the rate constants for cleavage are in the same ballpark (10^{-2} min^{-1}).[88] For the normal nucleobases guanine and adenine, as expected for natural nucleobases, cleavage is slowest; the attendant acidities are also the highest in value (so least acidic).

Table 16. Calculated N9−H Acidity Values in the Gas Phase (ε = 1), DMSO (ε = 48), and Water (ε = 78) and Experimental pK_a Values (in Water) for AlkA Substrates (Structures Shown in Figure 10)[a,b,c]

	acidity (kcal mol^{-1})[a,b]			
substrate	ε = 1	ε = 48	ε = 78	pK_a[c]
xanthine (**7b**)	315.6	16.6	24.5	7.3[h]
6-chloropurine (**6a**)	322.8 (7.2)	18.6 (2.0)	26.3 (1.8)	7.7−7.8[i] (0.5)
purine (**5a**)	329.8 (14.2)	21.0 (4.4)	28.2 (3.7)	8.9[j] (2.2)
hypoxanthine	330.5[d] (14.9)	21.7 (5.1)	28.2 (3.7)	8.9[k] (2.2)
ethenoadenine	330.7[e] (15.1)	22.7 (6.1)	29.7 (5.2)	9.9[e] (3.5)
guanine	334.3[f] (18.7)	24.7 (8.1)	30.2 (5.7)	10.0[l] (3.7)
adenine	334.8[g] (19.2)	24.3 (7.7)	30.4 (5.9)	9.8[m] (3.4)

[a]The values in parentheses are the differences between a substrate's acidity and that of xanthine, in kcal mol^{-1}. [b]Calculated using B3LYP/6-31+G(d). [c]pK_a values in water are all experimental, except for xanthine, which is calculated. [d]Ref 14. [e]Ref 13. [f]Ref 20. [g]Refs 16, 20. [h]Ref 89. [i]Refs 90, 91. [j]Refs 90−92. [k]Refs 91, 93. [l]Ref 94. [m]Refs 91, 95.

Our results showing correlation between excision rate constants and N9−H acidity values lend support to the argument that cleavage of the damaged base is dependent on the intrinsic stability of the *N*-glycosidic bond.

On the basis of the correlation we see herein, we would also predict that 6-chloropurine (as well as other halo-substituted purines) should (based on its high acidity) be easily cleaved by AlkA.

We also further postulate that the active site, by providing a hydrophobic environment, will aid in the discrimination of normal from damaged bases by *enhancing* the *relative* leaving group ability of the damaged bases. To examine that hypothesis, we compare the gas- and solution-phase N9−H acidities for those damaged bases whose pK_a values are known (Table 16). In an effort to "draw a line" from solution to the gas phase, we conducted dielectric medium calculations on the acidities in DMSO (ε = 48) and water (ε = 78) as well, to ascertain how acidities change with medium dielectric. The most acidic substrate, regardless of medium, is the damaged base xanthine, which has a gas-phase ΔH_{acid} of 315.6 kcal mol^{-1} and a pK_a of 7.3. The least acidic substrate in the gas phase is the normal base adenine, with a gas-phase acidity of 334.8. In the gas phase, xanthine is more acidic than adenine by 19 kcal mol^{-1}. In a dielectric of 48, that difference drops to 7.7; in a dielectric of water, it is even smaller (5.9 kcal mol^{-1}). When adenine and xanthine are actually fully solvated in water (experimental pK_a column), that acidity difference is only 3.4 kcal mol^{-1}. Overall, in comparing the pK_as and ΔH_{acid} values of the damaged bases (xanthine, 6-chloropurine, purine, hypoxanthine, ethenoadenine) versus the normal bases (adenine and guanine), the same trend is seen: the difference in acidity of the damaged versus normal bases is greatest in the gas phase and least in water. In fact, the solution phase pK_a values are so close that adenine is actually more acidic than its damaged counterpart, ethenoadenine. The intrinsically higher acidity of ethenoadenine is only evident in the gas-phase values. The nonpolar active site in AlkA could thus contribute to specificity by enhancing the differences in acidity among adenine, guanine, and damaged bases.

CONCLUSIONS

We have calculated the energies of the tautomers and calculated and measured the acidic and basic properties for a series of purines not heretofore studied. The results indicate that the damaged purines are all more acidic than the normal nucleobases adenine and guanine and would therefore be expected to be more easily cleaved (that is, their conjugate bases are better leaving groups). Furthermore, the gas-phase acidity trends track with the AlkA excision rates (Table 15). This is consistent with the proposal that AlkA provides a nonspecific active site and that the ease of nucleobase excision depends on the intrinsic stability of the *N*-glycosidic bond.

Our data also support our hypothesis that AlkA provides a hydrophobic site that enhances the discrimination of damaged from normal bases; in a nonpolar environment, the damaged bases are acidic by a greater amount over the normal bases than in aqueous solution.

We have also shown that accurate calculation of the thermochemical properties of several similar substrates by a given method and level does not guarantee reliable predictive power. In our case, although the acidity and proton affinity of many damaged purine bases (7-methyladenine, 3-methyladenine, 7-methylguanine, 3-methylguanine, purine, 6-chloropurine) are calculated accurately using both DFT and non-DFT methods, the proton affinity of xanthine is not. This highlights the importance of gas-phase experiments to benchmark computations.

Future studies will be directed toward designing substrates such as 6-chloropurine (**6**) that would be expected to be good AlkA substrates.

ASSOCIATED CONTENT

Supporting Information

Cartesian coordinates and energies for all calculated species (including higher energy tautomers), additional calculational data, and complete refs 48 and 49. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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