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Acidity of Adenine and Adenine Derivatives and Biological Implications. A Computational and Experimental Gas-Phase Study

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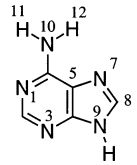
The gas-phase acidities of adenine, 9-ethyladenine, and 3-methyladenine have been investigated for the first time, using computational and experimental methods to provide an understanding of the intrinsic reactivity of adenine. Adenine is found to have two acidic sites, with the N9 site being 19 kcal mol^{-1} more acidic than the N10 site; the bracketed acidities are 333 ± 2 and 352 ± 4 kcal mol^{-1}, respectively. Because measurement of the less acidic site can be problematic, we benchmarked the adenine N10 measurement by bracketing the acidity of 9-ethyladenine, which has the N9 site blocked and allows for exclusive measurement of the N10 site. The acidity of 9-ethyladenine brackets to 352 ± 4 kcal mol^{-1}, comparable to that of the N10 site of the parent adenine. Calculations and experiments with 3-methyladenine, a harmful mutagenic nucleobase, uncovered the surprising result that the most commonly written tautomer of 3-methyladenine is not the most stable in the gas phase. We have found that the most stable tautomer is the "N10 tautomer" **10**, as opposed to the imine tautomer **3**. The bracketed acidity of **10** is 347 ± 4 kcal mol^{-1}. Since **10** is not a viable species in DNA, **3** is a likely tautomer; calculations indicate that this form has an extremely high acidity (320−323 kcal mol^{-1}). The biological implications of these results, particularly with respect to enzymes that cleave alkylated bases from DNA, are discussed.

Introduction

Accurate measurements of the acidities and basicities of nucleic bases and nucleic base derivatives are essential for understanding issues of fundamental importance in biological systems. Hydrogen bonding modulates recognition of DNA and RNA bases, and the interaction energy between two bonded complementary nucleobases is dependent on the intrinsic basicity and acidity of the acceptor and donor groups.[1,2] In addition, understanding the intrinsic reactivity of nucleic bases can shed light on key biosynthetic mechanisms for which nucleobases are substrates.[3−8]

The gas phase is a valuable environment in which to examine the properties and reactivity of biological molecules. Biological media, from intracellular environs to the interior of proteins, are seldom aqueous in nature. It has been shown that the interior of proteins is often nonpolar, causing shifts in acidity and basicity and changes in reactivity as compared to behavior in aqueous solution.[9−11] The gas phase is the "ultimate" nonpolar environment and therefore allows one to establish intrinsic reactivity in the absence of solvent, and extrapolate the effects of media.[9,12−14] In essence, gas-phase experiments can provide the link between calculations, which are most accurately conducted in the gas phase, and experimental data in other media.

Recently, our studies of nucleobases have focused on the purine base adenine (**1**). While the proton affinities



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of the most basic sites of the major nucleobases, deoxyribonucleosides, and deoxyribonucleotides have been

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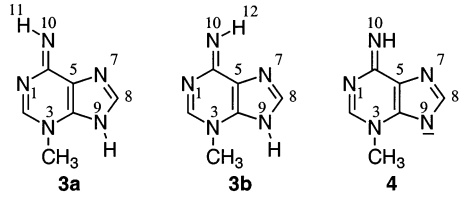
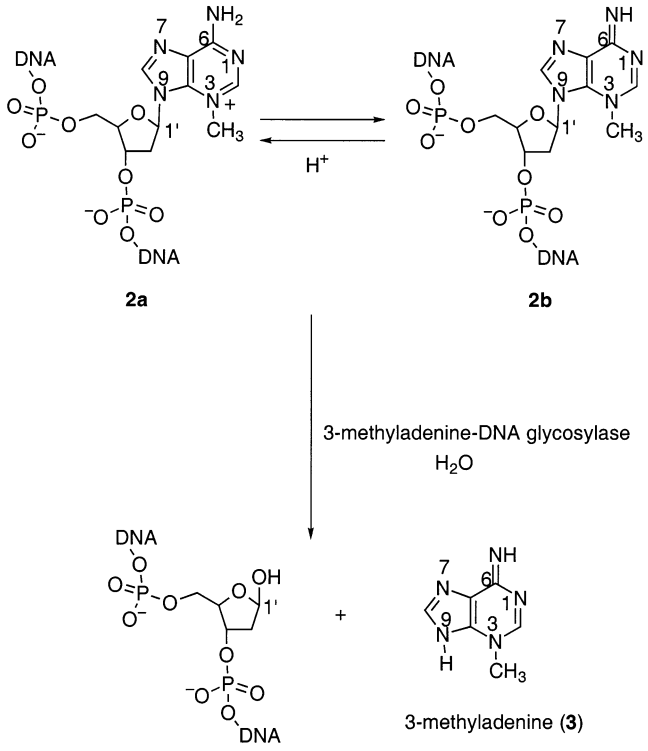
measured, gas-phase acidities of the nucleobases are largely unknown.[15−18] Our studies have also been motivated by our interest in a family of enzymes for which nucleobases are substrates, the glycosylases, which are responsible for the excision of mutated bases from DNA.[19−28] Adenine can be alkylated by cancer chemotherapeutics as well as environmental mutagens, and 3-methyladenine is the most common mutation.[26] 3-Methyladenine DNA glycosylase is an unusually *non*specific enzyme that will cleave a wide range of damaged bases, including hypoxanthine, 7-methylguanine, and 3-methyl- and 1,N^6-ethenoadenine from DNA (Scheme 1).[29,30] The proposed mechanism for excision of alkylated bases from DNA by human 3-methyladenine glycosylase involves nucleophilic attack at C1′ by some form of activated water (Scheme 1).[20,22] This prompts the question of how good of a leaving group 3-methyladenine is. It is not known whether the protonated substrate **2a** or the neutral substrate **2b** is the active site species.[31] The protonated substrate would undoubtedly be a good leaving group; in this paper, however, we focus on the possibility that the neutral **2b** is cleaved, with 3-methyladenine N9$^-$ (**4**) serving as a leaving group. A related question is how acidic the N9−H of 3-methyladenine **3** is. The more acidic



the site, the more stable the resultant N9$^-$ ion should be, and the better a leaving group it should be. The solution-phase pK_a of adenine is known, but acidity can change in different media; we have shown previously, for example, that the N1 and N3 sites in uracil differ in the gas phase by 14 kcal mol^{-1} but have the same pK_a in aqueous solution.[14,32] We thus became interested in

SCHEME 1



pursuing the intrinsic acidity of adenine and derivatives in the gas phase. The experimental gas-phase acidity of these nucleobases has until now not been determined. In this paper, we describe the experimental determination of the acidity of adenine, 9-ethyladenine, and 3-methyladenine and the biological and chemical implications of our results.

Experimental Section

All experiments were conducted on a dual-cell Fourier transform mass spectrometer (FTMS). Each side of the 2 in. cubic dual cell is pumped down to a baseline pressure of less than 1×10^{-9} Torr. The dual cell is positioned collinearly with the magnetic field produced by a 3.3 T superconducting magnet.

Neutral samples were introduced into the FTMS using a heated batch inlet system available commercially or a home-built heated batch inlet system, via a pulsed valve system, or by means of a heated solids probe. All chemicals were available commercially and were used as received. Most ions were produced by proton transfer to hydroxide. Hydroxide was generated by pulsing water into the cell and sending an electron beam (typically 6 eV, 8 μA, beam time 5 ms) through the center of the cell. A trapping potential of −2 V was applied to the cell walls perpendicular to the magnetic field at all times except when ions were transferred from one cell to another. Transfer is accomplished by temporarily grounding (50−150

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(31) In biological papers, 3-methyladenosine is most commonly shown as the protonated species **2a**. The pK_a of protonated 3-methyladenine has been measured (6.1), and the relatively low value in comparison to those of other purine derivatives led the authors to believe that the pK_a corresponds to protonation of the imidazole ring, implying that **10** may be the favored neutral tautomeric form of 3-methyladenine in solution. The pK_a of protonated 3-methyladenosine, where N9 is substituted with a ribose, is not, to our knowledge, known. Lawley, P. D.; Brookes, P. *Biochem. J.* **1963**, *89*, 127−138. Leonard, N. J. and Deyrup, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 4, 2148−2160.

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μs) the conductance limit plate, the trapping plate separating the two cells. The ions then can pass through a 2 mm hole in the center of the conductance limit plate. Transferred ions were cooled with argon.[33,34]

Acidity bracketing was utilized to measure the gas-phase acidities. Species of known acidities are allowed to react with the substrate of unknown acidity. The ability of the anionic conjugate base of the substrate of unknown acidity to deprotonate relatively stronger acids and the inability of the anion to deprotonate weaker acids (stronger bases) allow one to bracket the acidity of the unknown. Where possible, the reverse reaction is also explored. Rapid proton transfer (i.e., near the collision rate) was taken as evidence that the reaction was exothermic and is indicated by a "+" in the tables.

We have recently developed an FTMS method, building upon earlier work in the flowing afterglow, for the bracketing of less acidic sites in molecules that have multiple acidic sites; the experimental procedure and limitations have been described previously.[14,32,35−48]

Throughout the text, the term "gas-phase acidity" is used to refer to the enthalpic (ΔH) change associated with deprotonation.

Calculations were conducted at B3LYP/6-31+G* using Gaussian98.[49] Frequency calculations were conducted on all structures, and no scaling factor was applied. All calculated acidities are at 298 K.

Results and Discussion

Computational Results. The results of our acidity calculations at B3LYP/6-31+G* for adenine, 9-ethyladenine, and 3-methyladenine are shown in Table 1. These values represent the enthalpic requirement for deprotonating the neutral species at 298 K; therefore, the lower the value at a specific site of deprotonation, the more acidic that site.

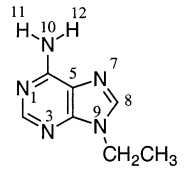
TABLE 1. Calculated Gas-Phase Acidities of the Different Sites of Adenine, 9-Ethyladenine, and 3-Methyladenine at B3LYP/6-31+G* (kcal mol^{-1})[a]

structure	N9	N10H11	N10H12
adenine, N9 tautomer (**1**)	333.3	352.7	352.0
9-ethyladenine (**5**)		353.2	352.6
3-methyladenine, imine tautomer	322.7 (**3a**) 320.6 (**3b**)		

[a] At 298 K.

The preferred gas-phase structure of adenine, by our calculations and others, is that of the N9 tautomer **1**.[50−54] This tautomer has three potentially acidic hydrogens: the N9−H, which has an acidity calculated to be 333.3 kcal mol^{-1}, and the N10 protons. The N10 protons, referred to as H11 and H12, have slightly differing acidities of 352.7 and 352.0 kcal mol^{-1}. Computational and experimental work indicates that the barrier for rotation of the C6−N10 bond is quite significant, on the order of 15−25 kcal mol^{-1}; the bond has partial double bond character from delocalization and does not freely rotate.[55,56] Regardless of the rotatability of this bond, the values (352.7 and 352.0) are sufficiently close to be indifferentiable by experiment. These calculations are also in agreement with earlier calculations conducted by Zeegers-Huyskens et al. at B3LYP/6-31+G**.[2]

9-Ethyladenine (**5**) is of interest because the more acidic N9 site is blocked by the ethyl moiety, allowing one to bracket the N10 site without interference from the N9 site (vide infra). 9-Ethyladenine is predicted to have a gas-phase acidity of 353 kcal mol^{-1}, for deprotonation of the N10 site.



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3-Methyladenine is of interest for biological reasons, as described in the Introduction. The calculated acidity of the imino form of 3-methyladenine is 320.6−322.7 kcal mol^{-1} (depending on which isomer, **3a** or **3b**, is considered).

Experimental Results

Adenine. Our results for the bracketing of the acidity of the adenine N9−H site are shown in Table 2. While

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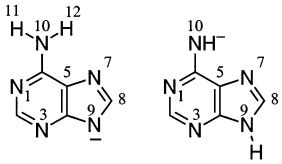
TABLE 2. Summary of Results of Proton Transfer from Reference Acids and Bases to Adenine N9

ref compd	ΔH_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conjugate base
CH_3COOH	348.1 ± 2.1	−	+
$CH_3COCH_2COCH_3$	343.8 ± 2.1	−	+
m-CF_3PhOH	339.3 ± 2.1	−	+
$CH_3CHClCOOH$	337.0 ± 2.1	−	+
$CH_3CHBrCOOH$	336.8 ± 2.1	−	+
$CH_3COCOOH$	333.5 ± 2.9	+	−
HCl	333.4 ± 0.1	+	+
CHF_2COOH	331.0 ± 2.2	+	−
$C_5H_5F_3O_2$	328.3 ± 2.9	+	−

[a] Acidities (kcal mol^{-1}) come from ref 58. [b] A "+" indicates the occurrence and a "−" denotes the absence of proton transfer.

the conjugate base of adenine deprotonates HCl (ΔH_{acid}(HCl) = 333.3 kcal mol^{-1}) and pyruvic acid (ΔH_{acid}(pyruvic acid, $C_3H_4O_3$) = 333.5 kcal mol^{-1}), it is unable to deprotonate 2-bromopropionic acid (ΔH_{acid}($C_3H_5O_2Br$) = 336.8 kcal mol^{-1}). The conjugate base of 2-bromopropionic acid deprotonates adenine, but pyruvate and difluoroacetate (ΔH_{acid} (difluoroacetic acid, $C_2H_2F_2O_2$) = 331.0 kcal mol^{-1}) do not. Furthermore, chloride also deprotonates adenine, implying a close to thermoneutral reaction, since the reactions proceed in both directions (Cl^- + adenine and adenine N9$^-$ + HCl). On the basis of these studies, we bracket the acidity of adenine to be 333 ± 2 kcal mol^{-1}.

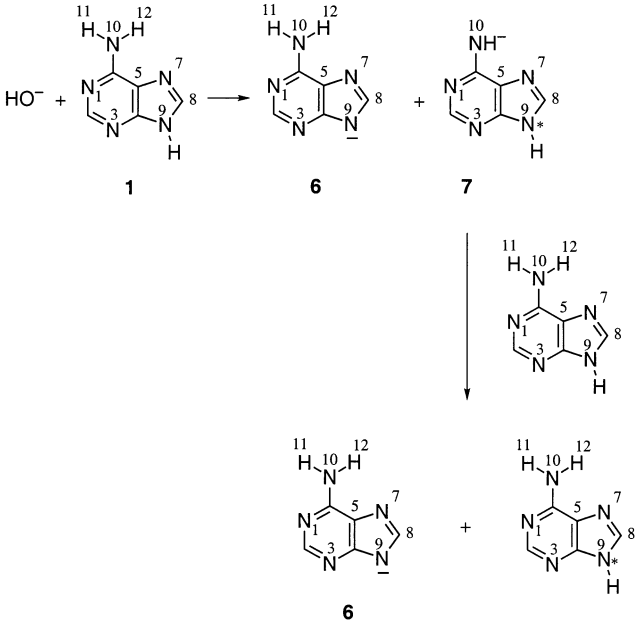
Bracketing the N9 adenine site is relatively straightforward. Bracketing the less acidic N10 site, however, is less so. Deprotonated adenine ions are formed via reaction of hydroxide with neutral adenine. Hydroxide has a proton affinity of 390.7 kcal mol^{-1}; since our calculations indicate that the ΔH_{acid} values for the N9 and N10 sites of adenine should be well below 390.7 kcal mol^{-1}, hydroxide should be basic enough to deprotonate both sites.[57,58] However, the adenine N10$^-$ ion (**7**) isomerizes in the presence of neutral adenine to the N9$^-$ ion (**6**; Scheme 2).[14,32,36] Therefore, to access the less acidic site,



6 **7**

we must operate under conditions that minimize the presence of neutral adenine. This experiment has been described previously, but briefly, the key is to remove the N9$^-$/N10$^-$ mixture from the neutral adenine environment as quickly as possible, which we accomplish by transferring ions from one reaction cell (which is flooded with neutral adenine) to the adjacent, adenine-free reaction cell. Under these "less acidic" conditions, we find that the N10$^-$ ion is able to deprotonate *m*-cresol (ΔH_{acid} (*m*-CH_3PhOH) = 349.6 kcal mol^{-1}) but is unable to deprotonate *p*-trifluoroaniline (ΔH_{acid} (*p*-CF_3PhNH_2) = 353.3 kcal mol^{-1}). Therefore, we bracket the N10−H of adenine to have a gas-phase acidity of 352 ± 4 kcal mol^{-1} (Table 3).

SCHEME 2



1 **6** **7** **6**

TABLE 3. Summary of Results of Proton Transfer from Reference Acids to Adenine N10

ref compd	ΔH_{acid}[a]	proton transfer[b] ref acid
$CHCl_3$	357.6 ± 2.1	−
$CH_3CHCHCHO$	354.7 ± 2.1	−
p-CF_3PhNH_2	353.3 ± 2.1	−
m-CH_3PhOH	349.6 ± 2.1	+
CF_3COCH_3	349.2 ± 2.1	+
CH_3COOH	348.1 ± 2.1	+
DCOOD	345.3 ± 2.2	+
$CH_3COCH_2COCH_3$	343.8 ± 2.1	+
m-CF_3PhOH	339.3 ± 2.1	+

[a] Acidities (kcal mol^{-1}) come from ref 58. [b] A "+" indicates the occurrence and a "−" denotes the absence of proton transfer.

TABLE 4. Summary of Results of Proton Transfer from Reference Acids and Bases to 9-Ethyladenine N10

ref compd	ΔH_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conjugate base
p-$C_6H_4NH_2F$	364.3 ± 2.1	−	+
o-$C_6H_4NH_2F$	362.6 ± 2.2	−	+
$CHCl_3$	357.6 ± 2.1	−	+
$CH_3CHCHCHO$	354.7 ± 2.1	−	+
p-CF_3PhNH_2	353.3 ± 2.1	−	+
CH_3PhOH	349.6 ± 2.1	+	−
CF_3COCH_3	349.2 ± 2.1	+	−
CH_3COOH	348.1 ± 2.1	+	−
HCOOH	345.3 ± 2.2	+	−
$CH_3COCH_2COCH_3$	343.8 ± 2.1	+	−

[a] Acidities (kcal mol^{-1}) come from ref 58. [b] A "+" indicates the occurrence and a "−" denotes the absence of proton transfer.

9-Ethyladenine. We have also conducted studies on 9-ethyladenine (**5**; Table 4). Unlike in adenine, deprotonation should only occur at N10. We therefore do not have isomerization issues and can benchmark our ad-

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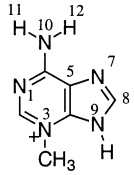
enine N10 measurements. Our calculations indicate that the ΔH_{acid} of this site should be near that of the N10−H of the parent adenine, at 352 kcal mol^{-1}. We find that the N10$^-$ ion of 9-ethyladenine deprotonates *m*-cresol (ΔH_{acid}(*p*-CH_3PhOH) = 349.6 kcal mol^{-1}), but is unable to deprotonate *p*-trifluoroaniline (ΔH_{acid}(*p*-CF_3PhNH_2) = 353.3 kcal mol^{-1}). Likewise, the conjugate base of *p*-trifluoroaniline deprotonates 9-ethyladenine, and the conjugate base of *m*-cresol does not. We therefore bracket the acidity of the N10 site of 9-ethyladenine to be 352 ± 4 kcal mol^{-1}.

3-Methyladenine. 3-Methyladenine is of especial interest to us due to its mutagenic properties in nature. It is most often drawn, in DNA, as the protonated species **2a**, which can be in equilibrium with the imine form **2b** (Scheme 1). The free base analogues of **2a** and **2b** are the protonated 3-methyladenine **8** and the imine **3**. Imine

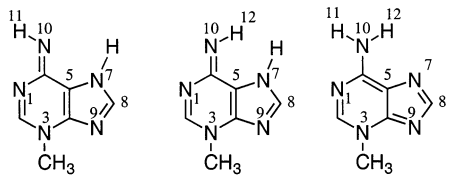


8

3 is predicted, computationally, to be extraordinarily acidic in the gas phase, comparable to HBr (Table 1). This highly acidic N9−H would imply that the corresponding conjugate base would be very stable, also implying that the N9$^-$ ion would serve as a good leaving group. This is of interest because if the deprotonated methylated adenine is a favorable leaving group, then it should be particularly facile for the 3-methyladenine glycosylase to excise.

In starting the bracketing studies, we allowed the conjugate base of 3-methyladenine to react with 1,1,1-trifluoro-2,4-pentadione, an acid that we had readily available in the laboratory, and the ΔH_{acid} of which is 328.3 ± 2.9 kcal mol^{-1}. We would not expect the conjugate base of 3-methyladenine, which has a predicted acidity of 320−323 kcal mol^{-1}, to deprotonate the pentadione. To our surprise, the conjugate base of 3-methyladenine *readily deprotonates* the dione reference acid, while the enolate of the pentadione is unable to deprotonate 3-methyladenine! Clearly, 3-methyladenine is far less acidic than calculations predicted; we ultimately found the gas-phase acidity to be 347 ± 4 kcal mol^{-1}, bracketing between those of acetic acid (ΔH_{acid}(CH_3COOH) = 348.1 kcal mol^{-1}) and formic acid (ΔH_{acid}(HCOOH) = 345.3 kcal mol^{-1}, Table 5).

This curious result, at odds with our calculations, led us to consider the various tautomers of 3-methyladenine. The possible tautomers are what we call the "N9 tautomers", which we initially assumed to be the most stable (**3a**, the N9H11 tautomer, and **3b**, the N9H12 tautomer), the "N7 tautomers" (**9a**, the N7H11 tautomer, and **9b**,



9a **9b** **10**

TABLE 5. Summary of Results of Proton Transfer from Reference Acids and Bases to 3-Methyladenine N10

		proton transfer[b]	
ref compd	ΔH_{acid}[a]	ref acid	conjugate base
$CH_3CHCHCHO$	354.7 ± 2.1	−	+
CF_3COCH_3	349.2 ± 2.1	−	+
CH_3COOH	348.1 ± 2.1	−	+
HCOOH	345.3 ± 2.2	+	−
$CH_3COCH_2COCH_3$	343.8 ± 2.1	+	−
$CH_3COCOOH$	333.5 ± 2.9	+	−
HCl	333.4 ± 0.1	+	−
$C_5H_5F_3O_2$	328.3 ± 2.9	+	−

[a] Acidities (kcal mol^{-1}) come from ref 58. [b] A "+" indicates the occurrence and a "−" denotes the absence of proton transfer.

TABLE 6. Calculated Gas-Phase Relative Optimized Energies and Acidities of the Different Tautomers of 3-Methyladenine at B3LYP/6-31+G* (kcal mol^{-1})[a]

structure	rel *E*	ΔH_{acid}
N9H11 tautomer **3a**	24.5	322.7 (N9−H)
N9H12 tautomer **3b**	24.7	320.6 (N9−H)
N7H11 tautomer **9a**	10.4	336.8 (N7−H)
N7H12 tautomer **9b**	17.8	327.5 (N7−H)
N10 tautomer **10**	0	345.3 (N10−H11)
		347.2 (N10−H12)

[a] At 298 K.

the N7H12 tautomer), and the "N10 tautomer" (**10**). The relative gas-phase energies for each of these species are shown in Table 6. The most stable tautomer appears to be the N10 tautomer **10**. The N9 tautomers **3a** and **3b** are about 25 kcal mol^{-1} less stable than the N10 tautomer. The N7H11 tautomer **9a** is 10 kcal mol^{-1} less stable and the N7H12 tautomer **9b** 18 kcal mol^{-1} less stable than the N10 tautomer **10**.

The corresponding acidities for all the tautomers are also given in Table 6. On the basis of our calculated results, we would expect the N10 tautomer **10** to be the major structure in the gas phase, and indeed, the calculated acidity of that tautomer correlates with our experimental result. The calculated values are 345.3 and 347.2 kcal mol^{-1} (depending on which proton is extracted); our experimental value is 347 kcal mol^{-1}.

While this result is of fundamental interest, biologically speaking, the N10 tautomer is probably not of importance. In DNA, the adenine N9 is substituted by a ribose moiety. Therefore, postalkylation, the N9 has no proton to lose to tautomerize to **10**. That is, the N10 tautomer of 3-methyladenine would only be accessible if there were a proton at N9; the ribose effectively blocks that site in DNA.[59]

Interestingly, if glycosylation of adenine in DNA yields the neutral 3-methyladenine tautomer **2b** (Scheme 1), the alkylated nucleobase may be a good leaving group. Our calculations show that the N9−H for **3** is highly acidic, 10 kcal mol^{-1} more so than for the parent adenine (Table 1).[27,29,60] Herein may lie a possible reason that 3-methyladenine is particularly susceptible to excision.

(59) Leonard, N. J.; Deyrup, J. A. *J. Am. Chem. Soc.* **1961**, *84*, 2148−2160.
(60) Lawley, P. D.; Brookes, P. *Biochem. J.* **1963**, *89*, 127−138.

Conclusions

In summary, we have measured the acidities of adenine and its alkylated derivatives for the first time. Our measurements and calculations of adenine, 9-ethyladenine, and 3-methyladenine have established that (1) the N9 site of adenine is 19 kcal mol^{-1} more acidic than the N10 site, with acidities at 333 ± 2 and 352 ± 4 kcal mol^{-1}, respectively, (2) the acidity of 9-ethyladenine, where the N9 is blocked, is 352 ± 4 kcal mol^{-1}, allowing us to benchmark our novel measurement of the less acidic N10 site of adenine, (3) the most stable tautomer of 3-methyladenine in the gas phase is the N10 tautomer **10**, as opposed to the more commonly written imine tautomer **3** (we have also bracketed the acidity of this species to 347 ± 4 kcal mol^{-1}), (4) if the imine tautomer of 3-methyladenine (**3**) is indeed the tautomer that exists in alkylated DNA, our calculations indicate that the high acidity of the N9 site will favor the excision of the alkylated base, and (5) B3LYP/6-31+G* is a relatively inexpensive and reliable method for predicting these acidities.

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Supporting Information Available: Cartesian coordinates for all calculated species. This material is available free of charge via the Internet at http://pubs.acs.org.

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