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Gas-Phase Acidity Studies of Multiple Sites of Adenine and Adenine Derivatives

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The acidities of multiple sites in the purine nucleobase adenine (**1**) and adenine alkyl derivatives 9-ethyladenine (**2**), 3-methyladenine (**3**), 1-methyladenine (**4**), and *N*,*N*-dimethyladenine (**5**) have been investigated for the first time, using computational and experimental methods to provide an understanding of adenine reactivity. We have previously measured two acidic sites on adenine, with the N9 site being 19 kcal mol^{-1} more acidic than the N10 site (333 ± 2 versus 352 ± 4 kcal mol^{-1}, respectively). In this work, we have established that 9-ethyladenine has two sites more acidic than water: the N10 (352 ± 4 kcal mol^{-1}) and the C8 (374 ± 2 kcal mol^{-1}). We have likewise measured two acidities for 3-methyladenine, the N10 (347 ± 4 kcal mol^{-1}) and the C2 (370 ± 3 kcal mol^{-1}). For 1-methyladenine and *N*,*N*-dimethyladenine, we measure the N9H acidity to be 331 ± 2 and 333 ± 2 kcal mol^{-1}, respectively. We believe that the bracketing of only one site for the latter species is a kinetic effect, which we discuss further in the paper. Computationally, we have found the interesting result that some of the vinylic C−H sites in these purine bases are predicted to be much more acidic than water (ΔH_{acid} = 390.7 kcal mol^{-1}) in the gas phase, on the order of 373 kcal mol^{-1}. The acidic vinylic C−H sites are always adjacent to an N−R group, and this pattern is maintained regardless of whether the site is on the five- or six-membered ring of the purine. Vinylic C−H sites elsewhere on the purine have calculated acidities of about 400 kcal mol^{-1}. The differing acidities are interpreted through electrostatic potential calculations. We also relate our results to the intriguing biochemical decarboxylation of orotate ribose monophosphate, which involves a vinylic anion adjacent to an N−R group; this decarboxylation is the last step in the de novo biosynthesis of pyrimidine nucleotides, and the enzyme that catalyzes the reaction, orotate ribose monophosphate decarboxylase, has been the subject of intense study recently, as its mechanism remains elusive.

Introduction

Accurate measurements of the acidities and basicities of nucleic bases and nucleic base derivatives are essential for greater understanding of fundamental biological issues. The recognition of DNA and RNA bases is modulated by hydrogen bonding; in turn, hydrogen bonding is dependent on the intrinsic acidity and basicity of acceptor and donor groups on the nucleic bases.[1,2] Furthermore, elucidating the intrinsic reactivity of nucleic bases can improve understanding of key biosynthetic mechanisms for which nucleobases are substrates.[3−8]

The gas phase is a particularly 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.[9−11] The gas phase is the "ultimate" nonpolar environment, where intrinsic reactivity can be explored and extrapolated to other media.[9,12−14]

Recently, our studies of nucleobases have focused on the purine base adenine.[15] Our interest in alkylated derivatives of adenine is motivated by their role in mutagenesis. Adenine can be alkylated by cancer chemotherapeutics as well as environmental mutagens, thereby damaging the genome.[16] In an effort to understand the electronic structure and reactivity of these purine bases, we embarked on an experimental and computational study of adenine and alkyl adenine derivatives. The experimental gas-phase acidities of the

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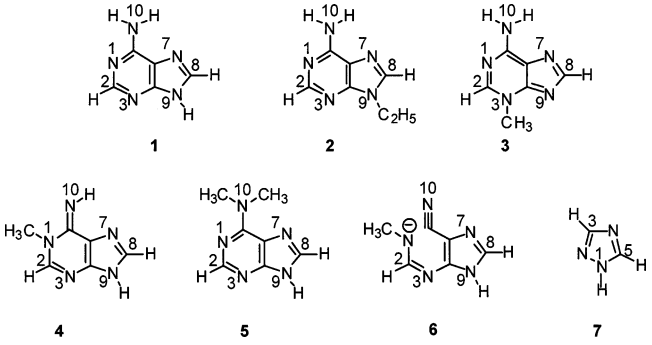
alkylated species have until now not been determined. In our systematic study, we have also discovered some interesting acidity patterns, the most intriguing of which is the fact that some purine C−H sites are as acidic as HF or acetone in the gas phase. In this paper, we describe experimental and computational determinations of the multiple acidities of adenine (**1**) and adenine alkyl derivatives 9-ethyladenine (**2**), 3-methyladenine (**3**), 1-methyladenine (**4**), and *N*,*N*-dimethyladenine (**5**).



Experimental Section

All chemicals were purchased from Sigma-Aldrich and used as received. Experiments were conducted on a Finnigan 2001 Fourier Transform Mass Spectrometer (FTMS) with a dual-cell setup, which has been described previously.[15] Briefly, the setup consists of two adjoining 2 in. cubic cells that are pumped 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. A heated batch inlet system or a heated solids probe is used to introduce neutral samples into the FTMS. A trapping potential of −2 V is applied to the cell walls perpendicular to the magnetic field at all times except when ions are being transferred from one cell to another. Transfer is accomplished by temporarily grounding (50−150 μs) the trapping plate separating the two cells. The ions can then be transferred into the next cell through a 2 mm hole in the center of the trapping plate. Transferred ions are cooled by a pulse of argon that raises the cell pressure to 10^{-5} Torr.[17,18]

Acidity bracketing was utilized to measure the gas-phase acidities. Hydroxide ions are generated by pulsing water into the FTMS cell and sending an electron beam (typically 6 eV, 8 μA, beam time 5 ms) through the center of the cell. The hydroxide is then used to deprotonate the molecules of interest, producing, for example, the $[M - H]^-$ of adenine. The $[M - H]^-$ anions are transferred into the second cell and allowed to react with reference acids having known gas-phase acidities.[19] For acidity bracketing of the most acidic site in a molecule, we also allow the conjugate bases of different reference acids to react with the neutral of unknown acidity. Rapid proton transfer (i.e., near the collision rate) was taken as evidence that the reaction is exothermic and is indicated by a "+" in Tables 2−5.[20] Throughout this paper, acidity is reported as ΔH_{acid} and is the change in enthalpy associated with deprotonation of a molecule HA to form H^+ and A^-. Reporting an experimentally bracketed ΔH_{acid} is valid because for all the reference acids used herein, the ΔS_{acid} values at 298 K are within 0.006 kcal mol^{-1}.[19,21,22] The term "$[M - H]^-$" refers to the ion formed by deprotonation of a neutral molecule. Also, the deprotonation of a given site to form the corresponding ion is referred to by the atom number of the deprotonated site; for example, "adenine N9$^-$" refers to the $[M - H]^-$ ion formed by deprotonation of the N9−H of adenine.

In our experiments, the nucleobase (neutral) is present in excess. Because the pressure as read by the instrument gauge is not a reliable measure of the amount of neutral present, we conduct a control experiment to ascertain nucleobase pressure. The pseudo-first-order rate constant is measured for the reaction of hydroxide with the neutral nucleobase. Assuming that this control reaction takes place at the collision rate, we can then obtain an estimate of the nucleobase pressure.

We have recently developed an FTMS method for the bracketing of less acidic sites in molecules that have multiple acidic sites; the experimental procedure and limitations have been described previously.[14,23] Briefly, using adenine as an example, when hydroxide is used to deprotonate adenine, multiple ions can be formed, including the N9-deprotonated adenine and the N10-deprotonated adenine. The N9−H is more acidic than the N10−H. Therefore, when the ions are allowed to stay in an environment where there is a constant pressure of neutral adenine, the more basic N10$^-$ ion isomerizes to N9$^-$. We then transfer the N9$^-$ ion to the second cell, where the reference acid is added at a constant pressure, and we monitor proton transfer. We refer to these conditions as "more acidic" conditions, because we allow for isomerization to the more acidic site before transfer. If, instead, the $(M - 1)^-$ of adenine (which is some mixture of N9$^-$ and N10$^-$) is transferred from the neutral adenine environment immediately to the second cell, then the N10$^-$ will not isomerize and that ion can be bracketed. We refer to this method as "less acidic" conditions.

Gas-phase acidity calculations were conducted at B3LYP/6-31+G* using Gaussian98.[24,25] Previous work has established the general accuracy of using this method and level to calculate nucleobase acidity.[14,15,23] Acidities are reported as ΔH_{acid} at 298 K.[26] Electrostatic potentials were calculated at B3LYP/6-31+G* using Gaussian98; figures were generated with GaussView 3.0 (isodensity setting 0.0004, electrostatic potential range ± 0.16 au).[27]

Computational Results

The calculated gas-phase acidities for all the ring protons of adenine (**1**), 9-ethyladenine (**2**), 3-methyl-

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(26) Calculated values reported herein differ from those reported at the same level in our previous work on adenine (Sharma, S.; Lee, J. K. *J. Org. Chem.* **2002**, *67*, 8360−8365); in the previous work, we reported the difference in the electronic energies of the neutral M and the anion $(M - H)^-$ at 298 K (ΔE_{298}). In this work, we account for the translational energy of H^+ and for enthalpic changes such that our reported value is a true ΔH_{acid}.

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TABLE 1. Gas-Phase Acidities (ΔH_{acid}) of Multiple Sites in Adenine and Alkyl Adenine Derivatives Calculated at B3LYP/6-31+G* in kcal mol^{-1} [a]

substrate	N9	N10H11	N10H12	C8	C2
adenine (**1**)	334.8[b]	354.2[b]	353.5[b]	373.1	399.0
9-ethyladenine (**2**)		355.0	354.4	373.8	399.5
3-methyladenine (**3**)		346.8[b]	348.7[b]	399.9	368.8
1-methyladenine (**4**)	334.3	356.4[c]		375.6	374.6
N,*N*-dimethyladenine (**5**)	335.5			373.4	399.6

[a] At 298 K. Results correspond to the most stable gas-phase tautomer for each molecule. [b] Refs 15 and 26. [c] Represents the energy required to ring open to form **6**; see text.

adenine (**3**), 1-methyladenine (**4**), and *N*,*N*-dimethyladenine (**5**) calculated at B3LYP/6-31+G* are summarized in Table 1. For each neutral molecule, the most stable tautomer is used.

Adenine (1). Previous calculations by our group have predicted that the most acidic site in adenine (**1**) is the N9 site with a calculated acidity of 334.8 kcal mol^{-1} (Table 1). The N10 and C8 sites in adenine are also predicted to be more acidic than water (ΔH_{acid} = 390.7 kcal mol^{-1}) in the gas phase.[19] The two (amino) protons at the N10 site, referred to as H11 and H12, have calculated acidities of 354.2 and 353.5 kcal mol^{-1}, while the C8 proton is less acidic, with a calculated acidity of 373.1 kcal mol^{-1}. The C2−H of adenine is the least acidic site on adenine, with a ΔH_{acid} of 399.0 kcal mol^{-1}.

9-Ethyladenine (2). We calculated the acidities of three sites on 9-ethyladenine: the N10, C2, and C8 (Table 1). The N10 amino protons are the most acidic, with calculated acidities of 355.0 and 354.4 kcal mol^{-1}, respectively. The C8 proton has a calculated acidity of 373.8 kcal mol^{-1}, and the C2−H, like in adenine, is the least acidic, at 399.5 kcal mol^{-1}.

3-Methyladenine (3). In 3-methyladenine (**3**), the N10 amino protons are the most acidic, with calculated acidities of 346.8 and 348.7 kcal mol^{-1}, respectively (Table 1). The C2 proton has a calculated acidity of 368.8 kcal mol^{-1}. The least acidic site is calculated to be the C8−H, at 399.9 kcal mol^{-1}.

1-Methyladenine (4). Unlike with adenine and the other alkyl adenine derivatives, our calculations predict that 1-methyladenine exists predominantly as an imino tautomer (**4**) in the gas phase; the most stable imino tautomer is 6 kcal mol^{-1} lower in energy than the amino tautomer. The three acidic sites in this structure are predicted to be the N9, C2, and C8 sites. The N9−H is the most acidic, with a calculated acidity of 334.3 kcal mol^{-1} (Table 1). The C2 and C8 sites are calculated to be comparable in acidity: 374.6 and 375.6 kcal mol^{-1}, respectively. The N10 site is quite interesting; deprotonation of the N10−H yields the ring-opened structure **6** as the product; we could not locate a stable ring-closed structure by calculation. The energy required to deprotonate the N10−H to form **6** is 356.4 kcal mol^{-1} (Table 1).

***N*,*N*-Dimethyladenine (5).** For *N*,*N*-dimethyladenine (**5**), the N9 site has a calculated acidity of 335.5 kcal mol^{-1}, and the C8 site has a calculated acidity of 373.4 kcal mol^{-1} (Table 1). The C2−H is the least acidic, calculated to be 399.6 kcal mol^{-1}.

TABLE 2. Summary of Results of Proton Transfer from Reference Acids to 9-Ethyladenine C8$^-$ Ion

reference compound	ΔH_{acid}[a]	proton transfer[b] reference acid
CH_3CH_2OH	378.3 ± 1.0	−
$CH_3CH_2CH_2OH$	375.7 ± 1.3	−
$CH_3CH_2CH_2CH_2OH$	375.3 ± 2.0	−
$CH_3CHOHCH_3$	375.1 ± 1.0	−
$CH_3CH_2CHOHCH_3$	374.1 ± 2.0	+
CH_2CHCH_2OH	373.5 ± 2.9	+
CH_2FCH_2OH	371.2 ± 2.9	+
CH_3COCH_3	369.1 ± 2.1	+
$CH_3CH_2COCH_2CH_3$	368.6 ± 2.2	+

[a] Acidities are in kcal mol^{-1} and were taken from ref 19. [b] "+" indicates the occurrence of proton transfer; "−" denotes the absence of proton transfer.

TABLE 3. Summary of Results of Proton Transfer from Reference Acids to 3-Methyladenine C2$^-$ Ion

reference compound	ΔH_{acid}[a]	proton transfer[b] reference acid
CH_3CH_2OH	378.3 ± 1.0	−
$CH_3CH_2CH_2CH_2OH$	375.3 ± 2.0	−
$CH_3CHOHCH_3$	375.1 ± 1.0	−
$CH_3CH_2CH_2CH_2CH_2OH$	374.1 ± 2.1	−
$CH_3CH_2CHOHCH_3$	374.1 ± 2.0	−
CH_2CHCH_2OH	373.5 ± 2.9	−
$CH_3CH_2CH(CH_2CH_3)CH_2OH$	373.1 ± 2.0	−
$(CH_3)_3CCH_2CH_2OH$	371.6 ± 2.1	−
CH_2FCH_2OH	371.2 ± 2.9	−
$CH_3CH_2N(OH)CH_2CH_3$	370.6 ± 2.1	−
$CH_2CHCHOHCH_2CH_2CH_3$	369.4 ± 2.1	+
CH_3COCH_3	369.1 ± 2.1	+
$CH_3CH_2COCH_2CH_3$	368.6 ± 2.2	+
$CH_3COCH_2CH_3$	367.2 ± 2.4	+

[a] Acidities are in kcal mol^{-1} and were taken from ref 19. [b] "+" indicates the occurrence of proton transfer; "−" denotes the absence of proton transfer.

Experimental Results

Adenine (1). We have previously bracketed two acidic sites in adenine, the N9 site at 333 ± 2 kcal mol^{-1} and the N10 site at 352 ± 4 kcal mol^{-1}.[15]

9-Ethyladenine (2). We have previously bracketed the most acidic N10 site in 9-ethyladenine as 352 ± 4 kcal mol^{-1}.[15] Since calculations indicate that the next most acidic site, the C8, would have an acidity near 374 kcal mol^{-1}, we allowed the [M − H]$^-$ ions of 9-ethyladenine to react with reference acids in the acidity range of 369 to 378 kcal mol^{-1}, under our "less acidic" conditions (Table 2). We find that the conjugate base of 9-ethyladenine does not deprotonate 2-propanol (ΔH_{acid} = 375.1 ± 1.0), but it does deprotonate 2-butanol (ΔH_{acid} = 374.1 ± 2.0). Therefore, we bracket the gas-phase acidity of the less acidic site in 9-ethyladenine to be 374 ± 2 kcal mol^{-1}. We believe the bracketing result corresponds to the C8 site in 9-ethyladenine, as this is consistent with our theoretical prediction (373.8 kcal mol^{-1}).

3-Methyladenine (3). We have previously bracketed the most acidic N10 site in 3-methyladenine as 347 ± 4 kcal mol^{-1}.[15] To bracket the next most acidic site in 3-methyladenine, predicted to be 369 kcal mol^{-1}, we allowed the [M − H]$^-$ ions of 3-methyladenine to react with reference acids in the acidity range of 367−378 kcal mol^{-1}, under our less acidic conditions (Table 3). We find that the conjugate base of 3-methyladenine does not

TABLE 4. Summary of Results of Proton Transfer from Reference Acids and Bases to 1-Methyladenine N9 Site

		proton transfer[b]	
reference compound	ΔH_{acid}[a]	reference acid	conjugate base
HCOOH	345.3 ± 2.2	−	+
$CH_3COCH_2COCH_3$	343.8 ± 2.1	−	+
m-CF_3PhOH	339.3 ± 2.1	−	+
$CH_3CHClCOOH$	337.0 ± 2.1	−	+
$CH_3CHBrCOOH$	336.8 ± 2.1	−	+
HCl	333.4 ± 0.1	−	+
$CH_3COCOOH$	333.5 ± 2.9	+	+
CHF_2COOH	331.0 ± 2.2	+	−
$C_5H_5F_3O_2$	328.3 ± 2.9	+	−

[a] Acidities are in kcal mol^{-1} and were taken from ref 19. [b] "+" indicates the occurrence of proton transfer; "−" denotes the absence of proton transfer.

deprotonate diethyl-hydroxylamine (ΔH_{acid} = 370.6 ± 2.1) but does deprotonate 1-hexen-3-ol (ΔH_{acid} = 369.4 ± 2.1). Therefore, we bracket the less acidic site in 3-methyladenine as 370 ± 3 kcal mol^{-1}. We believe the bracketing result corresponds to the C2 site in 3-methyladenine, as this is in agreement with the calculated acidity for the C2 site (368.8 kcal mol^{-1}).

1-Methyladenine (4). Experimental results for the acidity bracketing of 1-methyladenine are summarized in Table 4. Reference acids in the acidity range 328−345 kcal mol^{-1} were used to conduct these experiments. There is no proton-transfer reaction between deprotonated 1-methyladenine and HCl (ΔH_{acid} = 333.4 ± 0.1 kcal mol^{-1}); however, proton transfer does occur between 1-methyladenine and chloride. The lower limit for 1-methyladenine acidity is thus bracketed as 333.4 ± 0.1 kcal mol^{-1}. In another set of experiments, proton transfer is observed when deprotonated 1-methyladenine is allowed to react with difluoroacetic acid (ΔH_{acid} = 331.0 ± 2.2 kcal mol^{-1}), while no proton transfer is observed when neutral 1-methyladenine is allowed to react with difluoroacetate ion. This gives an upper bracketing limit of 331.0 ± 2.2 kcal mol^{-1}. Proton transfer reactions are observed when deprotonated 1-methyladenine is allowed to react with pyruvic acid (ΔH_{acid} = 333.5 ± 2.9 kcal mol^{-1}) and also when neutral 1-methyladenine is allowed to react with pyruvate ion. We therefore bracket the most acidic site in 1-methyladenine to be 331 ± 2 kcal mol^{-1}. We believe we have bracketed the N9 acidity since our experimental bracketing result agrees with the calculated acidity of 334.3 kcal mol^{-1} for the most acidic N9 site in 1-methyladenine.

We have not been able to experimentally bracket less acidic sites in 1-methyladenine. To bracket a less acidic site, we need to remove the $[M - H]^-$ ions from the first cell, which is high in adenine concentration, within a short period of time or else neutral-catalyzed isomerization takes place and only the "most acidic" ions (that is, the ions resulting from deprotonation of the most acidic site) survive (see Experimental Section).[14,23] Transfer of $[M - H]^-$ ions under these conditions results in a loss of signal too great to conduct the bracketing experiments for 1-methyladenine.

***N*,*N*-Dimethyladenine (5).** Experimental results for *N*,*N*-dimethyladenine acidity bracketing are summarized in Table 5. Reference acids in the acidity range 328−344 kcal mol^{-1} were used to conduct these experiments. While

TABLE 5. Summary of Results of Proton Transfer from Reference Acids and Bases to *N*,*N*-Dimethyladenine N9 Site

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

[a] Acidities are in kcal mol^{-1} and were taken from ref 19. [b] "+" indicates the occurrence of proton transfer; "−" denotes the absence of proton transfer.

proton transfer is not observed between deprotonated *N*,*N*-dimethyladenine and 2-chloropropionic acid (ΔH_{acid} = 337.0 ± 2.1 kcal mol^{-1}), the reverse reaction does occur. Proton transfer is observed when deprotonated *N*,*N*-dimethyladenine is allowed to react with pyruvic acid (ΔH_{acid} = 333.5 ± 2.9 kcal mol^{-1}), while no proton transfer is observed when neutral *N*,*N*-dimethyladenine is allowed to react with pyruvate ion. Furthermore, the reaction of *N*,*N*-dimethyladenine N9$^-$ with HCl (ΔH_{acid} = 333.4 ± 0.1 kcal mol^{-1}) and the reverse reaction both proceed. These results allow us to bracket the acidity of *N*,*N*-dimethyladenine N9−H to be 333 ± 2 kcal mol^{-1}. This value is in agreement with the calculated N9−H acidity value of 335.5 kcal mol^{-1}.

We were unsuccessful in bracketing the less acidic site in *N*,*N*-dimethyladenine, which has a calculated acidity of 373.4 kcal mol^{-1}. We find that even with acids that *N*,*N*-dimethyladenine C8$^-$ should deprotonate such as *meta*-cresol (ΔH_{acid} = 349.6 ± 2.1 kcal mol^{-1}), we do not see proton transfer.

Discussion

Acidity Bracketing. The experimental acidities of the adenine derivatives are consistent with calculations (Table 6). For adenine (**1**), only two acidities are bracketed, although hydroxide (ΔH_{acid} = 390.7 kcal mol^{-1}) is sufficiently basic to deprotonate three sites (N9, N10, C8).[19] We have found this same pattern in previous studies with uracil and attribute the results to a kinetic effect.[23] Hydroxide probably removes the proton from the third least acidic site (C8) most infrequently.[28,29] Additionally, the anion formed from the least acidic site will be particularly prone to isomerization, because there are *two more* sites with which it could react. For example, with adenine, reaction with hydroxide should result in the least amount of C8$^-$. Because there is so little C8$^-$, it will be a difficult position to bracket. In addition, the C8$^-$ will also isomerize readily to the N9$^-$ and the N10$^-$ ions, which will further decrease the odds of bracketing the C8 position. We believe that these two effects, minimal formation of the C8$^-$ and facile isomerization to N9$^-$ and N10$^-$, make bracketing the third and fourth least acidic sites in a molecule improbable.

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TABLE 6. Summary of Experimental and Calculated Gas-Phase Acidities of the Different Sites of Adenine, 9-Ethyladenine, 3-Methyladenine, 1-Methyladenine, and *N*,*N*-Dimethyladenine in kcal mol^{-1} [a,b]

substrate	N9	N10	C8	C2
adenine (**1**)	333 ± 2[b](334.8)[b]	352 ± 4[b](353.5)[b]	(373.1)	(399.0)
9-ethyladenine (**2**)		352 ± 4[b](354.4)[b]	374 ± 2 (373.8)	(399.5)
3-methyladenine (**3**)		347 ± 4[b](346.8)[b]	(399.9)	370 ± 3 (368.8)
1-methyladenine (**4**)	331 ± 2 (334.3)		(375.6)	(374.6)
N,*N*-dimethyladenine (**5**)	333 ± 2 (335.5)		(373.4)	(399.6)

[a] Values in parentheses were calculated at 298 K at B3LYP/6-31+G*. [b] Refs 15 and 26.

3-Methyladenine (**3**) also has three acidic sites prone to removal by hydroxide, the N10 and C2 indicated on the table, plus the NCH_2−H site, which has a surprisingly low calculated acidity of 372.4 kcal mol^{-1}. Again, only two acidic sites are measured, at 347 ± 4 and at 370 ± 3 kcal mol^{-1}.

9-Ethyladenine (**2**) has two sites that hydroxide is basic enough to deprotonate, and we have bracketed both: the N10 at 352 ± 4 kcal mol^{-1} and the C8 at 374 ± 2 kcal mol^{-1}.

1-Methyladenine (**4**) has four possible acidic sites: N9, N10, C2, and C8. The most acidic site, the N9, brackets to 331 ± 2 kcal mol^{-1}. Experimentally, we found that we were not able to transfer enough ions from one cell to the other to bracket a second acidity. It is interesting, however, to consider what acidity we would have measured had we not suffered signal loss. As noted in the Results section, deprotonation of the N10−H results in a ring-opened structure (**6**). The energy required to deprotonate the N10−H to yield **6** is 356.4 kcal mol^{-1}. However, the proton affinity of the ion **6**, that is, the energy that would be released upon protonation of **6**, is 340.2 kcal mol^{-1}. Our protocol for bracketing less acidic sites actually involves isolating the various deprotonated species and protonating them with acids HA to bracket the acidity of their conjugate acids. Deprotonation of 1-methyladenine by hydroxide would presumably yield four ions, the N9$^-$, C8$^-$, C2$^-$, and **6**. Of these four ions, the conjugate acid of N9$^-$ is the most acidic; the next most acidic would be the conjugate acid of **6**. Therefore, had the ion signal been strong, we would have likely measured a second acidity near 340 kcal mol^{-1}, corresponding to the ion **6**.

N,*N*-Dimethyladenine (**5**) has three acidic sites, but we bracket only one, the N9 at 333 ± 2 kcal mol^{-1}. We believe that the same kinetic effects at play with our adenine studies preclude us from measuring more than one *N*,*N*-dimethyladenine acidity. In other molecules for which we have bracketed less acidic sites, the difference in acidity between the most and the less acidic sites has been about 20 kcal mol^{-1} or less. For *N*,*N*-dimethyladenine, the acidity difference is predicted to be more than 35 kcal mol^{-1}. The combination of producing few C8$^-$ ions and the probability of fast isomerization probably leads to our inability to bracket this acidity.[14,23]

One of the difficulties in dealing with purine bases is the large number of possible tautomers; the parent adenine, for example, has 12 possible structures. Computationally, we find that while adenine, 9-ethyladenine, 3-methyladenine, and *N*,*N*-dimethyladenine show a preference for a structure where the group off C6 is amino (**1**, **2**, **3**, and **5**, respectively), the most stable structure for 1-methyladenine is imino structure **4** (Supporting Information). The most stable neutral tautomer for each species is at least 4 kcal mol^{-1} lower in energy than the next most stable structure. A caveat, however, arises with the deprotonated structures. By deprotonation/reprotonation reactions with HO^-/H_2O, one can envision isomerization to a very large number of anionic structures. For example, Table 7 shows the calculated energies for all 12 adenine tautomers and the ΔH_{acid} for the most acidic site of each tautomer (corresponding structures in Figure 1). We have bracketed the most acidic site of adenine to be 333 ± 2 kcal mol^{-1}. We assume that the bracketed site corresponds to anion **1′**. However, since the bracketing experiment involves deprotonation of the reference acids by the conjugate base of adenine, it is conceivable that we have a mix of **1′**, **1f′** **1h′**, **1i′**, and **1j′**. By calculation, all these anions have a proton affinity of around 333 kcal mol^{-1}, so we cannot know for sure whether one or all of these ions are present in the bracketing experiments. The possibility that our [M − H]$^-$ ions are a mix of isomers is an experimental limitation of this mass spectrometry-based method. However, the many possible anions of varying proton affinity coupled with the fact that our bracketing experiments are very clean, that is, when measuring a given acidity, we reproducibly bracket to one value, not a spread of values, imply that we have just one species present (or by coincidence have only those isomers that have the same proton affinity).[30]

TABLE 7. Calculated Gas-Phase Relative Optimized Energies and Acidities for Adenine Tautomers at B3LYP/6-31+G* in kcal mol^{-1} [a]

tautomer	relative *E*	ΔH_{acid}
N9−H (**1**)	0	334.8 (N9−H)
N7−H (**1a**)	8.1	326.7 (N7−H)
N3−H (**1b**)	8.1	326.7 (N3−H)
N1−H (**1c**)	18.6	316.3 (N1−H)
N3−H,N9−H (syn) (**1d**)	31.3	322.2 (N3−H)
N3−H,N9−H (anti) (**1e**)	31.6	321.6 (N9−H)
N3−H,N7−H (syn) (**1f**)	17.4	332.3 (N3−H)
N3−H,N7−H (anti) (**1g**)	24.7	328.5 (N7−H)
N1−H,N9−H (syn) (**1h**)	19.1	334.5 (N1−H)
N1−H,N9−H (anti) (**1i**)	12.5	333.0 (N9−H)
N1−H,N7−H (syn) (**1j**)	16.7	333.0 (N1−H)
N1−H,N7−H (anti) (**1k**)	17.0	328.5 (N7−H)

[a] At 298 K.

Calculated Carbon Acidities. The acidities of the C−H protons on the C2 and C8 of each adenine derivative are of particular interest. The calculated C2−H and C8−H acidities are shown in Figure 2. We were intrigued to find that some of these vinylic protons are quite acidic (shown in red). In adenine (**1**), the C2−H is of a

(30) Complete tautomer calculations can be found in Supporting Information.

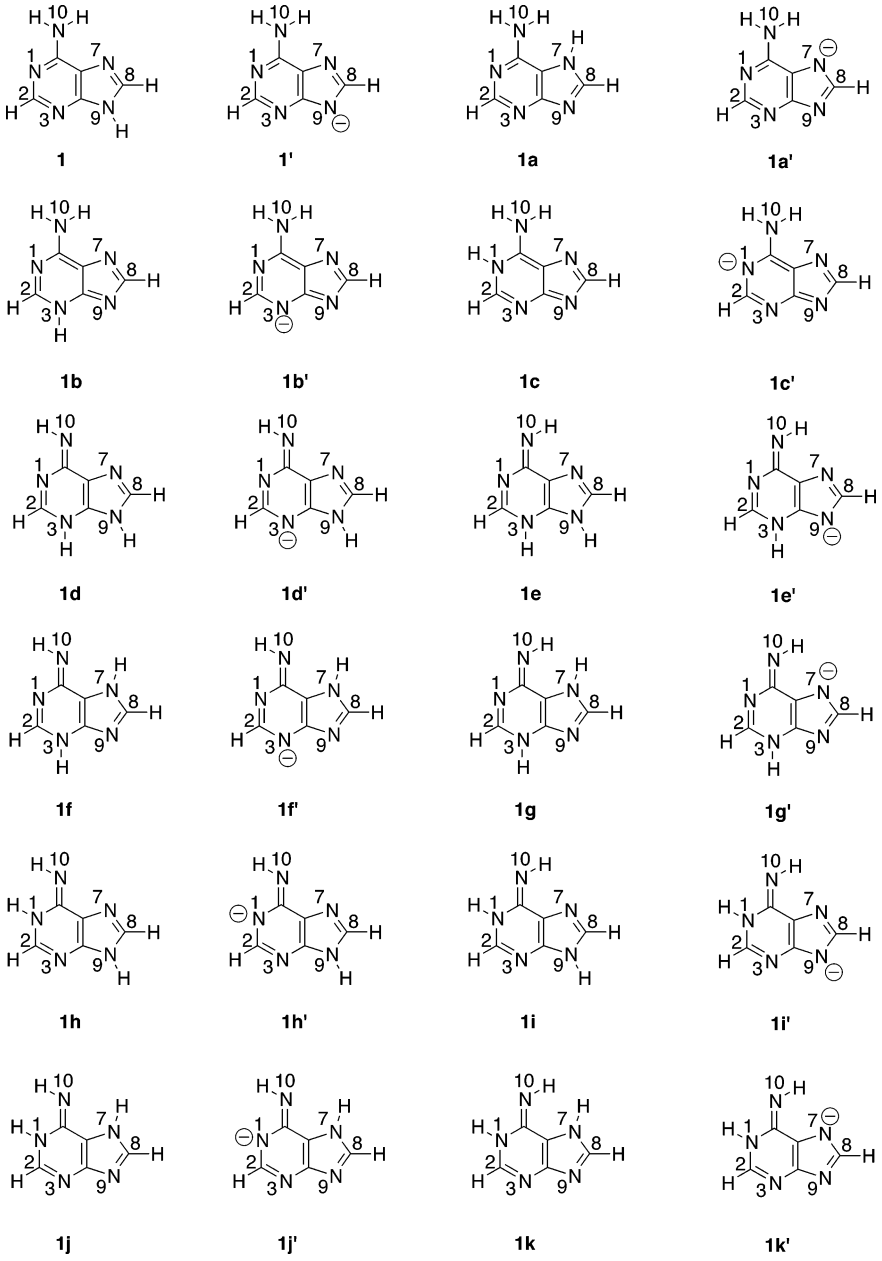


FIGURE 1. Adenine and deprotonated adenine tautomers.

typical aromatic proton acidity, 399.0 kcal mol^{-1} (the gas-phase acidity of benzene is 401.70 ± 0.50 kcal mol^{-1}).[19] The C8−H, however, is quite acidic, 373.1 kcal mol^{-1}, a value closer to that of HF (ΔH_{acid} = 371.3 kcal mol^{-1}) or acetone (ΔH_{acid} = 369.1 kcal mol^{-1}) than that of benzene.[19]

In 9-ethyladenine (**2**), like adenine, the C8−H acidity is calculated to be an acidic 373.8 kcal mol^{-1}, while the C2−H is much higher, at 399.5 kcal mol^{-1}. This pattern is repeated in *N*,*N*-dimethyladenine (**5**), where the C8−H is considerably more acidic than the C2−H. 3-Methyladenine (**3**), however, shows the opposite trend: the C2−H is the acidic one (368.8 kcal mol^{-1}, calculated), while the C8−H is the less acidic site, at 399.9 kcal mol^{-1}. 1-Methyladenine shows yet a different pattern, wherein both the C2−H and the C8−H are acidic (calculated values, 374.6 and 375.6 kcal mol^{-1}, respectively). These differing trends in C−H acidity among the adenine derivatives piqued our interest: why are some sites so much more acidic than others?

The pattern that emerges when one examines the five structures is that the more acidic vinylic C−H site in each molecule is always adjacent to an N−R moiety (R = H, alkyl). This appears to be true regardless of whether the proton is attached to a carbon on the five- or the six-membered ring of the purine. For example, in 1-methyladenine, both the C−H on the six-membered and on the five-membered rings are adjacent to N−R groups, and both are quite acidic. The values are also very consistent: all the C−H sites that are not adjacent to an N−R group essentially have a calculated acidity of 399−400 kcal mol^{-1}. The acidities of the C−H sites adjacent to an N−R group are quite consistent as well, ranging between 369 and 376 kcal mol^{-1}.

To test our hypothesis that vinylic C−H sites next to N−R moieties are more acidic than others, we calculated

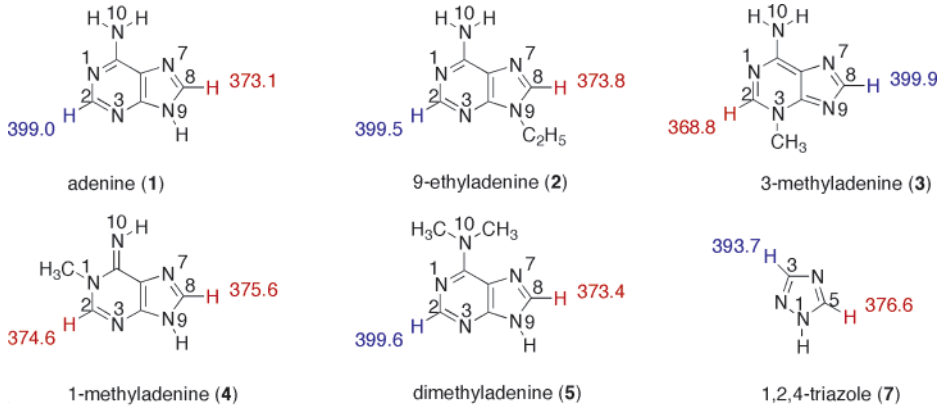


FIGURE 2. Calculated C2 and C8 acidities of adenine and alkyl derivatives using B3LYP/6-31+G*, at 298 K. Less acidic sites are indicated in blue, while more acidic sites are indicated in red.

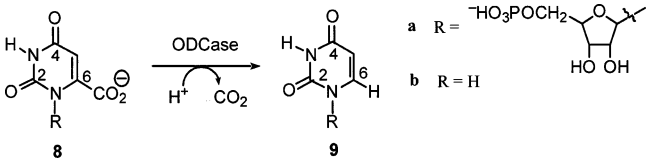
TABLE 8. Gas-Phase Acidities (ΔH_{acid}) of C3 and C5 Sites of 1,2,4-Triazole (7) Calculated at B3LYP/6-31+G* in kcal mol^{-1} [a]

substrate	C3	C5
triazole (**7**)	393.7	376.6

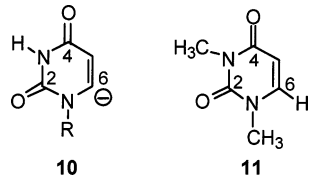
[a] At 298 K.

the C−H acidities on the model compound 1,2,4-triazole (**7**). Within this molecule, there are two C−H sites; by our hypothesis, the site between the two imine nitrogens (C3−H) should be less acidic than the site adjacent to the N−H (C5−H). The calculated acidity values are shown in Table 8 and Figure 2 and are consistent with our hypothesis. The C3−H is less acidic than water, at 393.7 kcal mol^{-1}, while the C5−H is significantly more acidic, 376.6 kcal mol^{-1}.

Acidities are a feature of reactivity that tend to figure largely in biological mechanisms. One mechanism that has long been of interest to us is that of orotate ribose monophosphate decarboxylase (ODCase), an enzyme that catalyzes the transformation of orotate ribose monophosphate (**8a**) to uracil ribose monophosphate (**9a**).[7,31−3631−36]



The mechanism by which the enzyme catalyzes this last step in the de novo biosynthesis of pyrimidines has long been in question. A particularly intriguing feature of the transformation is that decarboxylation of the orotate **8** results in a vinylic anion (**10**) that has no π-system into which to delocalize, which is unusual for a biological decarboxylation. The C6−H of uracil (**9b**) is calculated to be unusually acidic (362−365 kcal mol^{-1}, depending on level of calculation), and the acidity of the C6−H of 1,3-dimethyluracil (**11**) has been measured at 369.9 ± 3.1 kcal mol^{-1}.[23,37]



This unusually high gas-phase acidity is of interest because it may imply that the decarboxylation is not so difficult to execute in a nonpolar environment. That is, if the uracil C6−H is acidic, the resultant anion **10** may be quite stable and the decarboxylation may proceed more rapidly. Wolfenden and co-workers measured the acidity of 1,3-dimethyluracil (**11**) in water and found it to have a pK_a of 34, comparable to that of the C2−H in indole (pK_a = 36) but much lower than the pK_a of benzene (pK_a = 43).[38−40] Why is the uracil C6−H so acidic? With these purine calculations, we speculate that perhaps the adjacent N−R group at the N1-position might have more of an influence than we previously thought. This hypothesis is consistent with Wolfenden's findings; the C−H to which he compares the uracil acidity is adjacent to the N−H group in indole.

Why are the C−H sites adjacent to N−R groups particularly acidic? One possibility is that for those carbanions with an adjacent N−R group, the nitrogen lone pair can delocalize into the π-system, making that N more "positive", which could in turn electrostatically stabilize the adjacent carbanion (Scheme 1). One reviewer of our original manuscript also noted that, for example, the C2 carbanion of adenine must experience significant electrostatic repulsion from the two in-plane nitrogen lone pairs that flank it; in contrast, the C8

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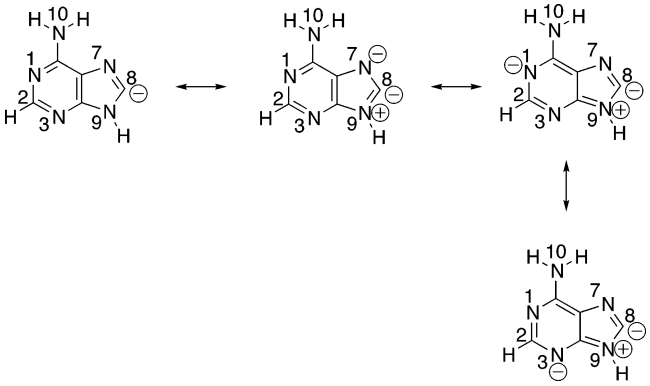
SCHEME 1



carbanion is adjacent to only one in-plane lone pair. To computationally explore these possibilities, we calculated the electrostatic potential for the relevant species. The electrostatic potential surfaces for adenine C2$^-$ and adenine C8$^-$ are shown in Figure 3. The color at each

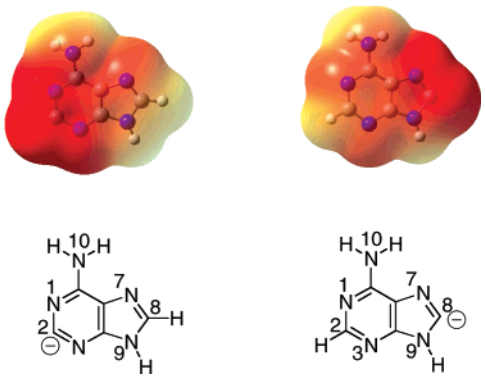
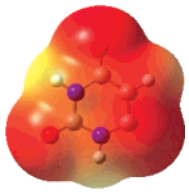


FIGURE 3. Calculated electrostatic potential surfaces for adenine C2$^-$ and adenine C8$^-$. Red indicates negative potential, while blue indicates positive potential.

point on these surfaces reflects the interaction energy between the molecule and a positive test charge at that point. A red color indicates attractive potential, while blue represents a repulsive potential. These anions have an attractive potential to a positive test charge, so the overall surface is quite red. The areas of pale red indicate a less "negative" region; yellow/green indicates a more neutral or positive region, depending on the bluishness of the color. The C2 site of the C2$^-$ ion appears to be flanked by quite a large negative cloud, consistent with the argument that the two in-plane nitrogen lone pairs provide substantial electrostatic repulsion. The C8 site of the C8$^-$ ion, on the other hand, is surrounded by a very red N7 but a less red N9. Furthermore, the proton on N9 is almost green, indicating a less negative environment. The slightly positive charge on the N−H group is consistent with delocalization of the N lone pair into the π-system. This pattern of electrostatic repulsion of carbanions flanked by two imine nitrogens and stabilization of carbanions with an adjacent N−R group holds true for all the calculated adenine derivatives and the deprotonated triazole anions. We have also found that the uracil C6$^-$ ion, discussed earlier in the context of OMP decarboxylase, has an electrostatic potential surface that implies stabilization of the C6 site by the adjacent N1−H group (Figure 4).



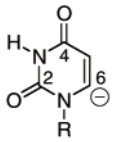


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FIGURE 4. Calculated electrostatic potential surface for uracil C6$^-$ ion. Red indicates negative potential, while blue indicates positive potential.

Conclusions

We have calculated and measured the acidities of multiple sites on a series of alkyl adenine derivatives. Calculations indicate that carbon-H sites adjacent to N−R groups are surprisingly acidic, with values comparable to that of HF and alcohols in the gas phase. In contrast, carbon-H groups situated between two imine nitrogens on a purine ring are much less acidic, by more than 20 kcal mol^{-1}. Our results are consistent with solution-phase observations such as that the C2 position in pyrimidine is the least acidic and that very strong bases abstract H-2 from *N*-alkyl imidazoles with total specificity.[41−45] Possible rationalization of these results include the N−R groups providing electrostatic stabilization of the adjacent carbanion and the imine nitrogen lone pairs providing electrostatic repulsion of the adjacent carbanion. Electrostatic potential calculations are consistent with these arguments. Furthermore, our results have implications in the biological decarboxylation of orotate ribose monophosphate.

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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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