JOC Article

pubs.acs.org/joc

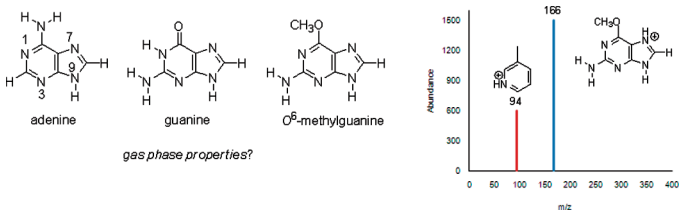
Gas-Phase Thermochemical Properties of the Damaged Base O^6-Methylguanine versus Adenine and Guanine

Anna Zhachkina, Min Liu, Xuejun Sun, F. Sedinam Amegayibor, and Jeehiun K. Lee*

Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08901

jeehiun@rci.rutgers.edu

Received July 10, 2009



The gas phase acidity (ΔH_{acid} and ΔG_{acid}) and proton affinity (PA, and gas phase basicity (GB)) of adenine, guanine, and O^6-methylguanine (OMG) have been examined using both theoretical (B3LYP/6-31+G*) and experimental (bracketing, Cooks kinetic) methods. We previously measured the acidity of adenine using bracketing methods; herein we measure the acidity of adenine by the Cooks kinetic method (ΔH_{acid} = 335 ± 3 kcal mol^{-1}; ΔG_{acid} = 329 ± 3 kcal mol^{-1}). We also measured the PA/GB of adenine using both bracketing and Cooks methods (PA = 224 and 225 kcal mol^{-1}; GB = 216 and 217 kcal mol^{-1}). Guanine is calculated to have several stable tautomers in the gas phase, in contrast to in solution, where the canonical tautomer predominates. Experimental measurements of gas phase guanine properties are difficult due to its nonvolatility; using electrospray and the Cooks kinetic method, we are able to measure a ΔH_{acid} of 335 ± 3 kcal mol^{-1} (ΔG_{acid} = 328 ± 3 kcal mol^{-1}). The proton affinity is 227 ± 3 kcal mol^{-1} (GB = 219 ± 3 kcal mol^{-1}). Comparison of these values to calculations indicates that we may have a mixture of the keto and enol tautomers under our conditions in the gas phase, although it is also possible that we only have the canonical form since in the Cooks method, we form the proton-bound dimers via electrospray of an aqueous solution, which should favor guanine in the canonical form. We also examined O^6-methylguanine (OMG), a highly mutagenic damaged base that arises from the alkylation of guanine. Our calculations indicate that OMG may exist as both the "N9" (canonical) and "N7" (proton on N7 rather than N9) tautomers in the gas phase, as both are calculated to be within 3 kcal mol^{-1} in energy. We have bracketed the acidity and proton affinity of OMG, which were previously unknown. The more acidic site of OMG has a ΔH_{acid} value of 338 ± 3 kcal mol^{-1} (ΔG_{acid} = 331 ± 3 kcal mol^{-1}). We have also bracketed the less acidic site (ΔH_{acid} = 362 ± 3 kcal mol^{-1}, ΔG_{acid} = 355 ± 3 kcal mol^{-1}) and the PA (229 ± 4 kcal mol^{-1} (GB = 222 ± 4 kcal mol^{-1})). We confirmed these results through Cooks kinetic method measurements as well. Our ultimate goal is to understand the intrinsic reactivity of nucleobases; gas phase acidic and basic properties are of interest for chemical reasons and also possibly for biological purposes, since biological media can be quite nonpolar. We find that OMG is considerably *less* acidic at N9 than adenine and guanine and *less* basic at O6 than guanine; the biological implications of these differences are discussed.

Introduction

The intrinsic, gas phase acidic and basic properties of nucleobases are of interest for purely chemical reasons but also could be of importance for biological reasons, since biological environs can be relatively nonpolar in nature.[1,2] Hydrogen bonding modulates recognition of DNA and RNA bases, and the interaction energy between two complementary nucleobases that are held together by NH−O and NH−N hydrogen bonds is dependent on the intrinsic

(1) Simonson, T.; Brooks, C. L. III *J. Am. Chem. Soc.* **1996**, *118*, 8452–8458.

(2) Jordan, F.; Li, H.; Brown, A. *Biochemistry* **1999**, *38*, 6369–6373.

DOI: 10.1021/jo901479m Published on Web 09/04/2009
© 2009 American Chemical Society

basicity of the acceptor atoms as well as on the acidity of the NH donor groups.[3,4]

Knowledge of and comparison of gas phase acidities and proton affinities to solution values can yield valuable information on intrinsic nucleobase reactivity and the role of solvent in affecting base reactivity.[5−13] In essence, gas phase experiments can provide the link between calculations and condensed phase data.

In previous work, we have reported the gas phase thermochemical properties of uracil and adenine, as well as several damaged purine nucleobases.[5−13] Damaged DNA bases differ in structure and properties from normal nucleobases and therefore intervene with gene replication and expression, leading to cell death, aging, and carcinogenesis.[14−17] Our studies are motivated by understanding the mechanisms by which mutated bases are cleaved.[5,6,8,9,11,13,18] The first step toward understanding how normal bases differ from damaged bases is to characterize the naturally occurring normal compounds.

The purine nucleobases have previously been the subject of both theoretical and experimental studies; to our knowledge, with the exception of work carried out in our lab, there have been two experimental measurements of the gas phase proton affinity of the most basic site of adenine, one of the proton affinity of guanine, and no experimental measurements of acidity of any purine.[4,19−31] Herein, we provide a comprehensive examination of the gas phase thermochemical properties of the naturally occurring purine bases adenine and guanine as well as of the damaged guanine base O^6-methylguanine. Where possible, we measure *multiple* acidities (more and less acidic sites, not heretofore accomplished) as well as proton affinities. We find OMG to be less basic at O6 than guanine and less acidic at N9 than adenine and guanine; these results have interesting biological implications, which are discussed.

Results

Adenine. Calculations: Adenine Tautomers. Adenine, as with all nucleobases, has several possible tautomeric forms (three most stable shown in Figure 1; see Supporting Information for higher energy tautomers).[4,25,32−36] At B3LYP/6-31+G*, we find that the canonical tautomer "N9H" (**1**) is the most stable. The next most stable tautomers are much higher in energy (by 8 kcal mol^{-1}): the "N7H" tautomer and the "N3H" tautomer. The next most stable tautomers are more than 12 kcal mol^{-1} less stable than the canonical.

Calculations: Adenine Acidity. The calculated values for the acidity of the three most stable tautomers of adenine are shown in red in Figure 1.[4,25,32−36] The ΔH_{acid} of the most acidic site for the canonical tautomer is calculated to be 334.8 kcal mol^{-1} (ΔG_{acid} = 327.9 kcal mol^{-1}). The two higher energy tautomers are more acidic, with the most acidic site on each (the N7-proton for the N7H tautomer **1a** and N3-proton for the N3H tautomer **1b**) having a ΔH_{acid} of ~327 kcal mol^{-1} (ΔG_{acid} ~ 320 kcal mol^{-1}).

Calculations: Adenine Proton Affinity. The calculated values for the proton affinity (PA; $-\Delta H$) and gas phase basicity (GB; $-\Delta G$) of the three most stable tautomers of adenine are shown in blue in Figure 1.[4,25,32−37] The PA/GB of the most basic site of adenine varies by tautomer. For the canonical tautomer **1**, there are two sites that are close in basicity: the N1 (PA = 223.7 kcal mol^{-1}; GB = 215.7 kcal mol^{-1}) and the N3 (PA = 222.2 kcal mol^{-1}; GB = 214.2 kcal mol^{-1}). The most basic site for the N7H tautomer **1a** is the N3 site (PA = 231.9 kcal mol^{-1}; GB = 224.3 kcal mol^{-1}). The N3H tautomer **1b** has two sites that are close in basicity to the N7H tautomer and to each other: the N7 (PA = 231.8 kcal mol^{-1}; GB = 223.9 kcal mol^{-1}) and the N9 (PA = 230.3 kcal mol^{-1}; GB = 222.3 kcal mol^{-1}).

Experiments: Adenine Acidity. Bracketing. The ΔH_{acid} of adenine was first measured in our lab in a previous study, using bracketing methods in our FTMS.[8,11,12] The results for the more acidic site are shown in Table 1, where "ref acid" refers to the reaction of deprotonated adenine with the neutral reference acid and "conj base" refers to the reaction of the conjugate base of the reference acid with neutral adenine. We found that the reaction with hydrochloric acid (ΔH_{acid} = 333.4 kcal mol^{-1}; ΔG_{acid} = 328.1 kcal mol^{-1}) proceeds in both directions; deprotonated adenine can

(3) Nguyen, M. T.; Chandra, A. K.; Zeegers-Huyskens, T. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 1277–1280.
(4) Chandra, A. K.; Nguyen, M. T.; Uchimaru, T.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **1999**, *103*, 8853–8860.
(5) Kurinovich, M. A.; Lee, J. K. *J. Am. Chem. Soc.* **2000**, *122*, 6258–6262.
(6) Kurinovich, M. A.; Lee, J. K. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 985–995.
(7) Kurinovich, M. A.; Phillips, L. M.; Sharma, S.; Lee, J. K. *Chem. Commun.* **2002**, 2354–2355.
(8) Lee, J. K. *Int. J. Mass Spectrom.* **2005**, *240*, 261–272.
(9) Liu, M.; Xu, M.; Lee, J. K. *J. Org. Chem.* **2008**, *73*, 5907–5914.
(10) Pan, S.; Sun, X.; Lee, J. K. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1383–1395.
(11) Sharma, S.; Lee, J. K. *J. Org. Chem.* **2002**, *67*, 8360–8365.
(12) Sharma, S.; Lee, J. K. *J. Org. Chem.* **2004**, *69*, 7018–7025.
(13) Sun, X.; Lee, J. K. *J. Org. Chem.* **2007**, *72*, 6548–6555.
(14) O'Brien, P. J.; Ellenberger, T. *J. Biol. Chem.* **2004**, *279*, 9750–9757.
(15) Speina, E.; Kierzek, A. M.; Tudek, B. *Mutat. Res.* **2003**, *531*, 205–217.
(16) Stivers, J. T.; Jiang, Y. L. *Chem. Rev.* **2003**, *103*, 2729–2759.
(17) Berti, P. J.; McCann, J. A. B. *Chem. Rev.* **2006**, *106*, 506–555, and references therein.
(18) Bennett, M. T.; Rodgers, M. T.; Hebert, A. S.; Ruslander, L. E.; Eisele, L.; Drohat, A. C. *J. Am. Chem. Soc.* **2006**, *128*, 12510–12519.
(19) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1979**, *101*, 2396–2403.
(20) Greco, F.; Liguori, A.; Sindona, G.; Uccella, N. *J. Am. Chem. Soc.* **1990**, *112*, 9092–9096.
(21) Chen, E. C. M.; Chen, E. S. *J. Phys. Chem. B* **2000**, *104*, 7835–7844.
(22) Chen, E. C. M.; Herder, C.; Chen, E. S. *J. Mol. Struct.* **2006**, *798*, 126–133, and references therein.
(23) *NIST Chemistry WebBook, NIST Standard Reference Database Number 69, June 2005*; Linstrom, P. J., Mallard, W. G., Eds.; National Institute of Standards and Technology: Gaithersburg, MD, 2005; http://webbook.nist.gov.
(24) Huang, Y.; Kenttamaa, H. *J. Phys. Chem. A* **2003**, *107*, 4893–4897, and references therein.
(25) Podolyan, Y.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2000**, *104*, 7346–7352, and references therein.
(26) Yang, Z.; Rodgers, M. T. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2749–2757, and references therein.
(27) Yao, C.; Cuadrado-Peinado, M. L.; Polášek, M.; Turecek, F. *J. Mass. Spec.* **2005**, *40*, 1417–1428, and references therein.
(28) Yao, C.; Turecek, F.; Polce, M. J.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **2007**, *265*, 106–123.
(29) Turecek, F.; Yao, C. *J. Phys. Chem. A* **2003**, *107*, 9221–9231.
(30) Kobayashi, R. *J. Phys. Chem. A* **1998**, *102*, 10813–10817.
(31) Wolken, J. K.; Yao, C.; Turecek, F.; Polce, M. J.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **2007**, *267*, 30–42.
(32) Hanus, M.; Kabelac, M.; Rejnek, J.; Ryjacek, F.; Hobza, P. *J. Phys. Chem. B* **2004**, *208*, 2087–2097.
(33) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18–29.
(34) (a) Russo, N.; Toscano, M.; Grand, A.; Jolibois, F. *J. Comput. Chem.* **1998**, *19*, 989–1000. (b) Del Bene, J. E. *J. Phys. Chem.* **1983**, *87*, 367–371.
(35) Huang, Y.; Kenttämaa, H. *J. Phys. Chem. A* **2004**, *108*, 4485–4490.
(36) Colominas, C.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1996**, *118*, 6811–6821, and references therein.
(37) McConnell, T. L.; Wheaton, C. A.; Hunter, K. C.; Wetmore, S. D. *J. Phys. Chem. A* **2005**, *109*, 6351–6362.

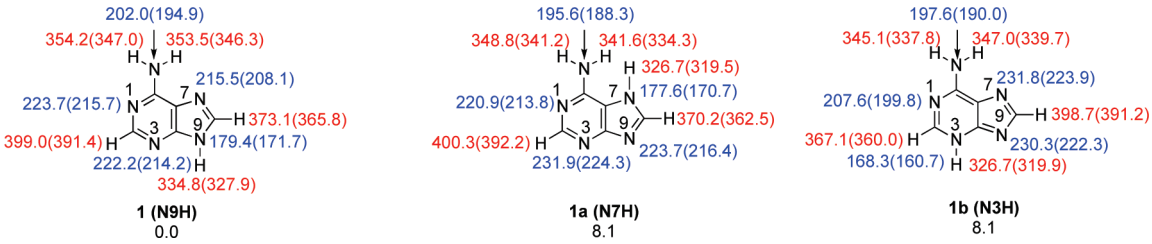


FIGURE 1. Relative enthalpies (ΔH in kcal mol^{-1}) of three possible tautomers of adenine and the acidities (red values; ΔH_{acid}, with ΔG_{acid} in parentheses; all values in kcal mol^{-1}) and proton affinities (blue values; PA, with GB in parentheses; all values in kcal mol^{-1}), calculated at B3LYP/6-31+G* (298 K).

TABLE 1. Summary of Results for Acidity Bracketing of More Acidic Site of Adenine[11,38]

ref compound	ΔH_{acid}[a]	ΔG_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conj base
acetic acid	347.4 ± 0.5	341.2 ± 0.5	−	+
2,4-pentadione	343.8 ± 2.1	336.7 ± 2.0	−	+
trifluoro-*m*-cresol	339.3 ± 2.1	332.4 ± 2.0	−	+
2-chloropropanoic acid	337.0 ± 2.1	330.4 ± 2.0	−	+
2-bromopropanoic acid	336.8 ± 2.1	329.8 ± 2.0	−	+
hydrochloric acid	333.4 ± 0.1	328.1 ± 0.2	+	+
pyruvic acid	333.5 ± 2.9	326.5 ± 2.8	+	−
difluoroacetic acid	331.0 ± 2.2	323.8 ± 2.0	+	−
1,1,1-trifluoro-2,4-pentadione	328.3 ± 2.9	322.0 ± 2.0	+	−

[a]Acidities are in kcal mol^{-1}.[23] [b]"+" indicates the occurrence and "−" indicates the absence of proton transfer.

deprotonate pyruvic acid (ΔH_{acid} = 333.5 kcal mol^{-1}; ΔG_{acid} = 326.5 kcal mol^{-1}) but not 2-bromopropanoic acid (ΔH_{acid} = 336.8 kcal mol^{-1}; ΔG_{acid} = 329.8 kcal mol^{-1}); pyruvate does not deprotonate adenine, but 2-bromopropanoate does. We therefore bracket the ΔH_{acid} value for the more acidic site in adenine as 333 ± 2 kcal mol^{-1} (ΔG_{acid} = 328 ± 2 kcal mol^{-1}) (Table 1).[5−9,11−13]

We also used methodology developed in our lab to measure the less acidic site of adenine (previously published, Table 2).[5−9,11−13] We find that deprotonated adenine under "less acidic conditions" (see Experimental Section) cannot deprotonate acids equal to and less acidic than 4-(trifluoromethyl)-aniline (ΔH_{acid} = 353.3 kcal mol^{-1}; ΔG_{acid} = 346.0 kcal mol^{-1}) but can deprotonate acids equal to and more acidic than *m*-cresol (ΔH_{acid} = 349.6 kcal mol^{-1}; ΔG_{acid} = 342.7 kcal mol^{-1}). We therefore bracket the less acidic site of adenine as ΔH_{acid} = 352 ± 4 kcal mol^{-1} (ΔG_{acid} = 344 ± 4 kcal mol^{-1}).

Cooks Kinetic Method. We also measured the acidity of adenine using the Cooks kinetic method, in a quadrupole ion trap (see Experimental Section for details), to compare to our earlier bracketing measurements. The reference acids 3-trifluorotoluic acid (ΔH_{acid} = 332.2 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 325.2 ± 2.0 kcal mol^{-1}), pyruvic acid (ΔH_{acid} = 333.5 ± 2.9 kcal mol^{-1}; ΔG_{acid} = 326.5 ± 2.8 kcal mol^{-1}), 2-chlorobenzoic acid (ΔH_{acid} = 335.1 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 328.2 ± 2.0 kcal mol^{-1}), 4-hydroxybenzoic acid (ΔH_{acid} = 335.9 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 328.9 ± 2.0 kcal mol^{-1}), 2-bromopropanoic acid (ΔH_{acid} = 336.8 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 329.8 ± 2.0 kcal mol^{-1}), and 2-chloropropanoic acid (ΔH_{acid} = 337.0 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 330.4 ± 2.0 kcal mol^{-1}) were used, yielding a ΔH_{acid} = 335 ± 3 kcal mol^{-1} (ΔG_{acid} = 329 ± 3 kcal mol^{-1}).

TABLE 2. Summary of Results for Acidity Bracketing of Less Acidic Site of Adenine[11,38]

ref compound	ΔH_{acid}[a]	ΔG_{acid}[a]	proton transfer[b]
chloroform	357.6 ± 2.1	349.9 ± 2.0	−
2-butenal	354.7 ± 2.1	348.1 ± 2.0	−
4-(trifluoromethyl)-aniline	353.3 ± 2.1	346.0 ± 2.0	−
m-cresol	349.6 ± 2.1	342.7 ± 2.0	+
1,1,1-trifluoroacetone	349.2 ± 2.1	342.1 ± 2.0	+
acetic acid	347.4 ± 0.5	341.2 ± 0.5	+
formic acid-d_2	345.3 ± 2.2	338.3 ± 2.0	+
2,4-pentadione	343.8 ± 2.1	336.7 ± 2.0	+
trifluoro-*m*-cresol	339.3 ± 2.1	332.4 ± 2.0	+

[a]Acidities are in kcal mol^{-1}.[23] [b]"+" indicates the occurrence and "−" indicates the absence of proton transfer.

TABLE 3. Summary of Results for PA Bracketing of More Basic Site of Adenine

ref compound	PA[a]	GB[a]	proton transfer[b] ref base	proton transfer[b] conj acid
piperidine	228.0 ± 2.0	220.0 ± 2.0	+	−
4-picoline	226.4 ± 2.0	218.8 ± 2.0	+	−
3-picoline	225.5 ± 2.0	217.9 ± 2.0	+	−
tert-amylamine	224.1 ± 2.0	216.0 ± 2.0	+	+
cyclohexylamine	223.3 ± 2.0	215.0 ± 2.0	−	+
pyridine	222.3 ± 2.0	214.7 ± 2.0	−	+
N-methyl propanamide	220.0 ± 2.0	212.6 ± 2.0	−	+

[a]Values are in kcal mol^{-1}.[23] [b]"+" indicates the occurrence and "−" indicates the absence of proton transfer.

Experiments: Adenine Proton Affinity. Bracketing. The bracketing results for the proton affinity of the most basic site of adenine are shown in Table 3, where "ref base" refers to the reaction of the neutral reference base with protonated adenine and "conj acid" refers to the reaction of the protonated reference base with neutral adenine. We find that the reaction between adenine and *tert*-amylamine (PA = 224.1 kcal mol^{-1}; GB = 216.0 kcal mol^{-1}) proceeds in both directions (adenine deprotonates protonated *tert*-amylamine and *tert*-amylamine deprotonates protonated adenine); with 3-picoline (PA = 225.5 kcal mol^{-1}; GB = 217.9 kcal mol^{-1}), reaction with protonated adenine does occur, but the opposite reaction does not. With cyclohexylamine (PA = 223.3 kcal mol^{-1}; GB = 215.0 kcal mol^{-1}), adenine can deprotonate protonated cyclohexylamine, but the opposite reaction does not proceed. These data point to a PA for adenine of 224 ± 3 kcal mol^{-1} (GB = 216 ± 3 kcal mol^{-1}).

Cooks Kinetic Method. We also measured the proton affinity of adenine using the Cooks kinetic method. The references bases 1-octanamine (PA = 222.0 ± 2.0 kcal mol^{-1}; GB = 213.9 ± 2.0 kcal mol^{-1}), pyridine (PA = 222.3 ± 2.0 kcal mol^{-1}; GB = 214.7 ± 2.0 kcal mol^{-1}), cyclohexylamine

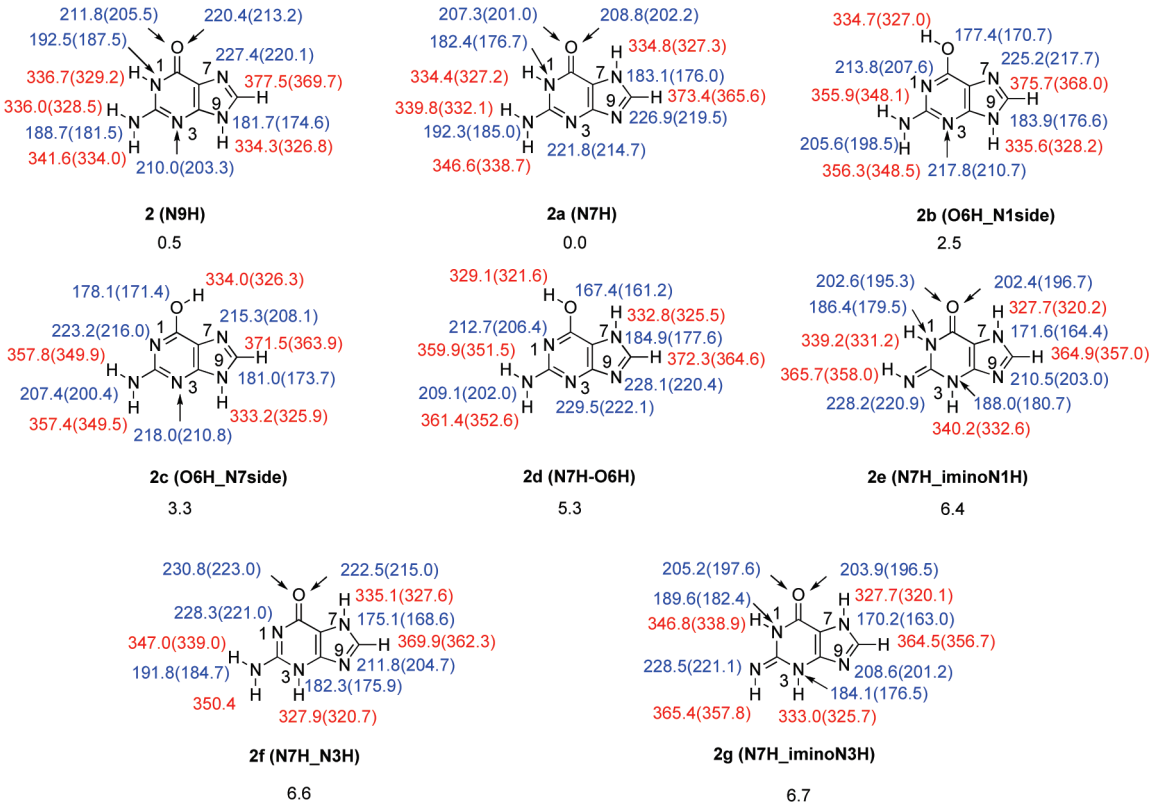


FIGURE 2. Relative enthalpies (ΔH in kcal mol^{-1}) of eight possible tautomers of guanine and acidities (red values; ΔH_{acid}, with ΔG_{acid} in parentheses; all values in kcal mol^{-1}) and proton affinities (blue values; PA, with GB in parentheses; all values in kcal mol^{-1}) of the eight most stable tautomers, calculated at B3LYP/6-31+G* (298 K).

(PA = 223.3 ± 2.0 kcal mol^{-1}; GB = 215.0 ± 2.0 kcal mol^{-1}), 3-picoline (PA = 225.5 ± 2.0 kcal mol^{-1}; GB = 217.9 ± 2.0 kcal mol^{-1}), and 4-picoline (PA = 226.4 ± 2.0 kcal mol^{-1}; GB = 218.8 ± 2.0 kcal mol^{-1}) were used, yielding a PA for adenine of 225 ± 3 kcal mol^{-1} (GB = 217 ± 3 kcal mol^{-1}).

Guanine. Calculations: Guanine Tautomers. Unlike adenine, guanine has more than one tautomer that is low-lying in energy (eight most stable tautomers are shown in Figure 2; all optimized tautomers are in Supporting Information).[4,25,32,34−36,39] At B3LYP/6-31+G*, the canonical structure **2** is slightly (0.5 kcal mol^{-1}) less stable than the "N7H" tautomer **2a**. The two enol tautomer forms of the canonical structure (rotamers **2b** and **2c**) are 2.5 and 3.3 kcal mol^{-1} less stable than **2a**. The next lowest-lying tautomer, an enol form of the N7H tautomer (**2d**), is 5.3 kcal mol^{-1} less stable than the N7H tautomer **2a**. The last three tautomers that are energetically within 10 kcal mol^{-1} of **2a** are the imino tautomers **2e** and **2g** and the N7H_N3H tautomer **2f**. All three are between 6 and 7 kcal mol^{-1} less stable than **2a**. The next lowest-lying tautomer is 13.4 kcal mol^{-1} higher in energy than **2a**; all other tautomers are even less stable.

Calculations: Guanine Acidity. The acidities of the eight lowest-lying tautomers of guanine are shown in Figure 2, in red.[40] The most acidic site of the four lowest energy tautomers are all in a narrow range: ΔH_{acid} = 333−335 kcal mol^{-1} (ΔG_{acid} = 326−327 kcal mol^{-1}).[4,25,32,34−36,39] For canonical structure **2**, the most acidic proton is attached to the N9 (ΔH_{acid} = 334.3 kcal mol^{-1}, ΔG_{acid} = 326.8 kcal mol^{-1}). For **2a**, the N1−H is the most acidic (ΔH_{acid} = 334.4 kcal mol^{-1}, ΔG_{acid} = 327.2 kcal mol^{-1}), though the N7−H is very close (ΔH_{acid} = 334.8 kcal mol^{-1}, ΔG_{acid} = 327.3 kcal mol^{-1}). For **2b**, the most acidic site is the enol proton (ΔH_{acid} = 334.7 kcal mol^{-1}, ΔG_{acid} = 327.0 kcal mol^{-1}), with the N9 proton being just 1 kcal mol^{-1} less acidic (ΔH_{acid} = 335.6 kcal mol^{-1}, ΔG_{acid} = 328.2 kcal mol^{-1}). For **2c**, the most acidic sites are also the enol and the N9 (N9 proton: ΔH_{acid} = 333.2 kcal mol^{-1}, ΔG_{acid} = 325.9 kcal mol^{-1}; enol proton: ΔH_{acid} = 334.0 kcal mol^{-1}, ΔG_{acid} = 326.3 kcal mol^{-1}). The tautomers **2d**-**2g** are all more than 5 kcal mol^{-1} less stable than the most stable structure **2a** so are unlikely to be found experimentally; these tautomers are more acidic than the four lower energy structures (ΔH_{acid} = 328−329 kcal mol^{-1}, ΔG_{acid} = 320−322 kcal mol^{-1}).

Calculations: Guanine Proton Affinity. The calculated values for the proton affinity and gas phase basicity of the eight most stable tautomers of guanine are shown in blue in Figure 2.[4,25,32,34−37,39] The PA/GB of the most basic site of guanine varies by tautomer. The canonical structure **2** has a

(38) Eyet, N.; Villano, S. M.; Bierbaum, V. M. *Int. J. Mass Spectrom.* **2009**, *283*, 26–29.

(38) Eyet, N.; Villano, S. M.; Bierbaum, V. M. *Int. J. Mass Spectrom.* **2009**, *283*, 26–29.

(39) (a) Jang, Y. H.; Goddard, W. A. III; Noyes, K. T.; Sowers, L. C.; Hwang, S.; Chung, D. S. *J. Phys. Chem. B* **2003**, *107*, 344–357. (b) Shukla, M. K.; Leszczynski, J. *Chem. Phys. Lett.* **2006**, *429*, 261–265. (c) Seefeld, K.; Brause, R.; Haber, T.; Kleinermanns, K. *J. Phys. Chem. A.* **2007**, *111*, 6217–6221.

(40) If more than one value is listed for an atom, the arrows show the site of protonation (for example, the O6 of guanine can be protonated on the "N1 side" and on the "N7 side").

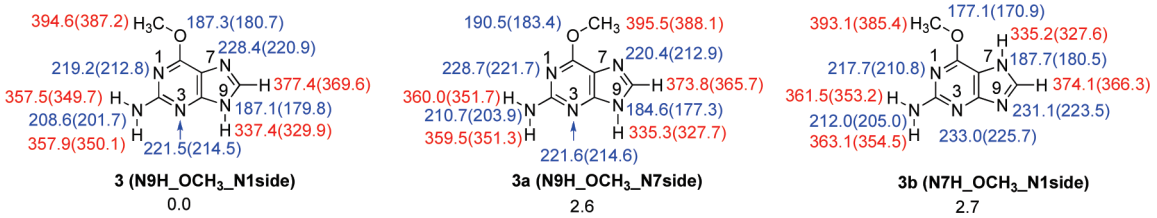


FIGURE 3. Relative enthalpies (ΔH in kcal mol^{-1}) of the three most stable OMG tautomers and acidities (red values; ΔH_{acid}, with ΔG_{acid} in parentheses; all values in kcal mol^{-1}) and proton affinities (blue values; PA, with GB in parentheses; all values in kcal mol^{-1}), calculated at B3LYP/6-31+G* (298 K).

calculated PA of 227.4 kcal mol^{-1} (GB = 220.1 kcal mol^{-1}), at the N7. The most basic site of **2a** is the N9, and has a similar value (PA = 226.9 kcal mol^{-1}; GB = 219.5 kcal mol^{-1}). The basicity of the enols **2b** and **2c** are slightly lower than that of the canonical (PA = 225.2 and 223.2 kcal mol^{-1}; GB = 217.7 and 216.0 kcal mol^{-1}, respectively). The four remaining tautomers **2d-2g** are similar in basicity to each other (PA = 228−231 kcal mol^{-1}; GB = 221−223 kcal mol^{-1}).

Experiments: Guanine Acidity. Cooks Kinetic Method. We could not conduct FTMS bracketing experiments with guanine as it is not readily vaporized via our solids probe. We therefore assessed the acidity of guanine using the Cooks kinetic method in our quadrupole ion trap, using electrospray to generate the proton-bound dimers (see Experimental Section). The reference acids 4-cyanophenol (ΔH_{acid} = 332.2 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 325.3 ± 2.0 kcal mol^{-1}), pyruvic acid (ΔH_{acid} = 333.5 ± 2.9 kcal mol^{-1}; ΔG_{acid} = 326.5 ± 2.8 kcal mol^{-1}), 4-hydroxybenzoic acid (ΔH_{acid} = 335.9 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 328.9 ± 2.0 kcal mol^{-1}), and 2-chloropropanoic acid (ΔH_{acid} = 337.0 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 330.4 ± 2.0 kcal mol^{-1}) were used, to yield a ΔH_{acid} for guanine of 335 ± 3 kcal mol^{-1} (ΔG_{acid} = 328 ± 3 kcal mol^{-1}).

Experiments: Guanine Proton Affinity. Cooks Kinetic Method. The proton affinity of guanine was measured, also using the Cooks kinetic method. The references bases cyclohexylamine (PA = 223.3 ± 2.0 kcal mol^{-1}; GB = 215.0 ± 2.0 kcal mol^{-1}), 3-picoline (PA = 225.5 ± 2.0 kcal mol^{-1}; GB = 217.9 ± 2.0 kcal mol^{-1}), 4-picoline (PA = 226.4 ± 2.0 kcal mol^{-1}; GB = 218.8 ± 2.0 kcal mol^{-1}), pyrrolidine (PA = 226.6 ± 2.0 kcal mol^{-1}; GB = 218.8 ± 2.0 kcal mol^{-1}), piperidine (PA = 228.0 ± 2.0 kcal mol^{-1}; GB = 220.0 ± 2.0 kcal mol^{-1}), and 2,4-lutidine (PA = 230.1 ± 2.0 kcal mol^{-1}; GB = 222.5 ± 2.0 kcal mol^{-1}) were used. The measured proton affinity (PA = ΔH) for guanine is 227 ± 3 kcal mol^{-1} (gas phase basicity = GB (ΔG) = 219 ± 3 kcal mol^{-1}).

***O*^{6}-Methylguanine (OMG). Calculations: OMG Tautomers.** At B3LYP/6-31+G*, the canonical "N9H" structure of OMG is the most stable (**3**, Figure 3; higher energy tautomers are in Supporting Information). The rotamer wherein the methyl group is "pointing" toward the N7 (rather than the N1, **3a**) is 2.6 kcal mol^{-1} higher in energy; the methyl should freely rotate (we calculate the barrier to rotation to be 6.9 kcal mol^{-1}) so at room temperature, the majority of molecules will be in the more stable minimum-energy structure.[41] The next most stable tautomer is the N7H structure (**3b**), which is 2.7 kcal mol^{-1} less stable than the canonical. All remaining tautomers are more than 10 kcal mol^{-1} less stable than the canonical N9H structure.

(41) Michalczyk, R.; Russu, I. M. *Biophys. J.* **1999**, *76*, 2679–2686.

TABLE 4. Summary of Results for Acidity Bracketing of More Acidic Site of OMG

ref compound	ΔH_{acid}[a]	ΔG_{acid}[a]	proton transfer[b] ref acid	proton transfer[b] conj base
2,4-pentadione	343.8 ± 2.1	336.7 ± 2.0	−	+
2-chlorophenol	343.4 ± 2.3	337.1 ± 2.0	−	+
ethoxyacetic acid	342.0 ± 2.2	335.0 ± 2.0	−	+
trifluoro-*m*-cresol	339.3 ± 2.1	332.4 ± 2.0	−	+
2-chloropropanoic acid	337.0 ± 2.1	330.4 ± 2.0	+	−
2-bromopropanoic acid	336.8 ± 2.1	329.8 ± 2.0	+	−
pyruvic acid	333.5 ± 2.9	326.5 ± 2.8	+	−

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

Calculations: OMG Acidity. The most acidic site of the canonical tautomer **3** is the N9−H (ΔH_{acid} = 337.4 kcal mol^{-1}; ΔG_{acid} = 329.9 kcal mol^{-1}, Figure 3, in red). The rotamer **3a** is slightly more acidic at N9 (ΔH_{acid} = 335.3 kcal mol^{-1}; ΔG_{acid} = 327.7 kcal mol^{-1}) and comparable in acidity to the N7 tautomer (N7−H, ΔH_{acid} = 335.2 kcal mol^{-1}; ΔG_{acid} = 327.6 kcal mol^{-1}).

Calculations: OMG Proton Affinity. The most basic site of the most stable form of OMG (canonical form **3**) has a calculated PA of 228.4 kcal mol^{-1} (GB = 220.9 kcal mol^{-1}) at N7 (Figure 3, in blue). The rotamer **3a** has a comparable basicity (at N1, PA = 228.7 kcal mol^{-1}, GB = 221.7 kcal mol^{-1}). The most basic site on the N7H tautomer is the N3 (PA = 233.0 kcal mol^{-1}; GB = 225.7 kcal mol^{-1}).

Experiments: OMG Acidity. Bracketing. The acidity bracketing results for the more acidic site of OMG are shown in Table 4. We find that deprotonated OMG cannot deprotonate trifluoro-*m*-cresol (ΔH_{acid} = 339.3 kcal mol^{-1}; ΔG_{acid} = 332.4 kcal mol^{-1}) but that the opposite reaction does occur. Deprotonated OMG does react with 2-chloropropanoic acid (ΔH_{acid} = 337.0; ΔG_{acid} = 330.4 kcal mol^{-1}), but 2-chloropropanoate does not deprotonate OMG. We therefore bracket the acidity of OMG to be ΔH_{acid} = 338 ± 3 kcal mol^{-1} (ΔG_{acid} = 331 ± 3 kcal mol^{-1}).

We also used the methodology developed in our lab to bracket the less acidic site of OMG (Table 5).[5−9,11−13] Deprotonated OMG under "less acidic" conditions does not deprotonate 2-fluoroaniline (ΔH_{acid} = 362.6 kcal mol^{-1}; ΔG_{acid} = 355.3 kcal mol^{-1}) but does deprotonate 2,2,2-trifluoroethanol (ΔH_{acid} = 361.7 kcal mol^{-1}; ΔG_{acid} = 354.1 kcal mol^{-1}). We therefore bracket the less acidic site of OMG to be ΔH_{acid} = 362 ± 3 kcal mol^{-1} (ΔG_{acid} = 355 ± 3 kcal mol^{-1}).

Cooks Kinetic Method. We also measured the acidity of OMG using the Cooks kinetic method. The reference acids 4-hydroxybenzoic acid (ΔH_{acid} = 335.9 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 328.9 ± 2.0 kcal mol^{-1}), 3-(chloromethyl)benzoic acid (ΔH_{acid} = 336.8 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 329.8 ± 2.0

TABLE 5. Summary of Results for Acidity Bracketing of Less Acidic Site of OMG

ref compound	ΔH_{acid}[a]	ΔG_{acid}[a]	proton transfer[b] ref acid
acetone	368.8 ± 2.0	361.9 ± 2.0	−
aniline	366.4 ± 2.1	359.1 ± 2.0	−
4-fluoroaniline	364.3 ± 2.1	357.0 ± 2.0	−
2-fluoroaniline	362.6 ± 2.2	355.3 ± 2.0	−
2,2,2-trifluoroethanol	361.7 ± 2.5	354.1 ± 2.0	+
pyrrole	359.5 ± 0.3	350.9 ± 2.0	+
4-(trifluoromethyl)-aniline	353.3 ± 2.1	346.0 ± 2.0	+
butanoic acid	346.8 ± 2.0	339.5 ± 2.0	+

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

kcal mol^{-1}), 2-fluorobenzoic acid (ΔH_{acid} = 338.1 ± 2.2 kcal mol^{-1}; ΔG_{acid} = 330.6 ± 2.0 kcal mol^{-1}), and 3-hydroxybenzoic acid (ΔH_{acid} = 338.6 ± 2.1 kcal mol^{-1}; ΔG_{acid} = 331.6 ± 2.0 kcal mol^{-1}) were used, yielding a ΔH_{acid} = 337 ± 3 kcal mol^{-1} (ΔG_{acid} = 330 ± 3 kcal mol^{-1}).

Experiments: OMG Proton Affinity. Bracketing. The proton affinity results for OMG are summarized in Table 6. We find that 1-methylpyrrolidine (PA = 230.8 kcal mol^{-1}; GB = 223.4 kcal mol^{-1}) does deprotonate protonated OMG, but the opposite reaction does not occur. Piperidine (PA = 228.0 kcal mol^{-1}; GB = 220.0 kcal mol^{-1}), however, is not basic enough to deprotonate protonated OMG; OMG does deprotonate protonated piperidine. The PA of OMG is therefore bracketed to be 229 ± 4 kcal mol^{-1} (GB = 222 ± 4 kcal mol^{-1}).

Cooks Kinetic Method. We also measured the proton affinity and gas phase basicity of OMG using the Cooks kinetic method. The reference bases piperidine (PA = 228.0 ± 2.0 kcal mol^{-1}; GB = 220.0 ± 2.0 kcal mol^{-1}), 2,4-lutidine (PA = 230.1 ± 2.0 kcal mol^{-1}; GB = 222.5 ± 2.0 kcal mol^{-1}), 1-methylpyrrolidine (PA = 230.8 ± 2.0 kcal mol^{-1}; GB = 223.4 ± 2.0 kcal mol^{-1}), *N, N*-dimethylbenzylamine (PA = 231.5 ± 2.0 kcal mol^{-1}; GB = 224.0 ± 2.0 kcal mol^{-1}), *N, N*-dimethylisopropylamine (PA = 232.0 ± 2.0 kcal mol^{-1}; GB = 224.6 ± 2.0 kcal mol^{-1}), and 1-methylpiperidine (PA = 232.1 ± 2.0 kcal mol^{-1}; GB = 224.7 ± 2.0 kcal mol^{-1}) were used. By this method, the PA for OMG is measured to be 231 ± 3 kcal mol^{-1} (GB = 223 ± 3 kcal mol^{-1}).

Discussion

Adenine. Although as with all nucleobases adenine has many tautomeric forms, our gas phase calculations and others' indicate that the canonical form should be most stable by a significant amount (>8 kcal mol^{-1}) in vacuo.[4,25,32−36] In previous work, we measured the acidity of the most acidic site of adenine to be ΔH_{acid} = 333 ± 2 kcal mol^{-1} (ΔG_{acid} = 328 ± 2 kcal mol^{-1}), in agreement with calculations of the N9−H acidity.[4,11,25,32−36] In this present work, we also confirmed these bracketing experiments with a Cooks kinetic method measurement (ΔH_{acid} = 335 ± 3 kcal mol^{-1} (ΔG_{acid} = 329 ± 3 kcal mol^{-1})).

The acidity of the less acidic site brackets to 352 ± 4 kcal mol^{-1} (ΔH_{acid}; ΔG_{acid} = 344 ± 4 kcal mol^{-1}), which is in agreement with the calculated value for the exocyclic amine (ΔH_{acid} = 353.5 kcal mol^{-1}; ΔG_{acid} = 346.3 kcal mol^{-1}).[11]

(42) Sindona and co-workers[20] used the kinetic method to obtain an adenine PA of 224.2 kcal mol^{-1}. Mautner conducted high pressure mass spectrometry equilibrium measurements, obtaining an adenine PA of 224.6 kcal mol^{-1}. NIST subsequently evaluated these values (updating for changes in the reference acid and base scale) to report an evaluated PA of 225.3 kcal mol^{-1} (GB = 218.1 kcal mol^{-1}).

TABLE 6. Summary of Results for PA Bracketing of More Basic Site of OMG

ref compound	PA[a]	GB[a]	proton transfer[b] ref base	proton transfer[b] conj acid
triethylamine	234.7 ± 2.0	227.0 ± 2.0	+	−
1-methylpiperidine	232.1 ± 2.0	224.7 ± 2.0	+	−
1-methylpyrrolidine	230.8 ± 2.0	223.4 ± 2.0	+	−
piperidine	228.0 ± 2.0	220.0 ± 2.0	−	+
3-picoline	225.5 ± 2.0	217.9 ± 2.0	−	+
pyridine	222.3 ± 2.0	214.7 ± 2.0	−	+
propylamine	219.4 ± 2.0	211.3 ± 2.0	−	+

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

We measured the proton affinity of adenine by both bracketing and Cooks kinetic methods, which yield a value of PA = 224 and 225 kcal mol^{-1} (GB = 216 and 217 kcal mol^{-1}), respectively, in agreement with previous measurements.[19,20,23,42,43] Our measured PA value of 224−225 kcal mol^{-1} coincides with our calculated value of 223.7 kcal mol^{-1} for the N1 site of the N9H tautomer (Figure 1). The N1 site is also found to be most basic in aqueous solution.[44,45]

In summary, adenine appears to exist as the canonical structure in the gas phase. In DMSO, Fischer et al. have conducted NMR studies of $^{15}N_5$-labeled adenine derivatives and conclude that while the N9H tautomer **1** predominates, the N7H and N3H tautomers (**1a** and **1b**) are also present.[46] In aqueous solution, studies indicate the predominance of the N9H tautomer with, possibly, up to 20% of the N7H tautomer.[47,48] Different tautomer distributions are often found in the gas phase as compared to in solution; cytosine for example assumes the canonical tautomer in solution but our and others' work indicates several different tautomers coexist in the gas phase.[4,24−26,30,31,49−59]

(43) Wilson, M. S.; McCloskey, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 3436–3444.
(44) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(45) Christensen, J. J.; Izatt, R. M. *J. Phys. Chem.* **1962**, *66*, 1030–1034.
(46) Laxer, A.; Major, D. T.; Gottlieb, H. E.; Fischer, B. *J. Org. Chem.* **2001**, *66*, 5463–5481.
(47) Gonnella, N. C.; Nakanishi, H.; B., H. J.; Horowitz, D. S.; Kanamori, K.; Leonard, N. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 2050–2055.
(48) Dreyfus, M.; Dodin, G.; Bensaude, O.; Dubois, J. E. *J. Am. Chem. Soc.* **1975**, *97*, 2369–2376.
(49) Trygubenko, S. A.; Bogdan, T. V.; Rueda, M.; Orozco, M.; Luque, F. J.; Sponer, J.; Slavicek, P.; Hobza, P. *Phys. Chem. Chem. Phys.* **2002**, *4*, 4192–4203.
(50) Barker, D. L.; Marsh, R. E. *Acta Crystallogr.* **1964**, *17*, 1581–1587.
(51) McClure, R. J.; Craven, B. M. *Acta Crystallogr. B* **1973**, *29*, 1234–1238.
(52) Weber, H. P.; Craven, B. M.; McMullan, R. K. *Acta Crystallogr. B* **1980**, *36*, 645–649.
(53) Ueda, T.; Fox, J. J. *J. Am. Chem. Soc.* **1963**, *85*, 4024–4028.
(54) Dreyfus, M.; Bensaude, O.; Dodin, G.; Dubois, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 6338–6349.

In order to compare and contrast the normal purines to the damaged base OMG, we next interpret the guanine data.

Guanine. We have measured the most acidic site of guanine to have a ΔH_{acid} = 335 ± 3 kcal mol^{-1} (ΔG_{acid} = 328 ± 3 kcal mol^{-1}), which is in agreement with calculations and a value estimated previously by Chen and Chen.[21,22] The proton affinity is bracketed to 227 ± 3 kcal mol^{-1} (GB = 219 ± 3 kcal mol^{-1}). This value is in agreement with that measured by Greco et al. in a high pressure mass spectrometer.[20,23,43,60]

In aqueous solution and the solid state, the canonical tautomer of guanine (**2**) is believed to be the predominant form, with perhaps a small amount of the other keto-amino tautomer **2a** in solution.[39,44,61−63] In the gas phase, the situation is not as clear-cut. Our calculations and others' indicate that the canonical N9H (**2**) and N7H (**2a**) tautomers are very close in stability (only 0.5 kcal mol^{-1} apart).[4,25,32,34−36,39] The enol forms **2b** and **2c** (which are rotamers) are calculated to be only 2.5 and 3.3 kcal mol^{-1} less stable than the N7H structure. Therefore, it might be expected that guanine in the gas phase would be some mixture of keto and enol forms. The IR spectrum of guanine in inert matrices suggests that the tautomers **2**, **2a** and **2b** are present under those conditions.[64] LeBreton and co-workers compared the photoelectron spectroscopic properties of guanine with methyl-substituted derivatives and concluded that under their gas phase conditions, guanine is in the N7H (**2a**) form.[65] Last, Nir and co-workers used UV−UV and IR-UV hole burning spectroscopy to ascertain that laser desorbed and jet cooled guanine exists in both keto (N9H (**2**) and N7H (**2a**)) forms as well as in the enol form **2b**.[66]

We have in the past used our acidity and PA measurements, compared to calculations, to try to ascertain what tautomers might be present. In the case of guanine acidity, the calculated ΔH_{acid} value for the most acidic site for the keto and enol tautomers **2** and **2a**−**2c** falls within a narrow range of 333−335 kcal mol^{-1}. Given that the error bar on Cooks kinetic method measurements is at least that of the reference acids and bases used (2−3 kcal mol^{-1}), using acidity as a means of differentiating these tautomers is unlikely. In terms of PA, the four tautomers **2**, **2a**, **2b**, and **2c** have, respectively, calculated PA values of 227, 227, 225, and 223 kcal mol^{-1}. The measured value is 227 ± 3 kcal mol^{-1}. It would be difficult to discount the possible presence of **2**, **2a** and/or **2b** (calculated PA range 225−227 kcal mol^{-1}). Less likely, but not without question (depending on the accuracy of the calculation), is the presence of the least stable tautomer, **2c** (calculated PA = 223 kcal mol^{-1}), although as the less stable rotamer of **2b** it should not be preferred. It is also possible that we only have the canonical tautomer present due to the manner in which we generate guanine for this experiment. A proton-bound dimer of guanine and a reference acid or base is formed in solution and vaporized by electrospray. Details are in the Experimental Section, but the point of interest is that the guanine is electrosprayed from aqueous solution as opposed to sublimed from the solid state, so it could be argued that the form most stable in solution, the canonical structure **2**, is the predominant reactant.[59,67,68]

Regardless of tautomer composition, guanine appears to be comparable in acidity to adenine. Our adenine ΔH_{acid} measurement is slightly lower in value in the bracketing experiment than in the Cooks experiment (333 versus 335 kcal mol^{-1}). The guanine acidity measurement (using the Cooks kinetic method) yields the same value as that from the adenine Cooks experiment, 335 kcal mol^{-1}. In terms of PA, adenine is less basic than guanine; the measured PA for adenine (by respectively, bracketing and Cooks kinetic method) is 224 and 225 kcal mol^{-1}, whereas for guanine the value is 227 kcal mol^{-1}. The next question is how these values compare/contrast to OMG, and what insights we might gain into the mutagenicity of OMG.

***O*^{6}-Methylguanine (OMG).** To our knowledge, there have been no prior gas phase experimental studies of OMG. In the gas phase, the N9H and N7H tautomers of O^6-methylguanine are calculated to be somewhat close in energy (less than 3 kcal mol^{-1} apart), and thus both might coexist (Figure 3). In Figure 3, we also show the rotamer of the N9 tautomer **3** (**3a**), which is 2.6 kcal mol^{-1} higher in energy than the canonical form. The methyl group in structure **3** is referred to as "distal", whereas that in **3a** is "proximal" (relative to the N9). We would expect the methyl group to rotate fairly freely in this tautomer; our calculated transition structure for rotation is only 6.9 kcal mol^{-1} higher than the most stable ground state (Figure 4). The distal structure of the N9H-tautomer (**3**) is also found to be preferred in the solid state and aqueous phase (over the proximal structure (**3a**)), in agreement with our gas phase calculations.[69−75]

In terms of acidity, the N9H tautomer **3** has a calculated ΔH_{acid} of 337.4 kcal mol^{-1} (the N9 proton). Its rotamer **3a** and the N7H tautomer **3b** both have a calculated ΔH_{acid} of 335 kcal mol^{-1} (at, respectively, the N9 and N7 sites). The measured OMG ΔH_{acid} is 338 ± 3 kcal mol^{-1} by bracketing and 337 ± 3 kcal mol^{-1} by the Cooks kinetic method. While

(55) Sambrano, J. R.; de Souza, A. R.; Queralt, J. J.; Andrés, J. *Chem. Phys. Lett.* **2000**, *317*, 437–443.
(56) Chandra, A. K.; Michalska, D.; Wysokinsky, R.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **2004**, *108*, 9593–9600, and references therein.
(57) Nir, E.; Müller, M.; Grace, L. I.; de Vries, M. S. *Chem. Phys. Lett.* **2002**, *355*, 59–64.
(58) Brown, R. D.; Godfrey, P. D.; McNaughton, D.; Pierlot, A. P. *J. Am. Chem. Soc.* **1989**, *111*, 2308–2310.
(59) Liu, M.; Li, T.; Amegayibor, F. S.; Cardoso, D. S.; Fu, Y.; Lee, J. K. *J. Org. Chem.* **2008**, *73*, 9283–9291.
(60) Sindona and co-workers[20] used the kinetic method to obtain a guanine PA of 227.4 kcal mol^{-1}. NIST subsequently evaluated these values (updating for changes in the reference acid and base scale) to report an evaluated PA of 229.3 kcal mol^{-1} (GB = 221.7 kcal mol^{-1}).
(61) Shapiro, R. *Prog. Nucleic Acid Res. Mol. Biol.* **1968**, *8*, 73–112.
(62) Miles, H. T.; Howard, F. B.; Frazier, J. *Science* **1963**, *142*, 1458–1459.
(63) Thewalt, U.; Bugg, C. E.; Marsh, R. E. *Acta Crystallogr. B* **1971**, *27*, 2358.
(64) Szczepaniak, K.; Szczesniak, M. *J. Mol. Struct.* **1987**, *156*, 29–42.
(65) Lin, J.; Yu, C.; Peng, S.; Akiyama, I.; Li, K.; Lee, L.-K.; LeBreton, P. R. *J. Phys. Chem.* **1980**, *84*, 1006–1012.
(66) Nir, E.; Janzen, C.; Imhof, P.; Kleinermanns, K.; de Vries, M. S. *J. Chem. Phys.* **2001**, *115*, 4604–4611.
(67) Tian, Z.; Kass, S. R. *J. Am. Chem. Soc.* **2008**, *130*, 10842–10843.
(68) Tian, Z.; Kass, S. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 1321–1323.
(69) Parthasarathy, R.; Fridey, S. M. *Carcinogenesis* **1986**, *7*, 221–227.
(70) Patel, D. J.; Shapiro, L.; Kozlowski, S. A.; Gaffney, B. L.; Jones, R. A. *Biochemistry* **1986**, *25*, 1027–1036.
(71) Patel, D. J.; Shapiro, L.; Kozlowski, S. A.; Gaffney, B. L.; Jones, R. A. *Biochemistry* **1986**, *25*, 1036–1042.
(72) Cruzeiro-Hansson, L.; Parker, K.; Goodfellow, J. M. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 251–258.
(73) Parker, K.; Cruzeiro-Hansson, L.; Goodfellow, J. M. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 2637–2650.
(74) Pederson, L. G.; Darden, T. A.; Deerfield, D. W. II; Anderson, M. W.; Hoel, D. G. *Carcinogenesis* **1988**, *9*, 1553–1562.
(75) Nakayama, N.; Tanaka, S.; Kikuchi, O. *J. Theor. Biol.* **2002**, *215*, 13–22.

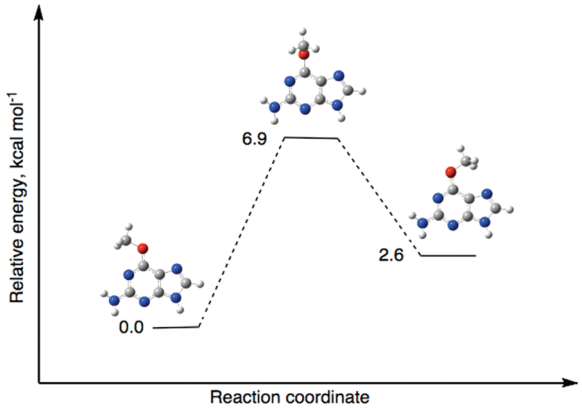


FIGURE 4. Rotation of the methyl group in the canonical O^6-methylguanine structure, calculated at 298 K at B3LYP/6-31+G*.

this value is certainly closest to the N9H tautomer **3**, it is still consistent with structures **3a** and **3b**, so we cannot discount their presence under our conditions.

We also measured a less acidic site on OMG; $\Delta H_{acid} = 362 \pm 3$ kcal mol^{-1}. This could correspond to the exocyclic -NH_2 protons on any of the three tautomers **3**−**3b**. Thus, on the basis of our calculations and experiments, we may have the OMG structures **3**, **3a**, and/or **3b** present under our gas phase conditions, though **3** should predominate. Also, the measurements from the bracketing and Cooks kinetic experiments are very close; in the FTMS bracketing experiment, OMG is heated in a solids probe in vacuo, whereas for the Cooks method the OMG is electrosprayed as a proton-bound dimer with a reference acid. We could therefore conceivably have different tautomeric mixtures under the different experimental conditions in which the substrate is generated.[67,68]

In terms of PA, we bracket the most basic site to be 229 ± 4 kcal mol^{-1} (GB = 222 ± 4 kcal mol^{-1}), which could correspond to **3** (the N7), **3a** (the N1), or **3b** (the N3). Again, on the basis of our calculations, **3** should predominate in the gas phase. By the Cooks kinetic method, we measure the most basic site to be 231 ± 3 kcal mol^{-1} (GB = 223 ± 3 kcal mol^{-1}); again, although this value could also correspond to any of the low-lying tautomers, we use electrospray to generate the OMG·reference-base protonated dimers from aqueous solution, so the canonical structure **3** favored in solution may predominate. These are the first studies probing tautomer composition of OMG in the gas phase.

Comparison of Adenine and Guanine Properties with O^6-Methylguanine (OMG). O^6-Methylguanine arises from the alkylation of guanine and is highly mutagenic.[76−85] Its mutagenicity arises from a "GC to AT" transition that occurs as a result of different hydrogen bonding preferences for normal guanine versus damaged OMG.[86−88] In DNA replication, guanine base pairs preferentially with cytosine. Once guanine is alkylated to form OMG, however, the preferred hydrogen bonding pattern is to *thymine* rather than cytosine.[89,90] The result is that a DNA sequence that originally contained a G·C base pair will become an OMG·T base pair; when the strand containing the "T" replicates, the end result will be an A·T base pair; this is the GC to AT transition. Since the exact sequence of DNA is necessary for proper life function (coding for proteins, signaling), the mutation to OMG is known to be highly carcinogenic.[91−95]

Two aspects in particular regarding OMG's biological activity intrigue us. First is the OMG preference for T over C, the provenance of which is unknown.[76,89,90] Jones et al. conducted aqueous studies of the stabilities of 9-mer duplexes containing OMG·C versus OMG·T base pairs and found that the OMG·C duplexes are more stable than those containing OMG·T.[96−98] We found this to be true in the gas phase as well in previously published work.[10,99] Thus, intrinsically, OMG binds more strongly to C, but in Nature, when replication occurs, OMG binds to T.

Because proton affinities and acidities are related to the hydrogen bonding acceptor and donor ability, we can use these values to try to understand OMG binding to C and to T. In Figure 5, we show the G·C, OMG·C, and OMG·T base pairs with the relevant proton affinity and acidity values.[100] Our goal here is to not focus on geometries but just to look at the proton affinities of acceptor atoms and the acidities of donor atoms of each individual substrate, in the context of hydrogen bonding.

The G·C base pair has three hydrogen bonds: from the O6 in guanine to the N4H in cytosine, from the N1−H in guanine to the N3 in cytosine, and from the N2H in guanine to the O2 in cytosine. The heteroatoms all have PA values higher than 220, and the acidic protons all have ΔH_{acid} values below 353 kcal mol^{-1}.

(76) Dimitri, A.; Burns, J. A.; Broyde, S.; Scicchitano, D. A. *Nucleic Acids Res.* **2008**, *36*, 6459–6471, and references therein.
(77) Beranek, D. T. *Mutat. Res.* **1990**, *231*, 11–30.
(78) Rydberg, B.; Lindahl, T. *EMBO J.* **1982**, *1*, 211–216.
(79) Sedgwick, B. *Carcinogenesis* **1997**, *18*, 1561–1567.
(80) Liu, J. G.; Li, M. H. *Carcinogenesis* **1989**, *10*, 617–620.
(81) Loechler, E. L. *Chem. Res. Toxicol.* **1994**, *7*, 277–280.
(82) Souliotis, V. L.; Kaila, S.; Boussiotis, V. A.; Pangalis, G. A.; Kyrtopoulos, S. A. *Cancer Res.* **1990**, *50*, 2759–2764.
(83) Taverna, P.; Catapano, C. V.; Citti, L.; Bonfanti, M.; D'Incalci, M. *Anticancer Drugs* **1992**, *3*, 401–405.
(84) De Bont, R.; van Larabeke, N. *Mutagenesis* **2004**, *19*, 169–185.
(85) Wyatt, M. D.; Pittman, D. L. *Chem. Res. Toxicol.* **2006**, *19*, 1580–1594.
(86) Toorchen, D.; Topal, M. D. *Carcinogenesis* **1983**, *4*, 1591–1597.
(87) Loechler, E. L.; Green, C. L.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6271–6275.
(88) Singer, B.; Dosanjh, M. K. *Mutat. Res.* **1990**, *233*, 45–51.
(89) Abbot, P. J.; Saffhill, R. *Biochim. Biophys. Acta* **1979**, *562*, 51–61.
(90) Kamiya, H.; Sakaguchi, T.; Fujimoro, M.; Miura, H.; Ishikawa, H.; Shimizu, M.; Inoue, H.; Matsukage, A.; Masutani, C.; Hanaoka, F.; Ohtsuka, E. *Chem. Pharm. Bull.* **1992**, *40*, 2792–2795.
(91) Galloway, S. M.; Greenwood, S. K.; Hill, R. B.; Bradt, C. I. *Mutat. Res.* **1995**, *346*, 231–245.
(92) Marchesi, F.; Turriziani, M.; Tortorelli, G.; Avvisati, G.; Torino, F.; De Vecchis, L. *Pharmacol. Res.* **2007**, *56*, 275–287.
(93) Voigt, J. M.; Topal, M. D. *Carcinogenesis* **1995**, *16*, 1775–1782.
(94) Kaina, B.; Fritz, G.; Coquerelle, T. *Environ. Mol. Mutagen.* **1993**, *22*, 283–292.
(95) Zhang, H.; Tsujimura, T.; Bhattacharyya, N. P.; Maher, V. M.; McCormick, J. J. *Carcinogenesis* **1996**, *17*, 2229–2235.
(96) Gaffney, B. L.; Jones, R. A. *Biochemistry* **1989**, *28*, 5881–5889.
(97) Gaffney, B. L.; Marky, L. A.; Jones, R. A. *Biochemistry* **1984**, *23*, 5686–5691.
(98) Kuzmich, S.; Marky, L. A.; Jones, R. A. *Nucleic Acids Res.* **1983**, *11*, 3393–3403.
(99) Pan, S.; Sun, X.; Lee, J. K. *Int. J. Mass Spectrom.* **2006**, *253*, 238–248.
(100) Note that we did not actually calculate the base pairs; we merely draw the configurations in Figure 5 to help the reader visualize the relevant hydrogen bonds.

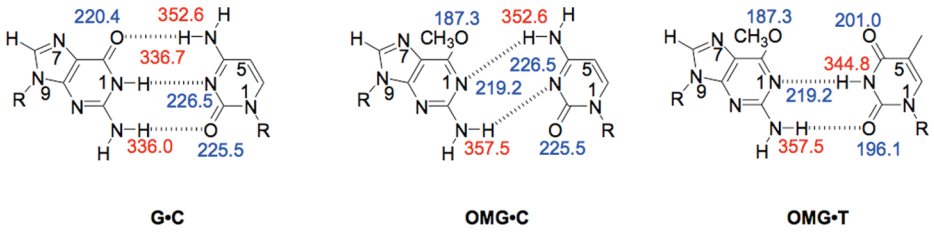


FIGURE 5. Calculated proton affinity (PA, blue) and acidity (ΔH_{acid}, red) values (B3LYP/6-31+G*, 298 K, in kcal mol^{-1}) for the atoms of the nucleobases involved in the G·C, OMG·C, and OMG·T base pairs.

The configuration we chose to draw of the OMG·C pair is based on crystallographic, NMR, and computational data (Figure 5).[70,72,73,101−104] The two main hydrogen bonds are the N1 on OMG to the exocyclic NH_2 on cytosine, and the exocyclic NH_2 on OMG to the N3 of cytosine. In both experiment and computation, it appears that sometimes the OMG methyl is proximal (pointing toward the "N7" side), and sometimes it is distal (toward the "N1" side). We utilized the PA associated with the latter, since that structure is the more stable by our calculations.

Alkylation of the O6 drops its basicity considerably, from 220.4 kcal mol^{-1} to 187.3 kcal mol^{-1}. Therefore, any hydrogen bond to this site would be fairly weak. Alkylation also decreases the acidity at the exocyclic -NH_2, from 336 kcal mol^{-1} to 357.5 kcal mol^{-1}, thus decreasing the ability of that proton to act as a hydrogen bond donor. The N1 changes from an acidic to a basic site, leading to the hydrogen bonding pattern shown in Figure 5. Two main hydrogen bonds versus three means that the OMG·C base pair will be less stable than the parent G·C. The proton donors in the OMG·C base pair have ΔH_{acid} values of 353 and 357.5 kcal mol^{-1} while the proton acceptors have PA values of 219 and 227 kcal mol^{-1}. Compared to the G·C base pair, the PA values are comparable, but the OMG -NH_2 acidity is considerably less than that of G (357.5 versus 336.0 kcal mol^{-1}), so not only does the OMG·C base pair have one less hydrogen bond than the G·C base pair, but depending on proximity, one of the hydrogen bonds may also be less stable.

Calculations and experiment (solid state and solution phase) are not wholly in agreement on the structure of the OMG·T base pair.[71−73,101,104,105] By NMR, the methyl group is distal (pointing toward N1); by X-ray, it is proximal. As we did with the OMG·C base pair, we utilize the PA associated with the distal configuration. Of the two hydrogen bonds, the one between the N1 of OMG and the N3−H of T seems reasonable on the basis of the associated PA (219.2 kcal mol^{-1}) and ΔH_{acid} (344.8 kcal mol^{-1}) values. However, the second one, between the rather nonacidic OMG N2H (ΔH_{acid} = 357.5 kcal mol^{-1}) to the only slightly basic O2 of thymine (PA = 196.1 kcal mol^{-1}) seems as if it would be much weaker, especially in comparison to the hydrogen bonds on the OMG·C pair. Our values are consistent with earlier studies in supporting that intrinsically, the OMG·C base pair is likely to be favored energetically over the OMG·T base pair. The preference *in vivo* for the OMG·T base pair must therefore be controlled by other features, such as the enzyme involved in replication.

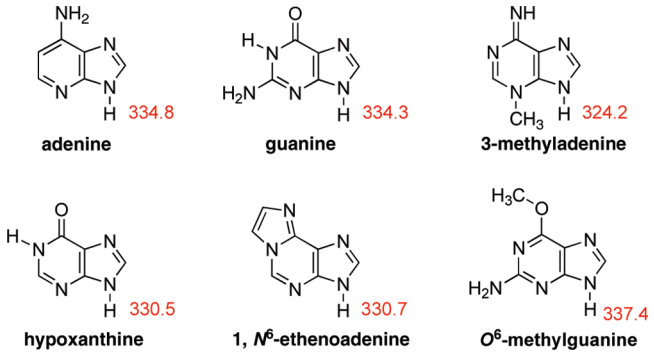


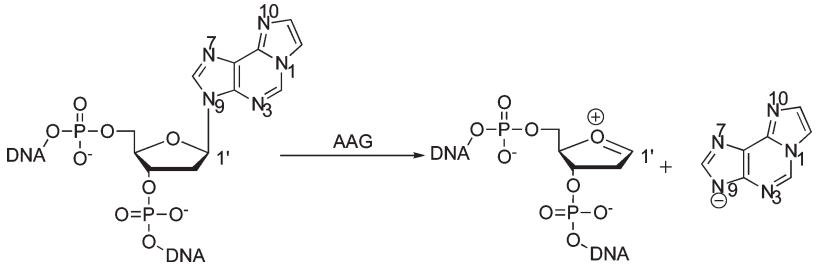
FIGURE 6. Calculated N9−H ΔH_{acid} values for normal and damaged purines (B3LYP/6-31+G*, 298 K).

The second aspect that intrigues us regarding OMG is how it is repaired in DNA. DNA is inevitably damaged, and Nature has devised various ways of repairing damaged nucleobases.[16,17] We have, for the past several years, been studying the glycosylase family of enzymes, which as part of the base excision repair (BER) pathway excises a wide range of oxidized and alkylated bases. In humans, alkyl adenine glycosylase (AAG) is responsible for excising a wide range of bases, including 3-methyladenine, hypoxanthine, and 1,N^6-ethenoadenine (structures shown in Figure 6; possible AAG mechanism shown in Scheme 1).[16,17] One mechanistic question is how AAG achieves this "broad specificity"; many of the damaged bases are very similar in structure to adenine and guanine, yet the latter normal nucleobases do not get cleaved. We have proposed that AAG excises the damaged nucleobases in deprotonated form (as the N9-deprotonated anions) and that discrimination is achieved because the damaged bases are more acidic at the N9 position and therefore are better anionic leaving groups and more prone to cleavage (Scheme 1). We have shown in previous studies that, in fact, the damaged bases 3-methyladenine, hypoxanthine, and 1,N^6-ethenoadenine are indeed more acidic than adenine and guanine, which provides support for our hypothesis.[9,11−13,59,106] Furthermore, we have shown that the differences in acidity between the normal and damaged bases is enhanced in the gas phase.[9,11−13,59] We therefore propose that AAG might provide a hydrophobic active site

(101) Spratt, T. E.; Levy, D. E. *Nucleic Acids Res.* **1997**, *25*, 3354–3361.
(102) Sriram, M.; van der Marel, G. A.; Roelen, H. L. P. F.; van Boom, J. H.; Wang, A. H.-J. *Biochemistry* **1992**, *31*, 11823–11834.
(103) Gaffney, B. L.; Goswami, B.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 12607–12608.
(104) Caldwell, J. W.; Kollman, P. A. *J. Biomol. Struct. Dyn.* **1985**, *3*, 57–66.
(105) Leonard, G. A.; Thomson, J.; Watson, W. P.; Brown, T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9573–9676.

(106) When adenosine is methylated on the 3-position, a positively charged species is formed, whose pK_a (to form the neutral structure) is to our knowledge unknown. In our previously published studies,[11] we probed the acidity at N9 of the neutral structure; that is the value reported herein.

SCHEME 1



that enhances the discrimination of normal from damaged bases.

We have long been intrigued by the fact that OMG is *not* excised by AAG.[107] Repair of an OMG lesion is carried out by a methylguanine methyl transferase (MGMT), which dealkylates OMG (Scheme 2).[108−110]

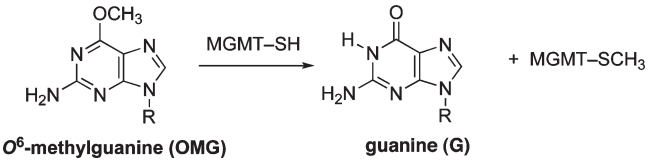
The studies herein show that OMG is markedly different from the other damaged bases we have studied (3-methyladenine, hypoxanthine, and 1, N^6-ethenoadenine) in that it is *less* acidic than adenine and guanine at the N9 position. The calculated values for the canonical structures indicate that while adenine and guanine have a ΔH_{acid} of about 335 kcal mol^{-1}, that of OMG is 337 kcal mol^{-1}. In contrast, the ΔH_{acid} values for 3-methyladenine, hypoxanthine, and 1,N^6-ethenoadenine are, respectively, 323, 331, and 331 kcal mol^{-1} (Figure 6).[9,11−13,59] We also confirmed the relative acidity ordering (hypoxanthine and 1,N^6-ethenoadenine are more acidic than adenine and guanine, which are more acidic than OMG) by conducting relative Cooks kinetic experiments among these substrates. That is, in addition to our Cooks experiments where we measure properties by dissociating proton-bound dimers of the nucleobases with reference compounds, we also did "relative" experiments where we generated proton-bound dimers of, for example, deprotonated adenine and deprotonated guanine ([$A^- \cdots H^+ \cdots G^-$]) in order to ascertain their relative acidities. These sets of experiments confirm that OMG is the least acidic nucleobase and that the other damaged bases are more acidic than adenine and guanine.

On the basis of these acidity values, it makes sense that AAG does not cleave OMG; if the enzyme achieves discrimination by cleaving those bases that are the best leaving groups, OMG would be even less likely than the normal bases to be excised. Because OMG has such a low acidity and therefore is not prone to anionic cleavage, Nature presumably devised an alternate method of repair, namely, dealkylating the OMG via a methyl transfer reaction (Scheme 2).

Conclusions

We have calculated and measured the acidity and proton affinity of adenine, guanine, and O^6-methylguanine (OMG) to probe the intrinsic reactivity of these purine nucleobases.

SCHEME 2



We are interested in particular in how damaged bases differ from normal bases. DNA is inevitably damaged by environmental mutagens as well as chemotherapeutics; such mutations are linked to carcinogenesis and aging.[15−17] Our lab studies the mechanisms by which enzymes might cleave damaged bases from DNA, thereby protecting our genome.[5,6,8,9,11,13,18] Previous results from our lab have shown that the properties of normal versus damaged bases lend insight into the mechanisms by which the damaged bases are cleaved.[5,6,8,9,11,13,18] The first step toward understanding how normal bases differ from damaged bases is to characterize the naturally occurring normal compounds, which motivates the study herein.

The acidity of the two most acidic sites of adenine have been measured using the acidity bracketing method: $\Delta H_{acid} = 333 \pm 2$ kcal mol^{-1} and 352 ± 4 kcal mol^{-1} ($\Delta G_{acid} = 328 \pm 2$ and 344 ± 4 kcal mol^{-1}); the acidity of the more acidic site was remeasured herein using the Cooks kinetic method ($\Delta H_{acid} = 335 \pm 3$ kcal mol^{-1}; $\Delta G_{acid} = 329 \pm 3$ kcal mol^{-1}). The PA of adenine, measured by both bracketing and Cooks kinetic method, is 224−225 kcal mol^{-1} (GB = 216−217 kcal mol^{-1}). Comparison of these values to theoretical data indicates that under our conditions we probably have the canonical tautomer of adenine.

Guanine has several low-lying tautomers in the gas phase. Using the Cooks kinetic method, the acidity ($\Delta H_{acid} = 335 \pm 3$ kcal mol^{-1}; $\Delta G_{acid} = 328 \pm 3$ kcal mol^{-1}) and proton affinity/gas phase basicity (PA = 227 ± 3 kcal mol^{-1}; GB = 219 ± 3 kcal mol^{-1}) were measured. While these values are consistent with both the keto and enol tautomers of guanine, we may have had predominantly the keto canonical tautomer, which prevails in aqueous solution (from which we electrospray the protonated dimers). We also examined the properties of O^6-methylguanine, a highly mutagenic and carcinogenic damaged form of guanine that has not been heretofore examined in the gas phase. We measure the ΔH_{acid} of the more acidic site to be 338 ± 3 kcal mol^{-1} ($\Delta G_{acid} = 331 \pm 3$ kcal mol^{-1}) and that of the less acidic site to be 362 ± 3 kcal mol^{-1} ($\Delta G_{acid} = 355 \pm 3$ kcal mol^{-1}), and the PA to be 229 ± 4 kcal mol^{-1} (GB = 222 ± 4 kcal mol^{-1}); these values are in agreement with Cooks kinetic method experiments that we also conducted. In terms of OMG

(107) Abner, C. W.; Lau, A. Y.; Ellenberger, T.; Bloom, L. B. *J. Biol. Chem.* **2001**, *276*, 13379–13387.
(108) Pegg, A. E.; Dolan, M. E.; Scicchitano, D. A.; Moriomoto, K. *Environ. Health Perspect.* **1985**, *62*, 109–114.
(109) Pegg, A. E.; Fang, Q.; Loktionova, N. A. *DNA Repair* **2007**, *6*, 1071–1078.
(110) Chen, J. M.; Zhang, Y. P.; Wang, C.; Sun, Y.; Fujimoto, J.; Ikenaga, M. *Carcinogenesis* **1992**, *13*, 1503–1507.

tautomerism, we may have a mix of the canonical N9H and the N7H structures present, though the N9H most likely predominates.

OMG is less acidic at the N9 position than both adenine and guanine and also less acidic than other damaged bases we have studied (3-methyladenine, hypoxanthine, 1,N^6-ethenoadenine).[9,11−13,59] This is interesting because unlike many other damaged bases, the OMG lesion is repaired in the genome by demethylation rather than enzyme-catalyzed excision at N9. The fact that OMG is not as acidic at the N9 position is consistent with the fact that OMG is repaired by a different method than the other damaged bases; that low acidity indicates that its conjugate base anion would be a worse leaving group, and demethylation might have evolved as a more efficient method for repair.

We also used the proton affinity and acidity of OMG to explore the issue of hydrogen bonding to cytosine (C) and thymine (T). Although the normal base guanine pairs preferentially with C in replication, OMG pairs with T. Experiments indicate that duplexes containing OMG·C base pairs are more stable than those containing OMG·T base pairs, so it is puzzling as to why OMG·T base pairs are formed in Nature.[10,96−99] Our examination of the proton donating and acceptor abilities are consistent with the in vitro experimental results; we would expect the OMG·C base pair to be more stable than the OMG·T base pair. Therefore, the preference of OMG to pair with T in replication is controlled by features other than pure intrinsic stability.

Experimental Section

All chemicals are commercially available and were used as received.

Acidity and proton affinity bracketing experiments were conducted using a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTMS) with a dual cell setup that has been described previously.[5,6,8] In our FTMS, two adjoining 1-in.^{3} cells are positioned collinearly with the magnetic field produced by a 3.3 T superconducting magnet. The pressure of the dual cell is pumped down to less than 1×10^{-9} Torr. The solid nucleobases are introduced to the cells via a heatable solids probe. Ions are generated via reaction with H_3O^+ or HO^- ions. Ions can be transferred from one cell to the second cell via a 2-mm hole in the center of the central trapping plate. Transferred ions are cooled by a pulse of argon that raises the cell pressure to 10^{-5} Torr.[111] Experiments are conducted at ambient temperature.

Acidity and proton affinity (and gas phase basicity) are assessed using bracketing experiments in the FTMS, which have been described previously.[5,6,8] Briefly, for acidity bracketing, hydroxide ions are generated first by pulsing water (via a pulsed valve system) into the FTMS cell and sending an electron beam (8 eV, 6 μA, beam time 0.5 s) through the center of the cell. The hydroxide ions deprotonate neutral molecules "M" (either nucleobases or reference bases) to yield the $[M - H]^-$ ions. The $[M - H]^-$ ion is allowed to react with the neutral nucleobase or reference base. The same procedure is used for bracketing proton affinity, where hydronium ions (20 eV, 6 μA, beam time 0.2 s) are used for protonation. The occurrence of proton transfer is regarded as evidence that the reaction is exothermic ("+" in tables). Because we can measure the acidity and basicity of multiple acidic and basic sites on a molecule (vide infra), we refer to these bracketing experiments as "more acidic" (or "more basic") conditions. Charged species are all either monodeprotonated (in acidity studies) or monoprotonated (in PA/GB studies).

We have developed an FTMS method for the bracketing of the acidity and basicity of less acidic and less basic sites in molecules that have multiple acidic and basic sites; the experimental procedure and limitations have been described previously.[5,6,11,12] Briefly, in this setup, nucleobase ions produced after reaction of the corresponding neutral with hydroxide ions are immediately removed from the first cell and transferred into the second cell. Reference acids are then introduced into the second cell via a batch inlet system and allowed to react with the nucleobase ions. The first reaction cell is high in neutral nucleobase concentration, and over time, neutral-catalyzed isomerization leads to survival of only the most acidic ions.[5] Transferring ions into the second cell immediately after their generation allows us to carry out the reaction between reference acids and nucleobase ions in the absence of neutral nucleobase. The same procedure can be applied to the bracketing of the basicity of less basic sites as well.[7] In our experience, we usually measure the acidity or basicity of two sites: the most acidic or basic site present and then a second site, which would be the least acidic or basic site present. The reasons for our measuring only two sites (and not more) have been previously discussed.[5−9,11−13] We refer to the conditions under which we run this experiment as "less acidic" or "less basic" conditions.[5−9,11−13]

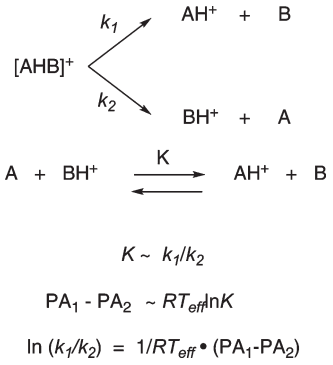
In our experiments, we have pseudo-first-order conditions, where the amount of the neutral substrate is in excess relative to the reactant ions. Reading the pressure from an ion gauge is not always an accurate measurement of the neutral pressure, because of both the gauge's remote location as well as varying sensitivity for different substrates.[12,112] We therefore "back out" the neutral pressure from a control reaction; this procedure has been described previously by us.[12] Briefly, we track the reaction of hydroxide and the neutral substrate. Because hydroxide is very basic, we assume this reaction proceeds at the theoretical collision rate.[11,12,113,114] We can then use the calculated collisional rate constant to "back out" the neutral pressure.

We also used the Cooks kinetic method in a quadrupole ion trap (LCQ) mass spectrometer[115−119] to measure the proton affinities and acidities. The procedure for conducting these experiments in our lab has been described previously.[9,13] Briefly, for PA experiments, this method involves the formation of a proton-bound complex, or dimer, of the unknown (for example, guanine) and a reference base of known proton affinity (Scheme 3, where "A" is guanine and "B" represents a series of reference bases). Collision-induced dissociation (CID) of this dimer leads to the formation of either the protonated unknown or the protonated reference base. The ratio of these two products yields the relative proton affinities of the two compounds of interest, assuming that the dissociation has no reverse activation energy barrier and that the dissociation transition structure is late and therefore indicative of the stability of the two protonated products. Both of these assumptions are generally true for proton-bound systems.[119−121] The same type of

(111) Amster, I. J. *J. Mass. Spec.* **1996**, *31*, 1325–1337, and references therein.

(112) Bartmess, J. E.; Georgiadis, R. M. *Vacuum* **1983**, *33*, 149–153.
(113) Su, T.; Chesnavich, W. J. *J. Chem. Phys.* **1982**, *76*, 5183–5185.
(114) Chesnavich, W. J.; Su, T.; Bowers, M. T. *J. Chem. Phys.* **1980**, *72*, 2641–2655.
(115) Cooks, R. G.; Kruger, T. L. *J. Am. Chem. Soc.* **1977**, *99*, 1279–1281.
(116) McLuckey, S. A.; Cameron, D.; Cooks, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 1313–1317.
(117) McLuckey, S. A.; Cooks, R. G.; Fulford, J. E. *Int. J. Mass Spectrom. Ion Processes* **1983**, *52*, 165–174.
(118) Brodbelt-Lustig, J. S.; Cooks, R. G. *Talanta* **1989**, *36*, 255–260.
(119) Green-Church, K. B.; Limbach, P. A. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 24–32.
(120) Ervin, K. M. *Chem. Rev.* **2001**, *101*, 391–444, and references therein.
(121) Gronert, S.; Feng, W. Y.; Chew, F.; Wu, W. *Int. J. Mass Spectrom.* **2000**, *196*, 251–258.

SCHEME 3



experiment can be done for acidity measurements (proton-bound dimer of deprotonated analyte and reference acid).

T_{eff} is the effective temperature of the dissociating proton-bound complex in Kelvin. A plot of $\ln(k_1/k_2)$ versus the proton affinity of a series of reference bases (PA_2) will yield T_{eff} from the slope; this value varied depending on the substrate. For all experiments, we utilized at least four reference bases or acids (where the limitation is often the formation of proton-bound dimers) and measured the product ion distributions three separate times to ensure reproducibility. In these experiments, we vary the collision energy only. The product ion ratio varies slightly as collision energy changes so we take an average of the product ratios at different energies and apply the "standard" Cooks kinetic method.[116] We estimate the error for the method to be that of the bracketing method.

Proton-bound complex ions are generated by electrospray (ESI).[122] For each experiment, a solution of the nucleobase and reference base (or acid) is prepared (10^{-3} to 10^{-4} M solutions in a 20% methanol or ethanol aqueous solution). The typical flow rate is 25 μL/min. An electrospray needle voltage of ~4500 V was used. The proton-bound complex ions were isolated and then dissociated by applying collision-induced dissociation (CID); the complexes were activated for about 30 ms. A total of 40 scans was averaged for the product ions.

The B3LYP method and the 6-31+G* basis set as implemented in Gaussian03 were used for all the gas phase calculations.[123−128] This level has been previously shown to be reasonably accurate for gas phase acidity and proton affinity calculations of nucleobases.[5−9,11−13] All the geometries are fully optimized and frequencies are calculated; no scaling factor is applied. Reported values herein are at 298 K.[129]

Acknowledgment. We gratefully acknowledge the support of NSF, the Alfred P. Sloan Foundation, and the National Center for Supercomputer Applications. J.K.L. thanks Drs. Gary and Alissa Kaye for helpful discussions.

Supporting Information Available: Cartesian coordinates for all calculated species (including higher energy tautomers) and full citations for references with greater than 16 authors. This material is available free of charge via the Internet at http://pubs.acs.org.

(122) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64–71.
(123) Frisch, M. J. et al. *Gaussian03, Rev. C*; Gaussian Inc.: Wallingford, CT, 2004.
(124) Kohn, W.; Becke, A. D.; Parr, R. G. *J. Phys. Chem.* **1996**, *100*, 12974–12980.
(125) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
(126) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372–1377.
(127) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
(128) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623–11627.
(129) To calculate the ΔH_{298}, we account for both the translational energy of the proton (3/2 RT) and the work term associated with the dissociation of one molecule into two (RT). For the ΔG_{298} values, we also account for the ΔS_{298} of the proton (experimental value = 26 eu).