Chemical Research in Toxicology

Article

pubs.acs.org/crt

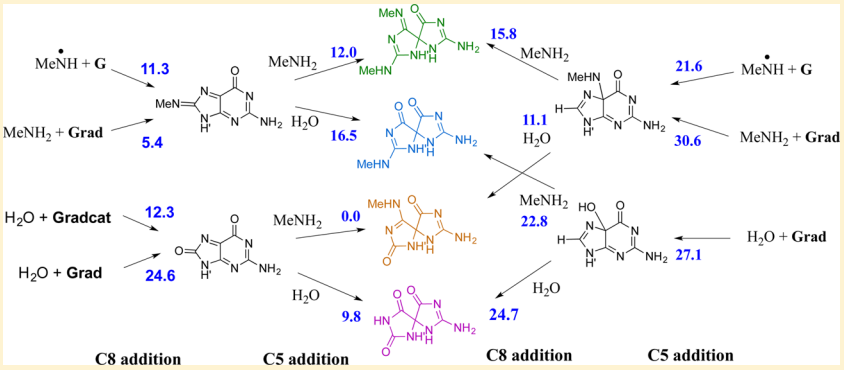
Computational Study of the Radical Mediated Mechanism of the Formation of C8, C5, and C4 Guanine:Lysine Adducts in the Presence of the Benzophenone Photosensitizer

Bishnu Thapa,[†] Barbara H. Munk,[†] Cynthia J. Burrows,[‡] and H. Bernhard Schlegel*[,†]

[†]Department of Chemistry, Wayne State University, Detroit, Michigan 48202, United States

[‡]Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States

Supporting Information



ABSTRACT: The oxidation of guanine by triplet benzophenone in the presence of lysine has been shown to produce mono- and dilysine-substituted spiroiminodihydantion products, 8-Lys-Sp and 5,8-diLys-Sp. The potential energy surfaces for C8, C5, and C4 nucleophilic addition have been mapped out using the B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p) level of density functional theory with the SMD solvation model and employing methylamine as a model for the side chain of lysine. Enthalpies, barrier heights, pK_a's, and reduction potentials were calculated for intermediates to find the lowest energy paths. For neutral methylamine plus guanine radical and neutral methylamine radical plus guanine, the barrier for addition at C8 is ca. 10 kcal/mol lower than that for addition at C5 and C4. The barriers for water addition at C8, C5, and C4 of guanine radical are 13−20 kcal/mol higher than that for methylamine addition at C8. Further oxidation and loss of a proton leads to 8-methylaminoguanine, the methylamino analogue of 8-oxo-7,8-dihydroguanine (8-oxoG). The barrier for the addition of a second methylamine at C5 of 8-methylaminoguanine is 4.5 kcal/mol lower than that for the corresponding addition of water. Nevertheless, if the concentration of methylamine (or lysine) is very low, water addition could be competitive with methylamine addition. This would lead to comparable fractions of 8-monosubstituted-Sp and 5−8-disubstituted-Sp, in agreement with the experimental observations.

INTRODUCTION

DNA−protein cross-links, DPCs, are common structural modifications that may affect the functions of DNA. These structural and/or functional modifications are generally accepted to be a major problem and can cause deleterious biological effects such as cellular aging, mutagenesis, and carcinogenesis.[1−5] DPCs are a common outcome of oxidatively generated damage of DNA and are formed under a wide range of conditions. The potential diversity of intermediate structures and reaction mechanisms could be the reason that DPC formation is the least understood DNA lesion despite being an abundant and significant type of DNA damage. Hence, it is highly desirable to develop a molecular level understanding of the chemical structures and mechanisms involved in DPC formation.

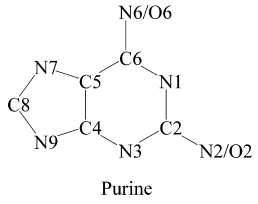
Because guanine has the lowest reduction potential among the DNA nucleobases, it is usually considered the first target of oxidatively generated damage to DNA.[4−8] Oxidation of guanine has been established to occur even in the presence of mild oxidative environments and results in products such as 8-oxo-7,8-dihydroguanine (8-oxoG), spiroiminodihydantoin (Sp), guanidinohydantoin (Gh), and imidazolone (Iz). Numerous experimental studies over the last four decades have explored the formation of protein cross-links with isolated nucleobases as well as single and double stranded DNA.[7,9−29] A wide range of amino acids including lysine, histamine, and arginine have been found to form cross-links with purine and pyrimidine bases in the presence of different oxidizing agents. Formation of DPCs has been observed in cellular DNA exposed to various chemical oxidizing agents such as hydroxyl radicals, singlet oxygen, sulfate radicals, carbonate radicals, and organic carcinogens such as aldehydes, carcinogenic metal ions such as Ni(II), Cr(VI), Fe(II),

Received: February 19, 2016
Published: August 1, 2016

ACS Publications © 2016 American Chemical Society

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

Scheme 1. Atomic Numbering for Purine Nucleobases



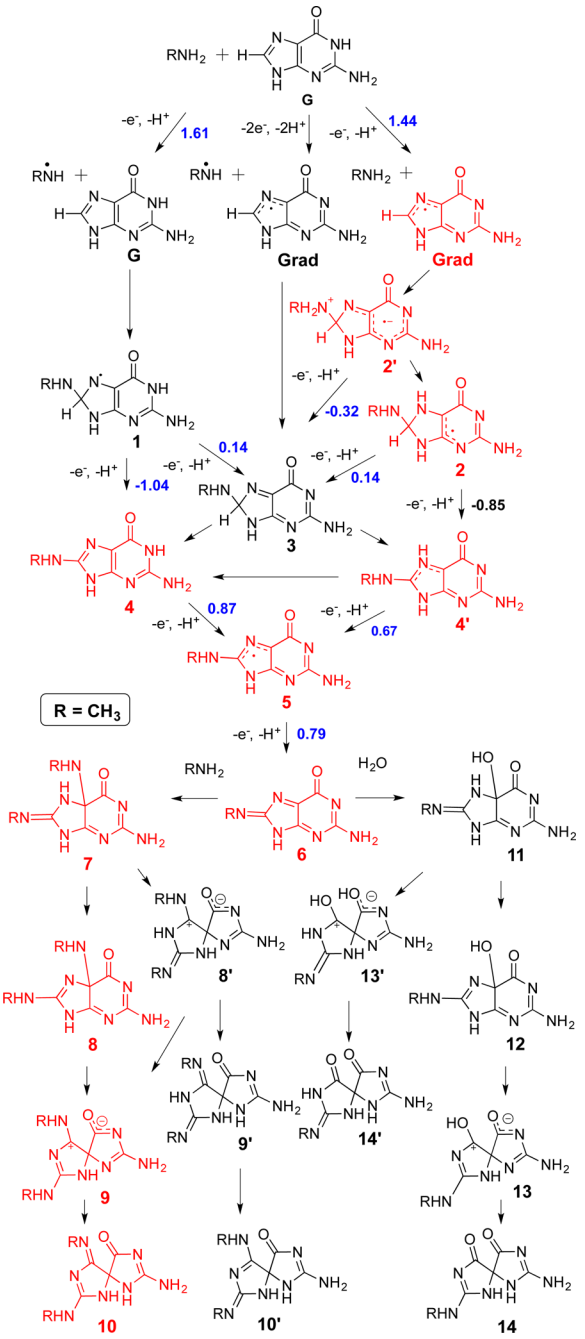
Purine

Fe(III) bleomycin, Ir(IV), ionizing radiation, UV light, and visible light with photosensitizers. Oxidation of nucleobases or protein residues produces electrophilic species which can then react with the nucleophilic groups to form cross-links. The nature of the DPCs formed depends on the type of oxidizing agent.[24] Morin and Cadet[13] developed a model system with lysine tethered to the sugar of 2′-deoxyguanosine that readily forms a lysine:guanine cross-link at C8 of guanine when oxidized by triplet benzophenone. In the presence of strong oxidizing agents such as HOCl, $ONOO^-$, and other one electron oxidants, Burrows and co-workers found that a covalent cross-link can be formed between C5 of 8-oxoG and the lysine side chains in single strand binding protein.[19] Perrier et al. observed the formation of a lysine:guanine cross-link at C8 of guanine upon one electron oxidation via riboflavin-mediated photosensitized oxidation of TGT oligonucleotide and trilysine.[21] Burrows and co-workers[24] have studied the formation of C5 and C8 cross-links between 2′-deoxyguanosine and lysine in the presence of a range of oxidizing agents such as type I and type II photosensitizers, sulfate radical and $Ir(Cl)_6^{2-}$, and have shown that the distribution of the final cross-linked products depends on the nature of the oxidizing agents. They have also examined the product profiles for the reaction of ammonia with guanine in the presence of a number of different oxidants.[30] Using riboflavin sensitized oxidation, Silerme et al.[28] demonstrated that the formation of C8 polyamine:guanine cross-links in double stranded DNA was more efficient than the addition of water to form 8-oxoG.

Computational and experimental studies on C-8 adducts of guanine have been reviewed recently.[31] Compared to the extensive experimental investigations, there are only a limited number of computational studies related to guanine:lysine cross-links.[32,33] Jena and Mishra compared the gas phase stabilities of noncovalently bound guanine:lysine complexes in different charge states using density functional and Møller−Plesset perturbation theories.[32] Their study showed that the spin and charge in a guanine:lysine radical cation complex were concentrated on the guanine subunit, which is consistent with the experimental observation that the ionization potential of guanine is lower than that of lysine.[34,35] The addition of ammonia to oxidized guanine forming 8-aminoguanine has been studied both computationally[36] and experimentally.[30] To the best of our knowledge, a full computational study of potential mechanisms for covalent guanine:lysine cross-link formation has not been reported yet.

Benzophenone is a type I photosensitizer[37] commonly used in photochemical studies and was one of the oxidants used by Burrows and co-workers in their study of guanine:lysine cross-link formation.[24] On excitation to the triplet state, benzophenone can oxidize nucleobases by abstracting a hydrogen atom or by proton coupled electron transfer (PCET).[38−40] The present study explores potential mechanisms for the formation of guanine:lysine cross-links in the presence of triplet benzophenone (^{3}**BP**) in aqueous solution near the physiological pH.

Scheme 2. Pathways for the Addition of Methylamine at C8 of Guanine Followed by Nucleophilic Addition of Methylamine or Water at C5[a]



[a] E^o is shown in blue, and the favored path is shown in red.

Under the conditions employed by Burrows and co-workers[24] (benzophenone, continuous irradiation at 365 nm for 20 h), triplet benzophenone could act as the oxidant for all four oxidation steps. Similarly, in the investigation by Perrier et al.[21] (riboflavin, a type I photosensitizer, irradiation by a halogen lamp for 30 min), all four oxidation steps could be mediated by riboflavin. Shafirovich and co-workers and Ravanat, Cadet, and co-workers have undertaken detailed studies of guanine oxidation and the formation of thymine and uracil cross-links with guanine.[41−51] In these studies, small quantities of oxidant were generated by photolysis with short laser pulses, resulting

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

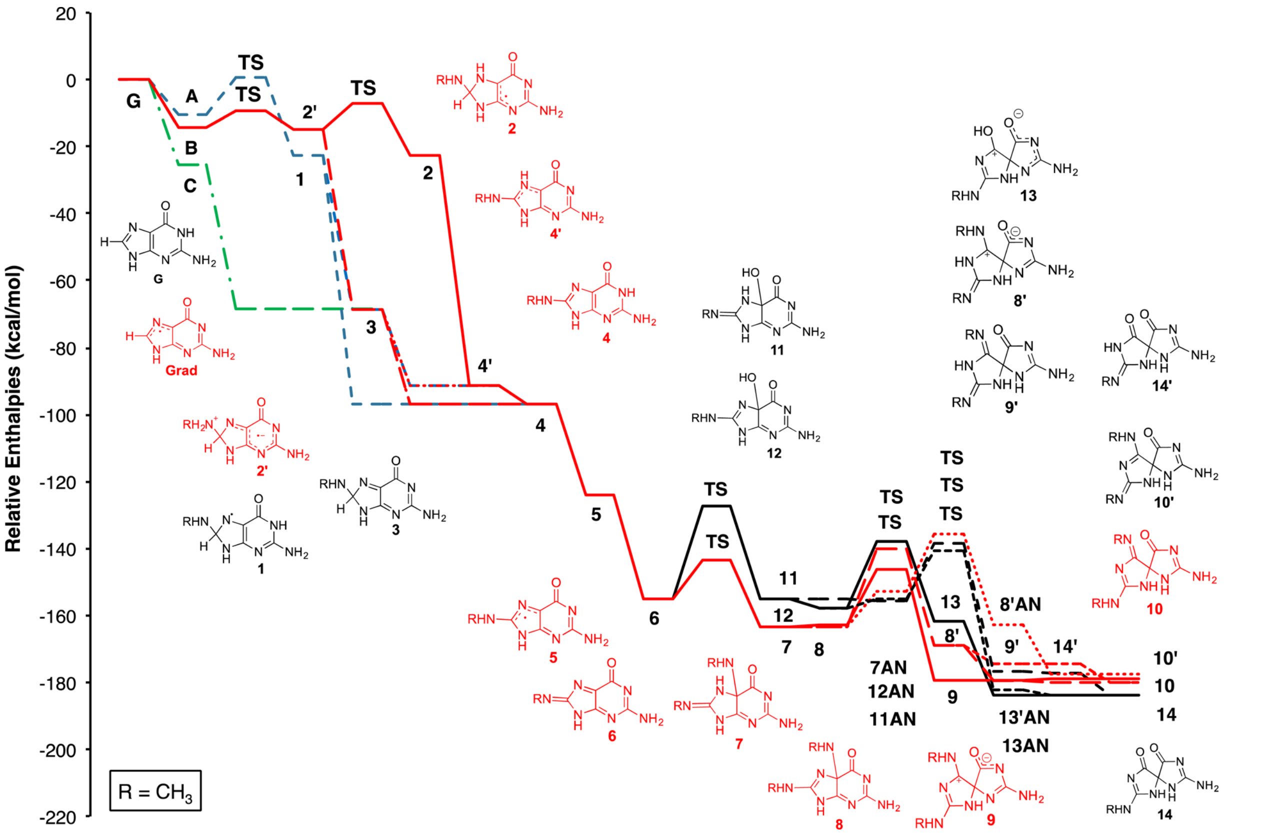


Figure 1. Comparison of the relative enthalpies (kcal/mol) of guanine adducts resulting from ^{3}**BP** oxidation and methylamine addition at the C8 position of guanine followed by nucleophilic addition of methylamine or water at the C5 position calculated at the SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) level of theory. On the left side of the figure, the dashed blue line (A) corresponds to the addition of methylamine radical to guanine, the solid red line (B) corresponds to the addition of methylamine to guanine radical, while the green dash-dot line (C) corresponds to methylamine radical addition to guanine radical. On the right side of the figure, the red line corresponds to the addition of a second methylamine, and the black line corresponds to the addition of water (pathways followed by the anion are shown in dashed or dotted lines). The solid red line represents the most favored pathway.

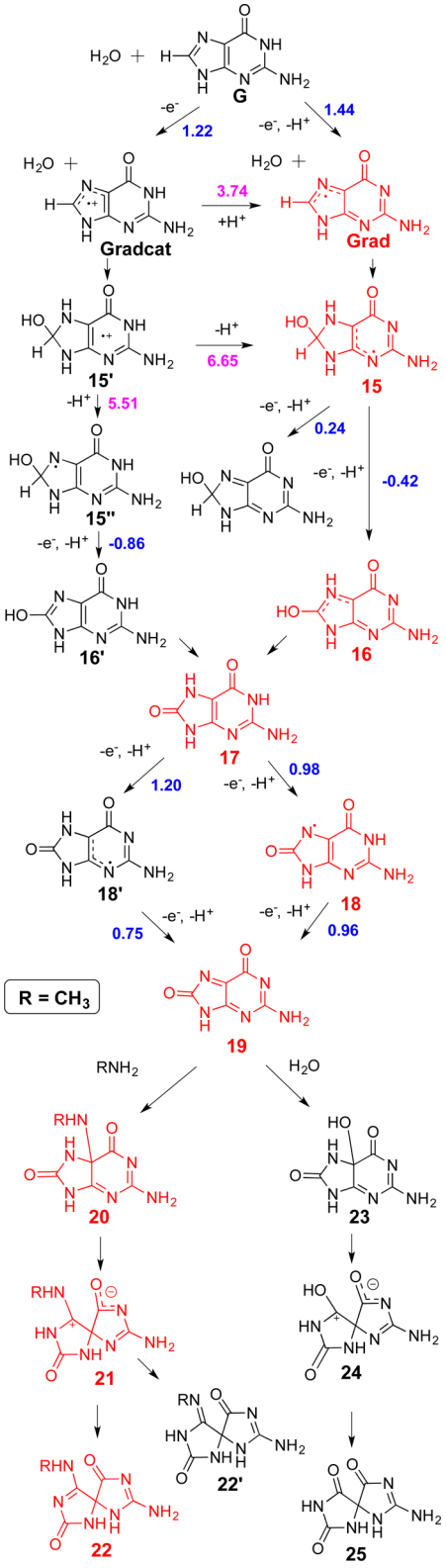
in localized one electron oxidation of guanine. This may be more relevant to *in vivo* conditions where oxidation by strong chemical oxidants would be isolated events. At low pH, water adds rapidly to guanine radical cation to form the 8-hydroxy-7,8-dihydroguanyl radical (8OHGrad); at higher pH, guanine radical cation can deprotonate and water adds more slowly, allowing other reactions such as guanine:thymine cross-links to dominate.[49,50] The second oxidation of 8OHGrad to form 8-oxoG can occur readily with O_2.[52] The generation of additional oxidants leads to the preferential oxidation of 8-oxoG[43] and subsequent formation of products such as Sp, Gh, and Iz. Rate constants are available for many of the initial oxidation steps.[41,42,50,53−55] Labeling studies show that the additional oxygen in 8-oxoG comes from water in riboflavin photosensitized oxidation of guanine.[52] Likewise, when $IrCl_6^{2-}$ is used to oxidize 8-oxoG, the additional oxygen in Sp and Gh comes from water.[56] However, when the carbonate radical anion is the oxidant, the additional oxygens come from the carbonate radical.[46] In previous work, we have used density functional theory to explore pathways for oxidation of guanine to form Sp and Gh, and to calculate the pK_a's and reduction potentials of intermediates in the oxidation of guanine.[57−63] In the present computational study, we have examined various pathways for the formation of C8, C5, and C4 guanine:lysine cross-links resulting from nucleophilic addition of lysine and water to oxidized forms of guanine. Since one of the goals of the present study is to understand the distribution of products seen by Burrows and co-workers, ^{3}**BP** is used as the oxidant, but oxidation by O_2 and O_2^- has also been examined for the appropriate steps. Lowest energy pathways have been considered based on the calculation of pK_a's, reduction potentials, changes in free energy, and reaction barriers. The formation of various DPCs agrees relatively well with reported experimental findings.

■ COMPUTATIONAL PROCEDURES

Calculations were performed with the Gaussian series of codes.[64] Geometries were optimized with B3LYP[65,66] density functional theory using the 6-31+G(d,p)[67−69] basis set with the SMD[70] implicit solvation method to model aqueous solution. Vibrational frequency calculations were used to check that optimized geometries were minima or transition states on the potential energy surface. Transition states had only one imaginary frequency and had a transition vector leading from reactants to products. While B3LYP calculations sometimes underestimate barriers by a few kcal/mol, test calculations on the addition of H_2O and CH_3NH_2 to guanine radical show that the B3LYP barriers are within about 2 kcal/mol of those calculated with ωB97XD. In key reactions, intrinsic reaction coordinate (IRC) calculations[71,72] were used to validate the connection of the transition state with the appropriate reactants and products. Thermal corrections for enthalpies were calculated by standard statistical thermodynamic methods using the unscaled B3LYP frequencies and the ideal gas/rigid rotor/harmonic oscillator approximations. To obtain higher accuracy, single point energies were calculated at the B3LYP/aug-cc-pVTZ[73] level of theory using the B3LYP/6-31+G(d,p)

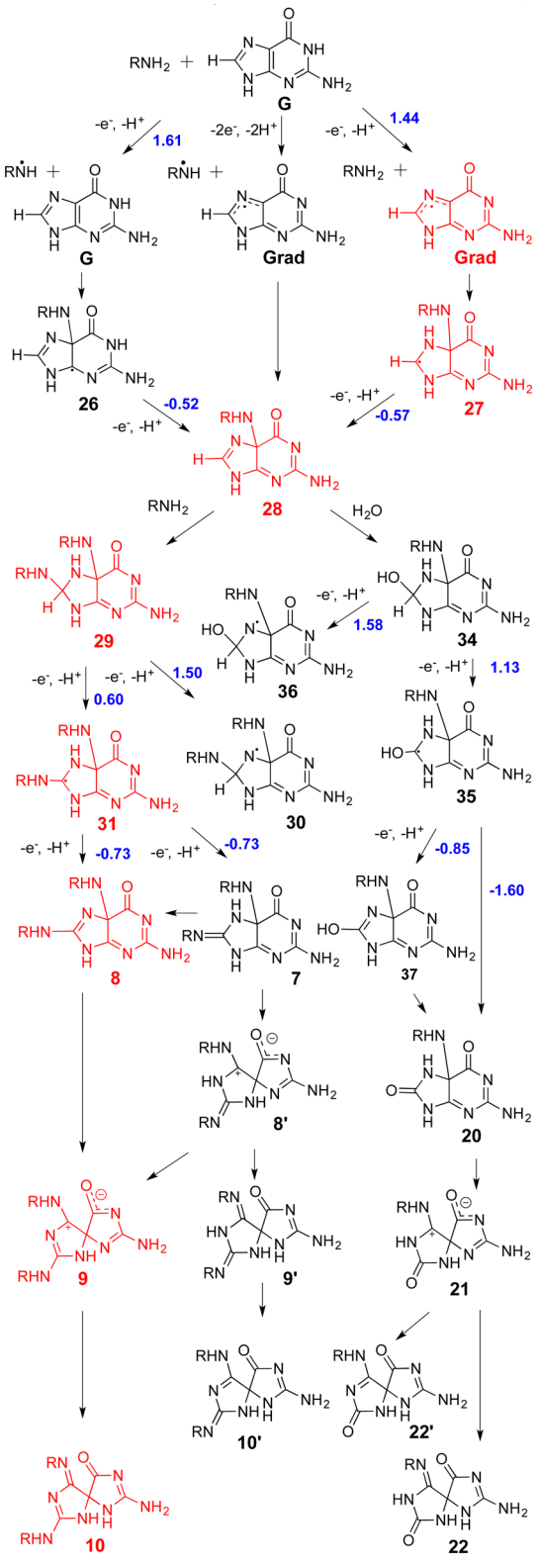
DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

Scheme 3. Pathways for the Addition of Water at C8 of Guanine Followed by Nucleophilic Addition of Methylamine or Water at C5[a]



[a] E^{o} is shown in blue, and the favored path is shown in red.

Scheme 4. Pathways for the Addition of Methylamine at C5 of Guanine Followed by Nucleophilic Addition of Methylamine or Water at C8[a]



[a] E^{o} is shown in blue, and the favored path is shown in red.

geometries optimized in solution. For each species, the enthalpy in solution is the sum of the electronic energy calculated at SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p), zero point energy (ZPE) at SMD/B3LYP/6-31+G(d,p), and thermal corrections at SMD/B3LYP/6-31+G(d,p). The numbering of the atoms in guanine is shown in Scheme 1.

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

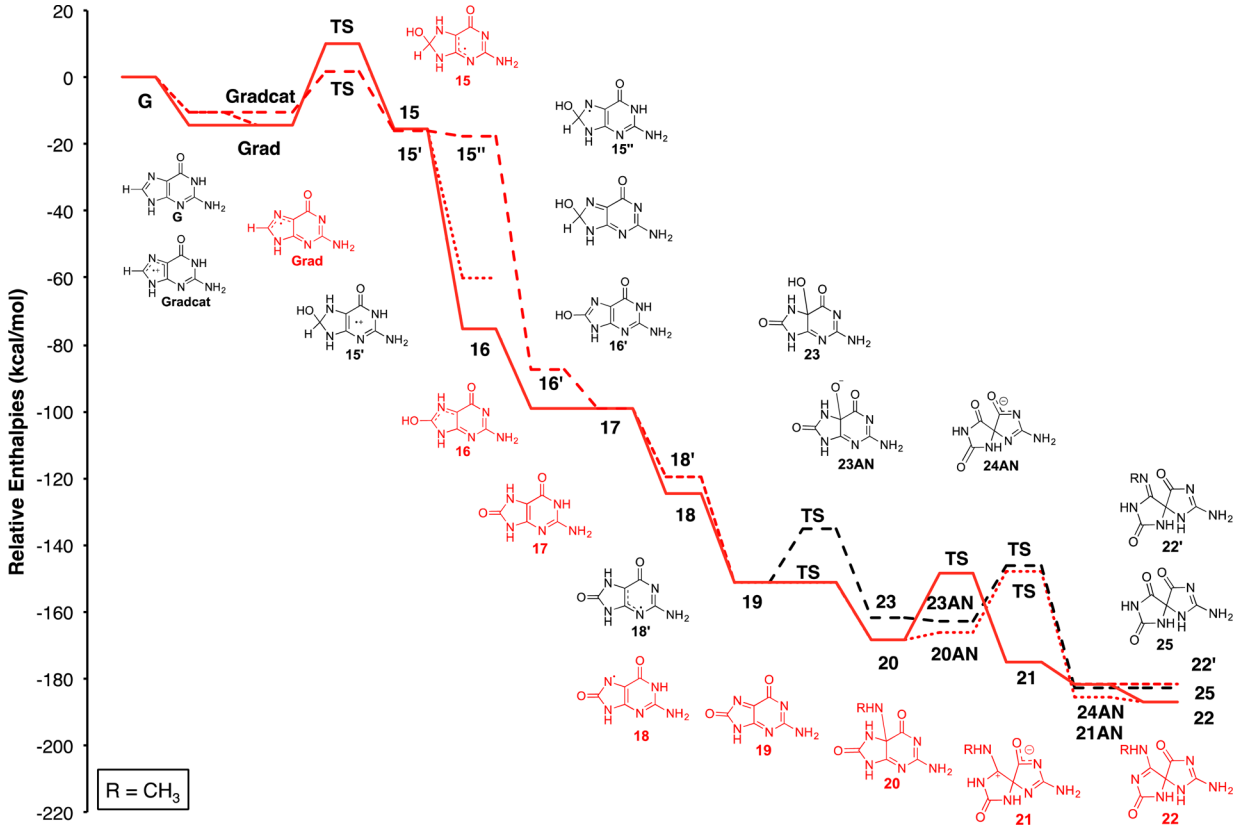


Figure 2. Comparison of the relative enthalpies (kcal/mol) of guanine adducts resulting from ^{3}**BP** oxidation and water addition at the C8 position of guanine radical followed by nucleophilic addition of methylamine or water at the C5 position calculated at the SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) level of theory. On the right side of the figure, the red line corresponds to the addition of the second methylamine, and the black line corresponds to water addition (pathways followed by the anion are shown by dashed or dotted lines). The solid red line represents the most favored pathway.

The pK_a and reduction potential calculations were performed as needed along each reaction pathway. For a reaction involving deprotonation,

$$BH_{(aq)} \rightleftharpoons B^-_{(aq)} + H^+_{(aq)} \quad (1)$$

the pK_a is given by

$$pK_a = \frac{\Delta G_{deprot(aq)}}{2.303RT} \quad (2)$$

where $\Delta G_{deprot(aq)}$ is the change in Gibbs free energy in solution for the deprotonation reaction, R and T are the gas constant (1.987 cal K^{-1} mol^{-1}) and temperature, respectively. The change in Gibbs free energy in solution for a deprotonation reaction is given by

$$\Delta G_{deprot(aq)} = G_{B^-_{(aq)}} + G_{H^+_{(aq)}} - G_{BH_{(aq)}} \quad (3)$$

The Gibbs free energy for protonated ($G_{BH_{(aq)}}$) and deprotonated species ($G_{B^-_{(aq)}}$) in solution can be obtained from optimization and single point energy calculations in solution. $G_{H^+_{(aq)}}$, the solution phase Gibbs free energy of the proton can be expressed as

$$G_{H^+_{(aq)}} = G^o_{H^+_{(g)}} + \Delta G^{1atm\rightarrow 1M} + \Delta G^*_{H^+_{(aq)}} \quad (4)$$

Here, $G^o_{H^+_{(g)}}$, the gas phase standard free energy of the proton, is equal to −6.287 kcal/mol at 298 K. This value is derived from $G^o_{H^+_{(g)}} = H^o_{H^+_{(g)}} - TS^o_{(g)}$ with $H^o_{H^+_{(g)}} = 5/2RT = 1.48$ kcal/mol and $S^o_{(g)} = 26.05$ cal/mol.[74,75] $\Delta G^{1\ atm\rightarrow 1M}$ is the correction for the change in free energy from the standard state of 1 atm to 1 M and is equal to 1.89 kcal/mol. $\Delta G^*_{H^+_{(aq)}}$, the solvation energy of proton, is equal to −265.9 kcal/mol and is taken from the literature.[76−79] Superscripts (°) and (*) represent the standard state in gas and solution phase, respectively.

The standard reduction potential for a reaction is calculated by

$$E^o_{red,(aq)} = -\frac{\Delta G^*_{red,(aq)}}{nF} - SHE$$

where $\Delta G^*_{red,(aq)}$ is the free energy change for the reduction reaction in solution, F is the Faraday constant (23.06 kcal/mol), n is the number of electrons involved in the reaction, and SHE is the absolute potential of the standard hydrogen electrode in an aqueous solution and is estimated to be 4.281 V.[77,78,80]

RESULTS AND DISCUSSION

The formation of guanine:lysine cross-links during oxidation by triplet benzophenone (^{3}**BP**) involves the addition of lysine to C8, C5, and/or C4 of guanine and leads to mono- and disubstituted spiroiminodihydantion (**Sp**) products.[24] Low energy pathways for the formation of these cross-links have been explored using the B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p) level of theory in aqueous solution with an SMD solvation model and are shown in Schemes 2−7. In order to reduce the cost and complexity of the calculations, the side chain of lysine (calc. E_7 = 1.39 V, calc. E_{10} = 1.03 V, exp. E_{10} ∼ 1.00 V[81]) was modeled by methylamine (calc. E_7 = 1.37 V, calc. E_{10} = 1.04 V). Thus, in all of the schemes and figures, R = CH$_3$. For the reactions involving deprotonation and protonation, imidazole and imidazolium were used as proton acceptor and donor, respectively. Since imidazole has a pK_a of 6.9, transferring a proton to/from imidazole/imidazolium is the computational

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

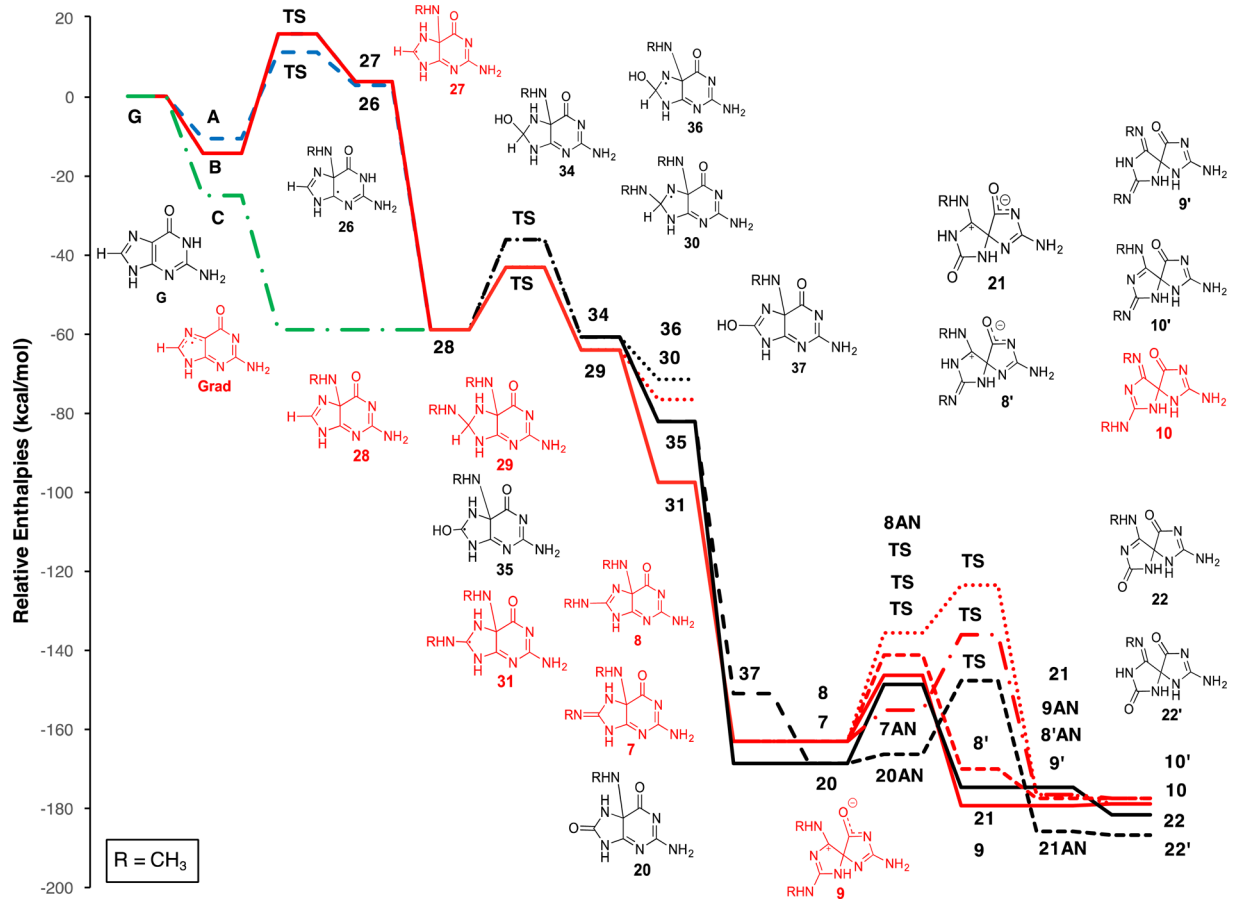


Figure 3. Comparison of the relative enthalpies (kcal/mol) of guanine adducts resulting from ^{3}**BP** oxidation and addition of methylamine at the C5 position of guanine followed by nucleophilic addition of methylamine or water at the C8 position calculated at the SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) level of theory. On the left side of the figure, the dashed blue line (A) corresponds to the addition of methylamine radical to guanine, the solid red line (B) corresponds to the addition of methylamine to guanine radical, while the green dash-dot line (C) corresponds to methylamine radical addition to guanine radical. On the right side of the figure, the red line corresponds to the addition of a second methylamine, and the black line corresponds to the addition of water (pathways followed by the anion are shown in dashed or dotted lines). The solid red line represents the most favored pathway.

equivalent of using a pH 7 buffer solution. Reactions involving the addition of N−H or O−H across a double bond are assisted by an explicit water molecule which reduces the barrier by forming a six-membered ring transition state.

The formation of guanine:methylamine cross-links starts with oxidation by ^{3}**BP** (via proton-coupled electron transfer or direct H abstraction). One-electron oxidation of guanine forms a guanine radical cation, **Gradcat**. Since the pK_a of guanine radical is 3.9,[53] near physiological pH **Gradcat** loses a proton to form a neutral guanine radical, **Grad**. The loss of an electron and a proton is equivalent to the loss of a hydrogen atom. Oxidation of methylamine by ^{3}**BP** forms methylamine radical cation (calculated pK_a = 2.3) which deprotonates to give a neutral methylamine radical, $CH_3NH^{\bullet}$, and this process is calculated to be 4.0 kcal/mol less exothermic than guanine oxidation. As a result, there are three low energy pathways that can initiate the formation of guanine:methylamine adducts at pH 7: (a) the oxidation of methylamine and the addition of neutral methylamine radical to guanine, (b) the oxidation of guanine and the addition of methylamine to neutral radical guanine, and (c) the oxidation of both methylamine and guanine, and the coupling of neutral methylamine and guanine radicals. For the oxidative formation of an initial water adduct, only the addition of water to guanine radical needs to be considered since water is not oxidized by ^{3}**BP**. In the schemes and figure, the lower energy pathways are indicated in red, but as is evident from the energetics in the figures, some of these paths may be favored by only a few kcal/mol.

C8 Addition of Methylamine. The low energy pathways for the formation of C8 guanine:methylamine adducts are shown in Scheme 2, and the thermodynamics related to these pathways for oxidation with ^{3}**BP** are presented in Figure 1. The initial oxidation by ^{3}**BP** can produce either guanine radical or methylamine radical or both. The addition of methylamine radical to C8 of neutral guanine (**G**) results in intermediate **1** and has a barrier height of 11.3 kcal/mol. Neutral methylamine can add to C8 of guanine radical, **Grad**, with a barrier of only 5.4 kcal/mol forming **2′**; tautomerization of **2′** to **2** is exothermic by 0.5 kcal/mol and has a barrier of 7.3 kcal/mol. Protonated methylamine could add across the C8−N7 bond of **Grad** to yield the protonated form of **2′**, but this process is endothermic by 4 kcal/mol. Thus, methylamine addition to **Grad** should be facilitated by higher pH where the equilibrium shifts toward neutral methylamine. At lower pH, the equilibrium moves away from neutral methylamine, and the addition of methylamine radical to guanine could become the dominant pathway, despite the somewhat higher barrier. At even lower pH, guanine radical (pK_a = 3.9) is a

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

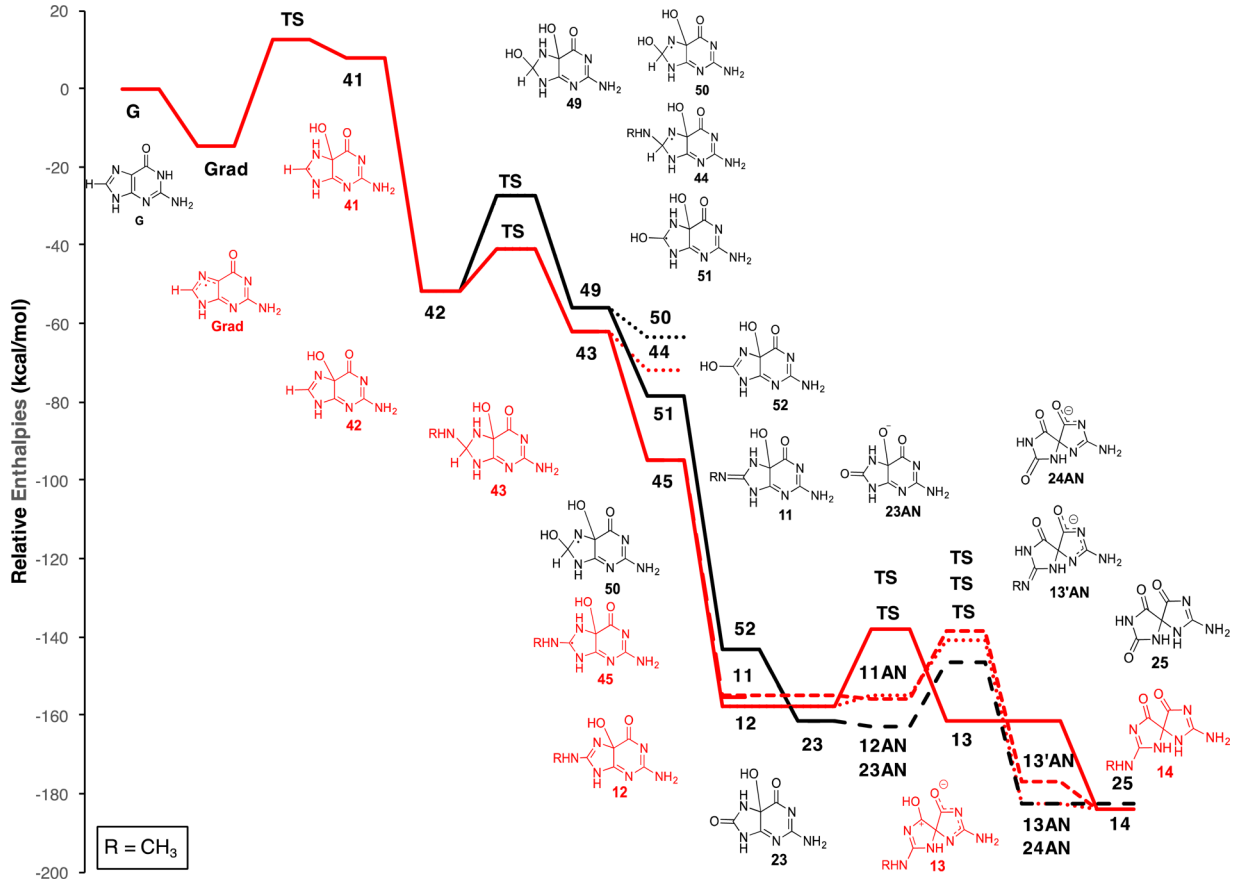


Figure 4. Comparison of the relative enthalpies (kcal/mol) of guanine adducts resulting from ^{3}**BP** oxidation and water addition at the C5 position of guanine radical followed by nucleophilic addition of methylamine or water at the C8 position calculated at the SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) level of theory. On the right side of the figure, the red line corresponds to the addition of a second methylamine, and the black line corresponds to the water addition (pathways followed by the anion are shown by dashed or dotted lines). The solid red line represents the most favored pathway.

cation, **Gradcat**; while addition of neutral methylamine is barrierless, the addition of protonated methylamine would be endothermic. In the third pathway, the coupling reaction between guanine radical and methylamine radical is barrierless and exothermic by 43.2 kcal/mol yielding **3** but is expected to occur at a lower rate because the concentration of radicals produced by ^{3}**BP** oxidation is expected to be low. Thus, in the first step, addition of methylamine to guanine radical is energetically preferred, having a barrier 4−6 kcal/mol lower than that for the addition of methylamine radical to guanine.

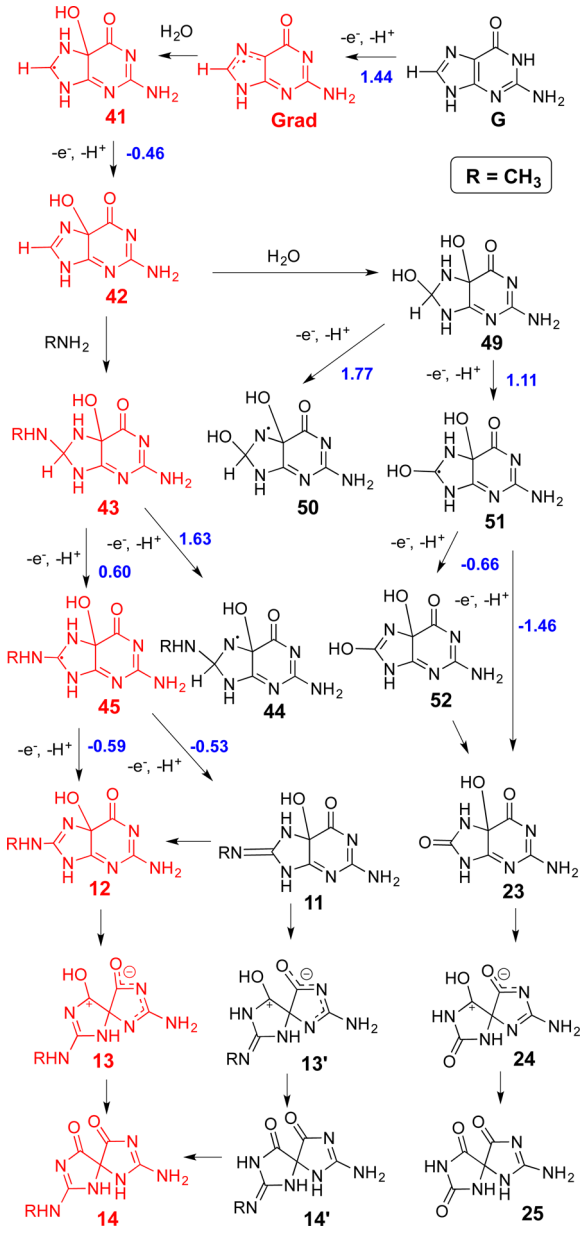
A second oxidation step results in the loss of a hydrogen from C8 in **1** to form **4** (E^o = −1.04 V) or from C8 in **2** to form **4′** (E^o = −0.85 V), rather than the loss of a hydrogen from N1 in **1** or N7 in **2** to form **3**. This oxidation can occur either by ^{3}**BP** if the concentration is high enough (exothermic by 74.0 and 69.2 kcal/mol, respectively) or by $^3O_2$ in aerobic environments (exothermic by 25.1 and 20.0 kcal/mol, respectively). Intermediate **4′** can be readily converted to the more stable tautomer **4** by shifting a proton from N7 to N1. Structure **4**, 8-methylaminoguanine, is the methylamino analogue of 8-oxoG and corresponds to product C observed by Perrier et al.[21] in riboflavin mediated oxidation of TGT oligonucleotide in the presence of trilysine. Morin and Cadet[12] also observed products from C8 addition of lysine in ^{3}**BP** oxidation of lysine tethered to **5′** of 2′-deoxyguanosine. Similarly, Silerme et al.[28] found polyamine:guanine cross-links at C8 of guanine in riboflavin sensitized oxidation of double stranded DNA in the presence of polyamines. Structure **3** resulting from the coupling of methylamine radical and guanine radical is 28.5 kcal/mol less stable than **4** but can be converted to **4** by the tautomerization of a proton from C8 to N1.

The third oxidation step, from **4** to **5**, is exothermic by 27.2 kcal/mol with ^{3}**BP** but endothermic with O_2 or superoxide, O_2^-. Since addition of methylamine or water to C5 of **5** is endothermic (25.9 or 29.2 kcal/mol, respectively), another oxidation is needed before the addition can occur. The oxidation of **5** is exothermic with ^{3}**BP** and HOO. (31.1 and 10.8 kcal/mol, respectively) but endothermic with O_2 and O_2^- (17.7 and 3.0 kcal/mol, respectively) and yields **6** (pK_a = −1.22 for protonation of **6**).

In the final addition step, methylamine or water can add across the C5−N7 double bond of **6**. Addition of neutral methylamine, assisted by an explicit water molecule, is calculated to have a barrier height of 12.0 kcal/mol and forms the 5,8-methylamine disubstituted guanine intermediate, **7**. Perrier et al.[21] observed a corresponding 5,8-lysine adduct in the oxidation of TGT and trilysine. This product was favored by higher pH where the NH_2 groups are not protonated. Addition of water across the C5−N7 double bond of **6** (also assisted by one water) is calculated to have a higher energy barrier, 27.6 kcal/mol. This barrier drops to 16.5 kcal/mol when assisted by two water molecules. In the methylamine and water adducts, **7** and **11**, can tautomerize to **8** and **12**, respectively.

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

Scheme 5. Pathways for the Addition of Water at C5 of the Guanine Followed by Nucleophilic Addition of Methylamine or Water at C8[a]

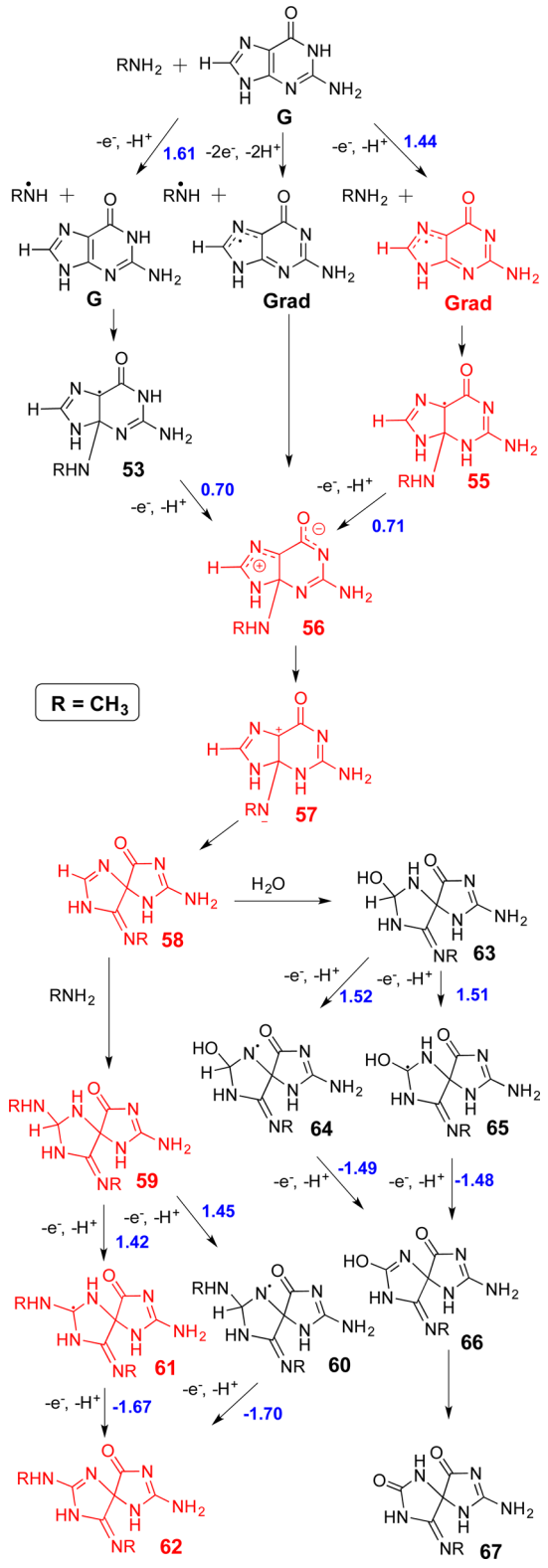


[a] E^{o} is shown in blue, and the favored path is shown in red.

Migration of the acyl group from C5 to C4 followed by tautomerization leads to the final products, 5,8-diNHCH$_3$-spiroiminodihydantoin, **5,8-diNHCH$_3$-Sp** (**10** or **10′**) and 8-NHCH$_3$-spiroiminodihydantoin, **8-NHCH$_3$-Sp** (**14** or **14′**), respectively.[82] As shown in Figure 1, the barrier for acyl migration in **7** is lower for the neutral form than for the deprotonated species. In solution near physiological pH, the acyl group migration of **11** occurs only in deprotonated species (pK_a = 6.6) as found in our earlier study.[58]

C8 Addition of Water. Scheme 3 shows the pathway for the addition of water at C8 of guanine radical, and the corresponding energetics are shown in Figure 2. ^{3}**BP** can oxidize guanine but not water. At low pH, guanine radical (pK_a = 3.9) remains protonated, and water addition to C8 of **Gradcat** has a barrier of 12.3 kcal/mol. At pH 7, guanine

Scheme 6. Pathways for the Addition of Methylamine at C4 of Guanine Followed by Nucleophilic Addition of Methylamine or Water at C8[a]



[a] E^{o} is shown in blue, and the favored path is shown in red.

radical is predominantly neutral, and addition of water to C8 of **Grad** assisted by an explicit water molecule has a barrier of 24.6 kcal/mol. Although the concentration of **Gradcat** at pH 7 is only about 10^{-3} times the concentration of **Grad**, the reaction

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

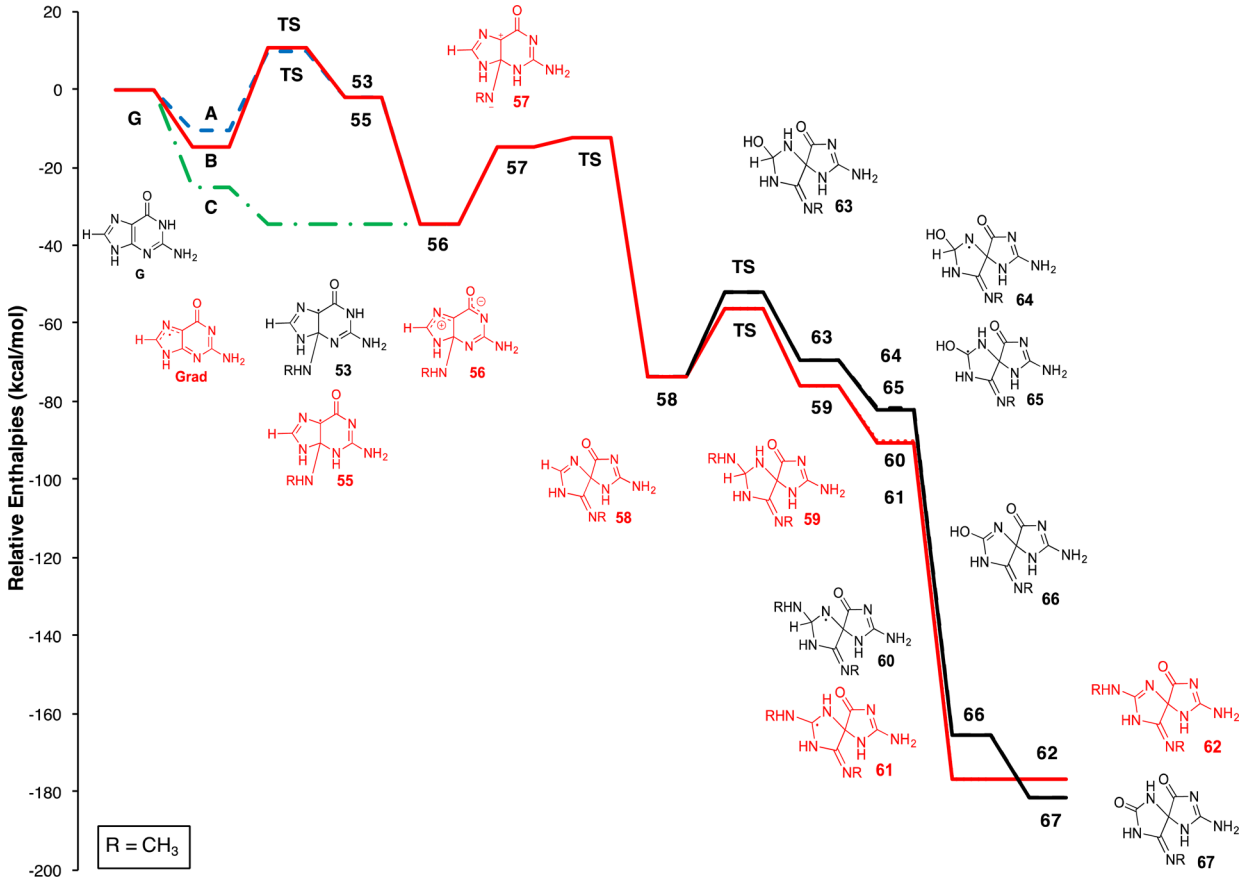


Figure 5. Comparison of the relative enthalpies (kcal/mol) of guanine adducts resulting from ^{3}**BP** oxidation and radical addition of methylamine at the C4 position of guanine followed by nucleophilic addition of methylamine or water at the C8 position calculated at the SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) level of theory. On the left side of the figure, the dashed blue line (A) corresponds to the addition of methylamine radical to guanine, the solid red line (B) corresponds to the addition of methylamine to guanine radical, while the green dash-dot line (C) corresponds to methylamine radical addition to guanine radical. On the right side of the figure, the red line corresponds to the addition of a second methylamine, and the black line corresponds to the addition of water. The solid red line represents the most favored pathway.

can proceed more rapidly by water addition to **Gradcat** because of the much lower barrier. The intermediate, 8-hydroxy-7,8-dihydroguanine (**8OHGrad**), **15**, can lose hydrogen from C8 of **15** due to oxidation by ^{3}**BP** or O_2 (ΔH = −59.8 and −11.0 kcal/mol, respectively) and produces **16** (E^o = −0.42). Tautomerization of **16** leads to the more stable form of 8-oxoG, **17**. The fact that the barrier for water addition is much smaller for **Gradcat** than for **Grad** is in agreement with Crean et al.[49] and Rokhlenko et al.[50] who observed that water adds readily to **Gradcat** at low pH to form 8-oxoG and its oxidation products but were unable to detect these products at high pH.

The third oxidation step is exothermic with ^{3}**BP** (ΔH = −25.5 kcal/mol) but endothermic with O_2 and O_2^- (ΔH = 23.4 and 8.6 kcal/mol, respectively). Oxidative loss of hydrogen from N7 to produce **18** is favored over loss of hydrogen from N1 to form **18′**. Addition of methylamine or water to C5 of **18** is endothermic (ΔH = 19.0 and 22.8 kcal/mol, respectively), so another oxidation is required before addition can take place. The fourth oxidation step is exothermic with ^{3}**BP** (ΔH = −26.4 kcal/mol) but endothermic with O_2 or superoxide (ΔH = 22.5 and 7.8 kcal/mol, respectively), and produces 8-oxoG^{ox}, **19**.

Addition of methylamine across the C5−N7 double bond of **19** to form **20** occurs without a barrier when assisted by an explicit water molecule. However, the corresponding addition of water to **19** to form **23** has a barrier of 16.2 kcal/mol assisted by one water and 9.8 kcal/mol assisted by two waters. Both **20** and **23** can undergo C5 to C4 acyl group migration followed by tautomerization to give final products, 5-NHCH$_3$-spiroiminodihydantoin, **5-NHCH$_3$-Sp** (**22** or **22′**) and spiroiminodihydantoin, **Sp** (**25**), respectively. For the C5 methylamine adduct, **20**, acyl migration is energetically more favorable in neutral form than in the deprotonated form. In agreement with our earlier study,[58] the doubly water substituted adduct, **23**, needs to be deprotonated (pK_a = 6.3), before it undergoes acyl migration to form the spirocyclic product, **25**.

From the computational results discussed in the previous paragraphs, addition of neutral methylamine at C8 of guanine radical, **Grad**, is energetically the most favored pathway among the various pathways discussed for pH 7. The barrier for the addition of methylamine radical to guanine is 4 kcal/mol higher. The addition of water to **Grad** has a significantly higher barrier than addition to **Gradcat**. Higher pH facilitates the addition of methylamine to **Grad**, while H_2O addition to **Gradcat** is promoted by lower pH. Oxidation by ^{3}**BP** or O_2 leads to 8-methylaminoguanine, **4**, the methylamino analogue of 8-oxoG, **17**. Two more oxidations of **4** produce **6**, the methylamino analogue of 8-oxoG^{ox}. For the second addition step, addition of methylamine across the C5−N7 double bond of **6** again has a lower barrier than water.

C5 Addition of Methylamine. As in the C8 additions, three pathways were considered for methylamine reacting with guanine to form C5 guanine:methylamine adducts, as shown in

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

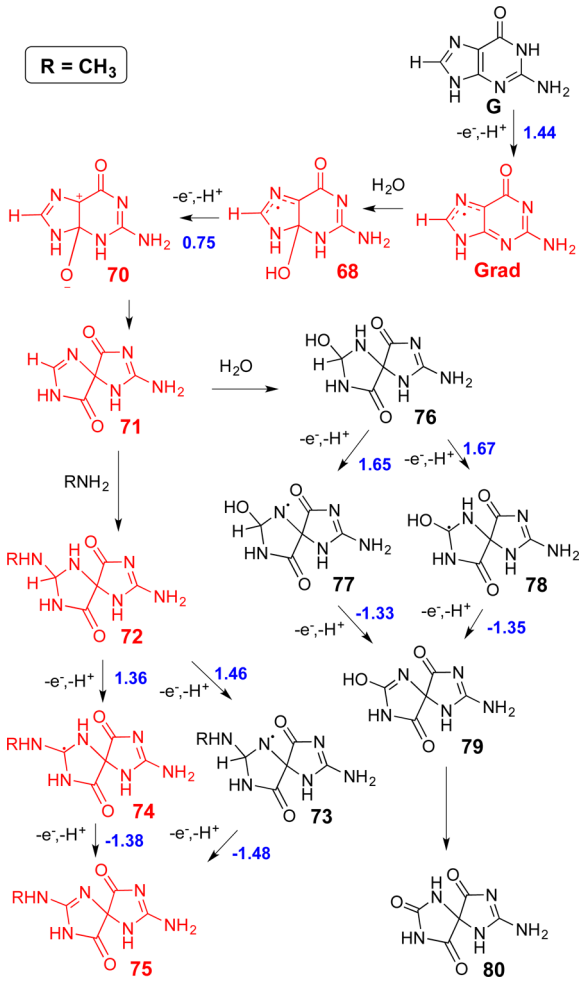
Scheme 4 and Figure 3. The addition of methylamine radical at C5 of neutral guanine (**G**) has a barrier of 21.6 kcal/mol and forms **26**. Water assisted addition of methylamine across the C5−N7 double bond of guanine radical has a barrier of 30.6 kcal/mol and forms **27** (the corresponding barrier for NH_3 addition is 31.7 kcal/mol). Oxidation of **26** and **27** can occur by either ^{3}**BP** (ΔH = −61.5 and −62.6 kcal/mol, respectively) or O_2 (ΔH = −12.7 and −13.8 kcal/mol, respectively). Hydrogen can be lost from N1 of **26** (E^o = −0.52 V) or N7 of **27** (E^o = −0.57 V), yielding **28**. Intermediate **28** can also be formed directly via the coupling of methylamine radical and guanine radical but would occur at a lower rate because the concentrations of these reactive species are expected to be low. A second addition can occur across the C8−N7 double bond of **28**. The barrier for methylamine addition to **28** forming **29** (15.8 kcal/mol) is 7.0 kcal/mol lower than the barrier for water addition forming **34**.

The third oxidation step is mediated by ^{3}**BP** and leads to the loss of the C8 hydrogen from **29** and **34** to produce **31** and **35**, respectively. The subsequent oxidation can occur by ^{3}**BP** (ΔH = −65 to −80 kcal/mol) or O_2 (ΔH = −16 to −37 kcal/mol). Loss of a hydrogen from **31** yields the oxidized intermediates **7** or **8**; loss of a hydrogen from **35** yields **20** or **37**, which can tautomerize to **20**. Intermediates **8**, **7**, and **20** can undergo acyl migration from C5 to C4 to produce the spiroimino intermediates, **9**, **9′**, and **21**, respectively. Acyl migration in the neutral form is favored over the anionic form (see Figure 3). Tautomerization of **9**, **9′**, and **21** forms the final products **5,8-diNHCH$_3$-Sp** (**10** and **10′**) and **5-NHCH$_3$-Sp** (**22** or **22′**), respectively.

C5 Addition of Water. Scheme 5 and Figure 4 show the pathways and energetics for C5 addition of water to guanine radical followed by C8 addition of methylamine or water. The barrier for water addition across the C5−N7 double bond to form **41** (ΔH = 27.1 kcal/mol) is 3.5 kcal/mol lower than that for the methylamine addition. Intermediate **41** is readily oxidized by either ^{3}**BP** or O_2 and loss of a proton leads to 5-OH guanine, **42** (ΔH = −59.5 and −10.7 kcal/mol, respectively, E^o = −0.46 V). In the second addition step, methylamine or water can add across the C8−N7 double bond of the **42**. Methylamine addition to form **43** has a much lower barrier (11.1 kcal/mol) than water addition to form **49** (24.7 kcal/mol). ^{3}**BP** is required for the oxidation of the 8-NHCH$_3$,5-OH guanine adduct, **43** (ΔH = −31.7 kcal/mol), and results in loss of a proton from C8 to form **45**. In turn, **45** can be readily oxidized by ^{3}**BP** or O_2 (ΔH = −62.8 and −13.9 kcal/mol, respectively) to form **11** and **12**. Near the physiological pH of 7.5, both **11** and **12** can easily lose another proton from the C5-OH group (pK_a 6.9 and 6.6, respectively) to form anions. These anionic intermediates of **11** and **12** undergo acyl group migration from C5 to C4 (barrier heights of 16.5 and 17.4 kcal/mol, respectively), followed by reprotonation to form final neutral products, **14′** and **14**, respectively. **14′** can tautomerize to form the lower energy structure, **14**. Nucleophilic addition of a second water at C8 of **42** follows a pathway similar to that of methylamine addition. The adduct **49** undergoes oxidation with ^{3}**BP** to form **51** and then oxidation by ^{3}**BP** or O_2 to form **23**. Acyl group migration of the anionic form of **23** (pK_a 6.3) yields the final product, spiroiminodihydantoin, **Sp** (**25**).

The lowest barriers for reactions at C5 are for methylamine radical addition to guanine (21.6 kcal/mol) and for water addition to guanine radical (27.1 kcal/mol); however, both barriers are higher than C8 addition of methylamine to guanine

Scheme 7. Pathways for the Addition of Water at C4 of Guanine Followed by Nucleophilic Addition of Methylamine or Water at C8[a]



[a] E^o is shown in blue, and the favored path is shown in red.

radical (5.4 kcal/mol). After the C5 addition, the barrier for the second nucleophilic addition at C8 is lower for methylamine than that for water, in part because the N−H bond in methylamine is weaker than the O−H bond in water.

C4 Addition of Methylamine. Three pathways for the C4 addition of methylamine to guanine are shown in Scheme 6 and Figure 5. Similar to C5, any addition at C4 of guanine removes the planarity of the molecule and interrupts the conjugation, resulting in higher energy barriers and intermediates than that for the corresponding steps in C8 addition. Addition of methylamine radical to C4 has a barrier of 20.3 kcal/mol to produce 4-NHCH$_3$-guanine radical, **53** (endothermic by 8.7 kcal/mol). Hydrogen loss from N1 of **53** can occur by ^{3}**BP** oxidation but not by O_2 oxidation (ΔH = −32.4 and 16.4 kcal/mol, respectively) and forms a zwitterionic intermediate, **56** (E^o = 0.70). The reaction between guanine radical and methylamine radical produces **56** as well. Intermediate **56** can also be formed via the water-assisted addition of methylamine across the C4−N3 double bond of **Grad** (26.1 kcal/mol barrier), followed by loss of the N3 hydrogen from **55** by ^{3}**BP** oxidation. Tautomerization of **56** to a different zwitterionic species, **57**, is required before the system can undergo the ring rearrangement reaction to form the spiro intermediate, **58**. In the second addition step, water or methylamine can add to

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

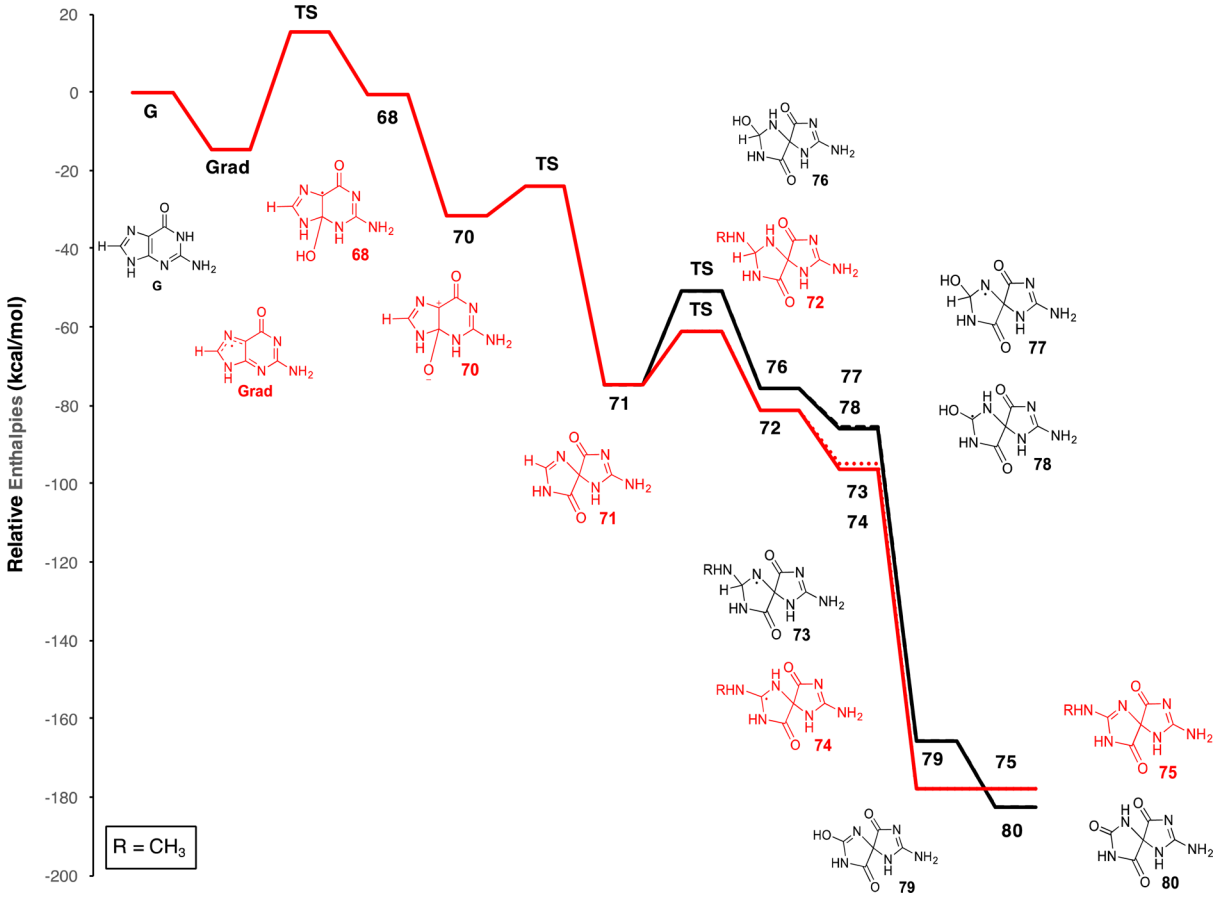


Figure 6. Comparison of the relative enthalpies (kcal/mol) of guanine adducts resulting from ^{3}**BP** oxidation and addition of water at the C4 position of guanine radical followed by nucleophilic addition of methylamine or water at the C8 position calculated at the SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) level of theory. On the right side of the figure, the red line corresponds to the addition of the second methylamine, and the black line corresponds to the water addition. The solid red line represents the most favored pathway.

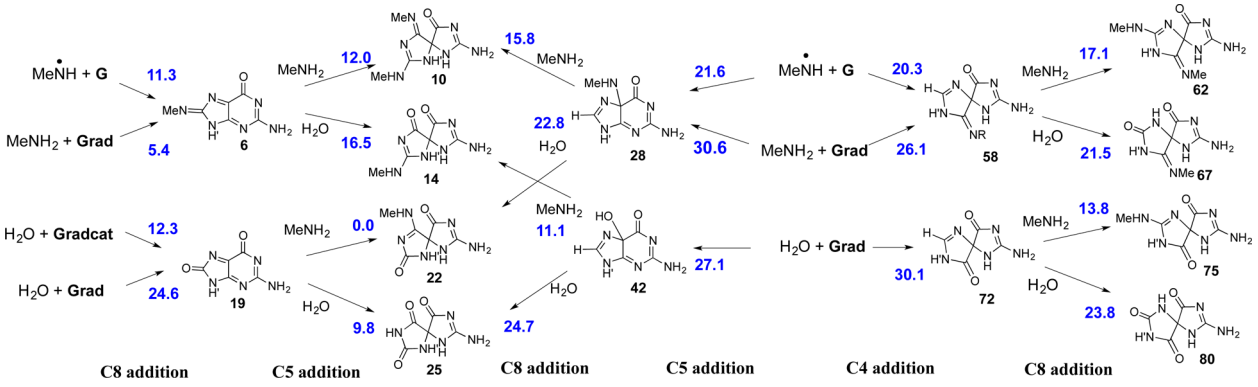


Figure 7. Summary of the pathways for the formation of the guanine:methylamine cross-link mediated by ^{3}**BP**. Numbers shown in blue correspond to the barrier for the addition reaction (in kcal/mol). For 2′-deoxyguanosine, the sugar would be attached to the position indicated by H′.

C8 of the spiro intermediate. Addition of a methylamine across the C8−N7 double bond of **58** has a barrier of 17.1 kcal/mol and leads to the formation of the 4-NCH$_3$, 8-NHCH$_3$ oxidized spiro intermediate, **59** (exothermic by 2.4 kcal/mol). Addition of methylamine to C8 could occur before ring rearrangement, but the barriers are 4−8 kcal/mol higher. Compared to methylamine, the barrier for water addition to the C8−N7 double bond of **58** is 4.4 kcal/mol higher in energy and forms the 4-NCH$_3$, 8-OH spiro intermediate, **63**. Oxidation of **59** and **63** requires ^{3}**BP**, leading to intermediates that are readily oxidized by ^{3}**BP** or O_2 to the final products, **4,8-diNHCH$_3$-Sp** (**62**) and **4-NHCH$_3$-Sp** (**67**), respectively.

C4 Addition of Water. Scheme 7 and Figure 6 show the pathways for the addition of water to the C4 of guanine radical followed by the second nucleophilic addition of methylamine or water. Addition of water to the C4−N3 double bond of guanine radical (**Grad**), assisted by another explicit water molecule has a barrier of 30.1 kcal/mol and forms **68** (endothermic by 14.1 kcal/mol). 4-OH-Guanine radical, **68**, can lose hydrogen by ^{3}**BP** oxidation to form a zwitterionic intermediate, 4-OH guanine, **70** (E^o = 0.75 V). Rearrangement of the ring in **70**

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

produces the 4-oxo spiro intermediate, **71**. In the second addition step, adding methylamine across the C8−N7 double bond to produce **72** has a barrier that is 10.2 kcal/mol lower than that for adding water to produce **76**. Oxidation of **72** and **76** by ^{3}**BP** forms intermediates **73**, **74**, and **77**, **78**, respectively, that are easily oxidized by ^{3}**BP** or O_2 to the final products, **8-NHCH$_3$-Sp** isomer, **75** and **Sp** isomer, **80**.

The results presented above indicate that neither methylamine nor water addition at C4 of guanine is favored over C8 addition. Although methylamine addition at C4 of guanine radical has a lower barrier than water addition, both processes are endothermic. Unlike in the C8 or C5 addition pathways, the rearrangement to the spirocyclic intermediate is predicted to occur before the addition of a second nucleophile.

SUMMARY

Potential energy surfaces for the triplet benzophenone initiated, radical mediated oxidation of guanine leading to the formation of the mono- and dimethylamine substituted spiroiminodihydantion products have been mapped out using DFT calculations with SMD solvation. In addition to barrier heights and enthalpies, pK_a's and reduction potentials were calculated for intermediates to find the lowest energy paths. These pathways are summarized in Figure 7. The product yields depend on the relative concentrations as well as barrier heights and reaction energies. The addition of protonated methylamine to C8 of guanine radical is endothermic, but addition of neutral methylamine is exothermic. C8 addition of neutral methylamine is kinetically preferred over C5 and C4 addition. Likewise, the addition of neutral methylamine radical at C8 of guanine is preferred over addition at C5 and C4. Even though the coupling of methylamine radical and guanine radical is barrierless, this rate should be lower because the radicals are expected to be short-lived and low in concentration. The barriers for water addition to C8, C5, and C4 of neutral guanine radical are higher than the corresponding barriers for methylamine addition. In the absence of other nucleophiles, water addition at C8 is kinetically favored over C5 or C4. The barrier for C8 addition of water is much lower for guanine radical cation than for neutral guanine radical. Further oxidations by triplet benzophenone or O_2 and loss of protons are needed before the second nucleophilic addition can take place. Again, methylamine addition has lower barriers than water addition. After the initial addition, all of the steps are exothermic and expected to proceed rapidly. Starting with the addition of methylamine at C8 of **Grad**, oxidation by ^{3}**BP** or O_2 leads to an 8-methylamine substituted oxidized guanine, **4**, which is the methylamino analogue of 8-oxoG, **17**. After oxidation of **4** or **17** by ^{3}**BP**, the barriers for the addition of methylamine across the C5−N7 double bond in **6** or **19** are significantly lower than that for the corresponding addition of water. Nevertheless, if the concentration of methylamine (or lysine) is low, water addition could be competitive with methylamine addition. This would lead to comparable fractions of **8-NHCH$_3$-Sp** and **5,8-diNHCH$_3$-Sp**, in agreement with the experimental observations.[24] For larger concentrations of methylamine, the fraction of 5,8-dimethylamine substituted products should increase, in agreement with experimental observations.[24] Higher pH, which shifts the equilibrium more toward unprotonated methylamine, should also increase the yield of the 5,8 methylamine adduct, as seen in related experiments.[21] However, low pH or hydrogen bonding in a base pair stabilizes guanine radical cation and favors the addition of water at C8, in agreement with experimental data at pH 2.5[50] and in double stranded DNA.[51]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.6b00057.

Optimized molecular geometries in Cartesian coordinates for all of the reactants, intermediates, transition states, and products (PDF)

Total and relative energies for various compounds, details of the calculation of the pK_a's and reduction potentials (XLSX)

AUTHOR INFORMATION

Corresponding Author

*Tel: 313-577-2562. Fax: 313-577-8822. E-mail: hbs@chem.wayne.edu.

Funding

This work was supported by grants from National Science Foundation (CHE1464450 to H.B.S. and CHE1507813 to C.J.B.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

B.T. thanks Wayne State University for a Thomas C. Rumble Fellowship. We also thank the Wayne State University computing grid for the computational time.

ABBREVIATIONS

DPC, DNA−protein cross-link; ^{3}BP, triplet benzophenone; G, guanine; Gradcat, guanine radical cation; Grad, guanine radical neutral; 8OHGrad, 8-hydroxy-7,8-dihydroguanine radical neutral; 8-oxoG, 8-oxo-7,8-dihydroguanine; Sp, spiroiminodihydantoin; Gh, guanidinohydantoin; Iz, 2,5-diamino-4*H*-imidazolone; 5-NHCH$_3$−Sp, 5-methylamino-spiroiminodihydantoin; 5,8-diNHCH$_3$−Sp, 5,8-dimethylamino-spirioiminodihydantoin; 8-NHCH$_3$−Sp, 8-methylamino-spiroiminodihydantoin

REFERENCES

(1) Breen, A. P., and Murphy, J. A. (1995) Reactions of oxyl radicals with DNA. *Free Radical Biol. Med. 18*, 1033−1077.

(2) Burrows, C. J., and Muller, J. G. (1998) Oxidative nucleobase modifications leading to strand scission. *Chem. Rev. 98*, 1109−1152.

(3) Hasty, P., Campisi, J., Hoeijmakers, J., van Steeg, H., and Vijg, J. (2003) Aging and genome maintenance: Lessons from the mouse? *Science 299*, 1355−1359.

(4) Gimisis, T., and Cismaş, C. (2006) Isolation, characterization, and independent synthesis of guanine oxidation products. *Eur. J. Org. Chem. 2006*, 1351−1378.

(5) Pratviel, G., and Meunier, B. (2006) Guanine oxidation: One- and two-electron reactions. *Chem. - Eur. J. 12*, 6018−6030.

(6) Neeley, W. L., and Essigmann, J. M. (2006) Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chem. Res. Toxicol. 19*, 491−505.

(7) Cadet, J., Douki, T., and Ravanat, J.-L. (2008) Oxidatively generated damage to the guanine moiety of DNA: Mechanistic aspects and formation in cells. *Acc. Chem. Res. 41*, 1075−1083.

(8) Cadet, J., Douki, T., and Ravanat, J.-L. (2010) Oxidatively generated base damage to cellular DNA. *Free Radical Biol. Med. 49*, 9−21.

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

(9) Strniste, G. F., and Rall, S. C. (1976) Induction of stable protein-deoxyribonucleic acid adducts in Chinese hamster cell chromatin by ultraviolet light. *Biochemistry 15*, 1712−1719.
(10) Ramakrishnan, N., Clay, M. E., Xue, L. Y., Evans, H. H., and Rodriguezantunez, A. (1988) Induction of DNA-protein cross-links in Chinese hamster cells by the photodynamic action of chloroaluminum phthalocyanine and visible light. *Photochem. Photobiol. 48*, 297−303.
(11) Morin, B., and Cadet, J. (1994) Benzophenone photosensitization of 2′-deoxyguanosine: Characterization of the 2R and 2S diastereoisomers of 1-(2-deoxy-beta-d-erythro-pentofuranosyl)- 2-methoxy-4,5-imidazolidinedione. A model system for the investigation of photosensitized formation of DNA-protein crosslinks. *Photochem. Photobiol. 60*, 102−109.
(12) Morin, B., and Cadet, J. (1995) Chemical aspects of the benzophenone-photosensitized formation of two lysine-2′-deoxyguanosine cross-links. *J. Am. Chem. Soc. 117*, 12408−12415.
(13) Morin, B., and Cadet, J. (1995) Type I benzophenone-mediated nucleophilic reaction of 5′-amino-2′,5′-dideoxyguanosine. A model system for the investigation of photosensitized formation of DNA-protein cross-links. *Chem. Res. Toxicol. 8*, 792−799.
(14) Altman, S. A., Zastawny, T. H., Randers-Eichhorn, L., Cacciuttolo, M. A., Akman, S. A., Dizdaroglu, M., and Rao, G. (1995) Formation of DNA-protein cross-links in cultured mammalian cells upon treatment with iron ions. *Free Radical Biol. Med. 19*, 897−902.
(15) Zhitkovich, A., Voitkun, V., and Costa, M. (1996) Formation of the amino acid−DNA complexes by hexavalent and trivalent chromium in vitro: Importance of trivalent chromium and the phosphate group. *Biochemistry 35*, 7275−7282.
(16) Nguyen, K. L., Steryo, M., Kurbanyan, K., Nowitzki, K. M., Butterfield, S. M., Ward, S. R., and Stemp, E. D. A. (2000) DNA−protein cross-linking from oxidation of guanine via the flash−quench technique. *J. Am. Chem. Soc. 122*, 3585−3594.
(17) Chakrabarti, S. K., Bai, C., and Subramanian, K. S. (2001) DNA−protein crosslinks induced by nickel compounds in isolated rat lymphocytes: Role of reactive oxygen species and specific amino acids. *Toxicol. Appl. Pharmacol. 170*, 153−165.
(18) Kurbanyan, K., Nguyen, K. L., To, P., Rivas, E. V., Lueras, A. M. K., Kosinski, C., Steryo, M., González, A., Mah, D. A., and Stemp, E. D. A. (2003) DNA−protein cross-linking via guanine oxidation: Dependence upon protein and photosensitizer†. *Biochemistry 42*, 10269−10281.
(19) Johansen, M. E., Muller, J. G., Xu, X., and Burrows, C. J. (2005) Oxidatively induced DNA−protein cross-linking between single-stranded binding protein and oligodeoxynucleotides containing 8-oxo-7,8-dihydro-2′-deoxyguanosine†. *Biochemistry 44*, 5660−5671.
(20) Barker, S., Weinfeld, M., and Murray, D. (2005) DNA−protein crosslinks: Their induction, repair, and biological consequences. *Mutat. Res., Rev. Mutat. Res. 589*, 111−135.
(21) Perrier, S., Hau, J., Gasparutto, D., Cadet, J., Favier, A., and Ravanat, J.-L. (2006) Characterization of lysine−guanine cross-links upon one-electron oxidation of a guanine-containing oligonucleotide in the presence of a trilysine peptide. *J. Am. Chem. Soc. 128*, 5703−5710.
(22) Sun, G., Fecko, C. J., Nicewonger, R. B., Webb, W. W., and Begley, T. P. (2006) DNA−protein cross-linking: Model systems for pyrimidine−aromatic amino acid cross-linking. *Org. Lett. 8*, 681−683.
(23) Xu, X., Fleming, A. M., Muller, J. G., and Burrows, C. J. (2008) Formation of tricyclic [4.3.3.0] adducts between 8-oxoguanosine and tyrosine under conditions of oxidative DNA−protein cross-linking. *J. Am. Chem. Soc. 130*, 10080−10081.
(24) Xu, X., Muller, J. G., Ye, Y., and Burrows, C. J. (2008) DNA−protein cross-links between guanine and lysine depend on the mechanism of oxidation for formation of C5 vs C8 guanosine adducts. *J. Am. Chem. Soc. 130*, 703−709.
(25) Solivio, M. J., Joy, T. J., Sallans, L., and Merino, E. J. (2010) Copper generated reactive oxygen leads to formation of lysine−DNA adducts. *J. Inorg. Biochem. 104*, 1000−1005.
(26) Wickramaratne, S., Mukherjee, S., Villalta, P. W., Schärer, O. D., and Tretyakova, N. Y. (2013) Synthesis of sequence-specific DNA−protein conjugates via a reductive amination strategy. *Bioconjugate Chem. 24*, 1496−1506.
(27) Petrova, K. V., Millsap, A. D., Stec, D. F., and Rizzo, C. J. (2014) Characterization of the deoxyguanosine−lysine cross-link of methylglyoxal. *Chem. Res. Toxicol. 27*, 1019−1029.
(28) Silerme, S., Bobyk, L., Taverna-Porro, M., Cuier, C., Saint-Pierre, C., and Ravanat, J.-L. (2014) DNA-polyamine cross-links generated upon one electron oxidation of DNA. *Chem. Res. Toxicol. 27*, 1011−1018.
(29) Uvaydov, Y., Geacintov, N. E., and Shafirovich, V. (2014) Generation of guanine-amino acid cross-links by a free radical combination mechanism. *Phys. Chem. Chem. Phys. 16*, 11729−11736.
(30) Fleming, A. M., Armentrout, E. I., Zhu, J., Muller, J. G., and Burrows, C. J. (2015) Spirodi(iminohydantoin) products from oxidation of 2′-deoxyguanosine in the presence of NH_4Cl in nucleoside and oligodeoxynucleotide contexts. *J. Org. Chem. 80*, 711−721.
(31) Millen, A. L., Sharma, P., and Wetmore, S. D. (2012) C8-linked bulky guanosine DNA adducts: Experimental and computational insights into adduct conformational preferences and resulting mutagenicity. *Future Med. Chem. 4*, 1981−2007.
(32) Jena, N. R., and Mishra, P. C. (2007) Interaction of guanine, its anions, and radicals with lysine in different charge states. *J. Phys. Chem. B 111*, 5418−5424.
(33) Jena, N. R., Mishra, P. C., and Suhai, S. (2009) Protection against radiation-induced DNA damage by amino acids: A DFT study. *J. Phys. Chem. B 113*, 5633−5644.
(34) Dougherty, D., Younathan, E. S., Voll, R., Abdulnur, S., and McGlynn, S. P. (1978) Photoelectron spectroscopy of some biological molecules. *J. Electron Spectrosc. Relat. Phenom. 13*, 379−393.
(35) Cannington, P. H., and Ham, N. S. (1985) He(II) photoelectron-spectra of esters. *J. Electron Spectrosc. Relat. Phenom. 36*, 203−205.
(36) Kaloudis, P., D'Angelantonio, M., Guerra, M., Spadafora, M., Cismaş, C., Gimisis, T., Mulazzani, Q. G., and Chatgilialoglu, C. (2009) Comparison of isoelectronic 8-HO-G and 8-NH2-G derivatives in redox processes. *J. Am. Chem. Soc. 131*, 15895−15902.
(37) Foote, C. S. (1991) Definition of type I and type II photosensitized oxidation. *Photochem. Photobiol. 54*, 659−659.
(38) Cuquerella, M. C., Lhiaubet-Vallet, V., Cadet, J., and Miranda, M. A. (2012) Benzophenone photosensitized DNA damage. *Acc. Chem. Res. 45*, 1558−1570.
(39) Zhao, J., Wu, W., Sun, J., and Guo, S. (2013) Triplet photosensitizers: From molecular design to applications. *Chem. Soc. Rev. 42*, 5323−5351.
(40) Marazzi, M., Wibowo, M., Gattuso, H., Dumont, E., Roca-Sanjuán, D., and Monari, A. (2016) Hydrogen abstraction by photoexcited benzophenone: Consequences for DNA photosensitization. *Phys. Chem. Chem. Phys. 18*, 7829−7836.
(41) Shafirovich, V., Cadet, J., Gasparutto, D., Dourandin, A., and Geacintov, N. E. (2001) Nitrogen dioxide as an oxidizing agent of 8-oxo-7, 8-dihydro-2′-deoxyguanosine but not of 2′-deoxyguanosine. *Chem. Res. Toxicol. 14*, 233−241.
(42) Joffe, A., Mock, S., Yun, B. H., Kolbanovskiy, A., Geacintov, N. E., and Shafirovich, V. (2003) Oxidative generation of guanine radicals by carbonate radicals and their reactions with nitrogen dioxide to form site specific 5-guanidino-4-nitroimidazole lesions in oligodeoxynucleotides. *Chem. Res. Toxicol. 16*, 966−973.
(43) Ravanat, J.-L., Saint-Pierre, C., and Cadet, J. (2003) One-electron oxidation of the guanine moiety of 2′-deoxyguanosine: Influence of 8-oxo-7, 8-dihydro-2′-deoxyguanosine. *J. Am. Chem. Soc. 125*, 2030−2031.
(44) Misiaszek, R., Crean, C., Joffe, A., Geacintov, N. E., and Shafirovich, V. (2004) Oxidative DNA damage associated with combination of guanine and superoxide radicals and repair mechanisms via radical trapping. *J. Biol. Chem. 279*, 32106−32115.

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409

(45) Misiaszek, R., Crean, C., Geacintov, N. E., and Shafirovich, V. (2005) Combination of nitrogen dioxide radicals with 8-oxo-7, 8-dihydroguanine and guanine radicals in DNA: Oxidation and nitration end-products. *J. Am. Chem. Soc. 127*, 2191−2200.
(46) Crean, C., Geacintov, N. E., and Shafirovich, V. (2005) Oxidation of guanine and 8-oxo-7, 8-dihydroguanine by carbonate radical anions: Insight from oxygen-18 labeling experiments. *Angew. Chem., Int. Ed. 44*, 5057−5060.
(47) Crean, C., Geacintov, N. E., and Shafirovich, V. (2008) Pathways of arachidonic acid peroxyl radical reactions and product formation with guanine radicals. *Chem. Res. Toxicol. 21*, 358−373.
(48) Crean, C., Uvaydov, Y., Geacintov, N. E., and Shafirovich, V. (2008) Oxidation of single-stranded oligonucleotides by carbonate radical anions: Generating intrastrand cross-links between guanine and thymine bases separated by cytosines. *Nucleic Acids Res. 36*, 742−755.
(49) Crean, C., Lee, Y. A., Yun, B. H., Geacintov, N. E., and Shafirovich, V. (2008) Oxidation of guanine by carbonate radicals derived from photolysis of carbonatotetramminecobalt(III) complexes and the pH dependence of intrastrand DNA cross-links mediated by guanine radical reactions. *ChemBioChem 9*, 1985−1991.
(50) Rokhlenko, Y., Geacintov, N. E., and Shafirovich, V. (2012) Lifetimes and reaction pathways of guanine radical cations and neutral guanine radicals in an oligonucleotide in aqueous solutions. *J. Am. Chem. Soc. 134*, 4955−4962.
(51) Rokhlenko, Y., Cadet, J., Geacintov, N. E., and Shafirovich, V. (2014) Mechanistic aspects of hydration of guanine radical cations in DNA. *J. Am. Chem. Soc. 136*, 5956−5962.
(52) Kasai, H., Yamaizumi, Z., Berger, M., and Cadet, J. (1992) Photosensitized formation of 7, 8-dihydro-8-oxo-2′-deoxyguanosine (8-hydroxy-2′-deoxyguanosine) in DNA by riboflavin: A nonsinglet oxygen-mediated reaction. *J. Am. Chem. Soc. 114*, 9692−9694.
(53) Candeias, L., and Steenken, S. (1989) Structure and acid-base properties of one-electron-oxidized deoxyguanosine, guanosine, and 1-methylguanosine. *J. Am. Chem. Soc. 111*, 1094−1099.
(54) Candeias, L. P., and Steenken, S. (2000) Reaction of HO with guanine derivatives in aqueous solution: Formation of two different redox-active OH-adduct radicals and their unimolecular transformation reactions. Properties of G(-H). *Chem. - Eur. J. 6*, 475−484.
(55) Steenken, S., Jovanovic, S. V., Bietti, M., and Bernhard, K. (2000) The trap depth (in DNA) of 8-oxo-7, 8-dihydro-2′deoxyguanosine as derived from electron-transfer equilibria in aqueous solution. *J. Am. Chem. Soc. 122*, 2373−2374.
(56) Luo, W., Muller, J. G., Rachlin, E. M., and Burrows, C. J. (2000) Characterization of spiroiminodihydantoin as a product of one-electron oxidation of 8-oxo-7,8-dihydroguanosine. *Org. Lett. 2*, 613−616.
(57) Munk, B. H., Burrows, C. J., and Schlegel, H. B. (2007) Exploration of mechanisms for the transformation of 8-hydroxy guanine radical to FAPyG by density functional theory. *Chem. Res. Toxicol. 20*, 432−444.
(58) Munk, B. H., Burrows, C. J., and Schlegel, H. B. (2008) An exploration of mechanisms for the transformation of 8-oxoguanine to guanidinohydantoin and spiroiminodihydantoin by density functional theory. *J. Am. Chem. Soc. 130*, 5245−5256.
(59) Verdolino, V., Cammi, R., Munk, B. H., and Schlegel, H. B. (2008) Calculation of pKa values of nucleobases and the guanine oxidation products guanidinohydantoin and spiroiminodihydantoin using density functional theory and a polarizable continuum model. *J. Phys. Chem. B 112*, 16860−16873.
(60) Ye, Y., Munk, B. H., Muller, J. G., Cogbill, A., Burrows, C. J., and Schlegel, H. B. (2009) Mechanistic aspects of the formation of guanidinohydantoin from spiroiminodihydantoin under acidic conditions. *Chem. Res. Toxicol. 22*, 526−535.
(61) Psciuk, B. T., Lord, R. L., Munk, B. H., and Schlegel, H. B. (2012) Theoretical determination of one-electron oxidation potentials for nucleic acid bases. *J. Chem. Theory Comput. 8*, 5107−5123.
(62) Psciuk, B. T., and Schlegel, H. B. (2013) Computational prediction of one-electron reduction potentials and acid dissociation constants for guanine oxidation intermediates and products. *J. Phys. Chem. B 117*, 9518−9531.
(63) Thapa, B., and Schlegel, H. B. (2015) Calculations of pKa's and redox potentials of nucleobases with explicit waters and polarizable continuum solvation. *J. Phys. Chem. A 119*, 5134−5144.
(64) Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Mennucci, B., and Petersson, G. A., et al. (2014) *Gaussian Development Version: Revision H.35*, Gaussian, Inc., Wallingford, CT.
(65) Lee, C., Yang, W., and Parr, R. G. (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B: Condens. Matter Mater. Phys. 37*, 785−789.
(66) Becke, A. D. (1993) Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys. 98*, 5648−5652.
(67) Hehre, W. J., Ditchfield, R., and Pople, J. A. (1972) Self—consistent molecular orbital methods. XII. Further extensions of Gaussian—type basis sets for use in molecular orbital studies of organic molecules. *J. Chem. Phys. 56*, 2257−2261.
(68) Hariharan, P. C., and Pople, J. A. (1973) The influence of polarization functions on molecular orbital hydrogenation energies. *Theor. Chim. Acta 28*, 213−222.
(69) Francl, M. M., Pietro, W. J., Hehre, W. J., Binkley, J. S., Gordon, M. S., DeFrees, D. J., and Pople, J. A. (1982) Self-consistent molecular orbital methods. XXIII. A polarization-type basis set for second-row elements. *J. Chem. Phys. 77*, 3654−3665.
(70) Marenich, A. V., Cramer, C. J., and Truhlar, D. G. (2009) Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B 113*, 6378−6396.
(71) Fukui, K. (1981) The path of chemical reactions - the IRC approach. *Acc. Chem. Res. 14*, 363−368.
(72) Hratchian, H. P., and Schlegel, H. B. (2005) Chapter 10 - Finding Minima, Transition States, and Following Reaction Pathways on Ab Initio Potential Energy Surfaces, in *Theory and Applications of Computational Chemistry* (Scuseria, E. G., Dykstra, C. E., Frenking, G., and Kim, K. S., Eds.) pp 195−249, Elsevier, Amsterdam.
(73) Kendall, R. A., Dunning, T. H., and Harrison, R. J. (1992) Electron affinities of the first-row atoms revisited. Systematic basis sets and wave functions. *J. Chem. Phys. 96*, 6796−6806.
(74) Bartmess, J. E. (1994) Thermodynamics of the electron and the proton. *J. Phys. Chem. 98*, 6420−6424.
(75) Bartmess, J. E. (1995) Thermodynamics of the electron and the proton. *J. Phys. Chem. 99*, 6755−6755.
(76) Camaioni, D. M., and Schwerdtfeger, C. A. (2005) Comment on "Accurate experimental values for the free energies of hydration of H^+, OH^-, and H_3O^+". *J. Phys. Chem. A 109*, 10795−10797.
(77) Kelly, C. P., Cramer, C. J., and Truhlar, D. G. (2006) Aqueous solvation free energies of ions and ion−water clusters based on an accurate value for the absolute aqueous solvation free energy of the proton. *J. Phys. Chem. B 110*, 16066−16081.
(78) Isse, A. A., and Gennaro, A. (2010) Absolute potential of the standard hydrogen electrode and the problem of interconversion of potentials in different solvents. *J. Phys. Chem. B 114*, 7894−7899.
(79) Marenich, A. V., Ho, J., Coote, M. L., Cramer, C. J., and Truhlar, D. G. (2014) Computational electrochemistry: Prediction of liquid-phase reduction potentials. *Phys. Chem. Chem. Phys. 16*, 15068−15106.
(80) Lewis, A., Bumpus, J. A., Truhlar, D. G., and Cramer, C. J. (2004) Molecular modeling of environmentally important processes: Reduction potentials. *J. Chem. Educ. 81*, 596−604. (2007) *84*, 934−934.
(81) Koppang, M. D., Witek, M., Blau, J., and Swain, G. M. (1999) Electrochemical oxidation of polyamines at diamond thin-film electrodes. *Anal. Chem. 71*, 1188−1195.
(82) The numbering for the spiroiminodihydantoin structures is abbreviated in such a way as to represent the carbon of guanine at which methylamine attacks. The correct IUPAC nomenclature for **5-NHCH$_3$-Sp** is 7-amino-4-(methylamino)-1,3,6,8-tetraazaspiro[4.4]nona-3,7-diene-2,9-dione, **8-NHCH$_3$-Sp** is 2-amino-7-(methylamino)-1,3,6,8-tetraazaspiro[4.4]nona-2,7-diene-4,9-dione, and **5,8-diNHCH$_3$-Sp** is 2-amino-7-(methylamino)-9-(methylimino)-1,3,6,8-tetraazaspiro[4.4]nona-2,7-dien-4-one.

DOI: 10.1021/acs.chemrestox.6b00057
Chem. Res. Toxicol. 2016, 29, 1396−1409