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Computational Study of the Formation of C8, C5, and C4 Guanine:Lysine Adducts via Oxidation of Guanine by Sulfate Radical Anion

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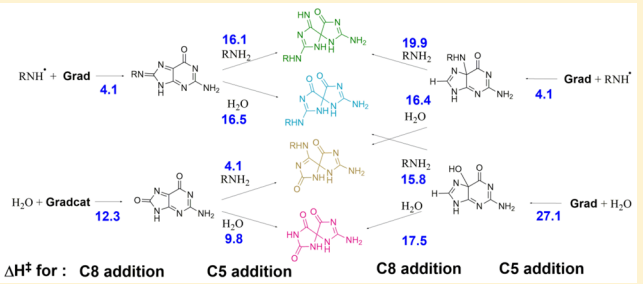
Supporting Information

ABSTRACT: Oxidative damage to DNA can lead to DNA−protein cross-links which can interfere with DNA transcription, replication, and repair. In experimental studies modeling oxidative damage to DNA, oxidation of guanosine by sulfate radical anion in the presence of lysine produced a mixture of lysine (Lys)-substituted spiroiminodihydantoins (Sp): ∼65% 5-Lys-Sp, ∼30% 8-Lys-Sp, and ∼5% 5,8-diLys-Sp. Pathways for formation of the lysine adducts during the oxidation of guanine by sulfate radical anions have been mapped out using B3LYP density functional theory and the SMD solvation model. Methylamine was used as a model for lysine, and imidazole served as a proton acceptor. The lowest barrier for methylamine reaction with guanine radical is addition at C8, yielding mainly 8-NHR-Sp and some 5,8-diNR-Sp. This is in good agreement with the cross-link ratios for mild oxidations mediated by type I photosensitizers such as benzophenone, but this is not in agreement with the product ratios for strong oxidants such as sulfate radical anion. The calculations explored pathways for oxidation of guanine by sulfate radical anion that produced guanine radical and radical cation and doubly oxidized guanine (G^{ox}) and its cation. Sulfate radical anion can also oxidize methylamine to produce neutral methylamine radical ($CH_3NH^{\bullet}$) after deprotonation. The calculations qualitatively reproduced the observed product ratio at pH 7 via a pathway involving the barrierless addition of methylamine radical at C5 and C8 of guanine radical. After C5 addition of methylamine radical, the lowest barrier is for H_2O addition at C8 leading exclusively to 5-NHR-Sp. After C8 addition of methylamine radical, H_2O and methylamine addition to C5 lead to 8-NHR-Sp and some 5,8-diNR-Sp.



INTRODUCTION

Oxidatively generated damage to DNA is thought to be responsible for a variety of biological effects including carcinogenesis, mutagenesis, cell aging, and cell death.[1−4] Oxidatively induced damage can result in a variety of changes to DNA including nucleobase modifications, nucleobase deletions, strand breaks, and the formation of DNA−protein cross-links (DPCs). DPCs may be produced via exposure to a variety of endogenous and exogenous agents such as formaldehyde, ultraviolet light, ionizing radiation, metalloids, and certain chemotherapeutic agents (e.g., cisplatin and mitomycin C).[5−8] DPCs are predicted to interfere with a number of DNA metabolic processes including transcription, replication, and repair.[5−8] In human white blood cells, DPC levels range from 0.5 to 4.5 cross-links per 10^7 bases.[9] Oxidative damage to cells resulting from oxidation induced by ionizing radiation is estimated to result in the formation of 150 DPCs per cell per Gy (1 Gy = 100 rad), significantly higher than the rates of DNA double strand breaks or DNA−DNA cross-links.[5] *In vivo* studies conducted in mice indicated that the level of DPCs found in the liver, brain, and heart increased with age and were correlated with the level of 8-oxo-7,8-dihydroguanine (**8-oxoG**), a biomarker for oxidative stress.[9]

Guanine is the most easily oxidized of the nucleobases, resulting in a number of oxidative products, including **8-oxoG**, guanidinohydantoin (**Gh**), spiroiminodihydantoin (**Sp**), 5-carboxamido-5-formamido-2-iminohydantion (**2Ih**), and 2,5-diaminoimidazolone (**Iz**).[2−4,10−14] Numerous *in vitro* studies on nucleosides and single-stranded and double-stranded DNA have shown that covalent adducts can be formed between guanine oxidation products and proteins such as histones or the amino acid lysine.[4,15−25] Morin and Cadet[15,16] have

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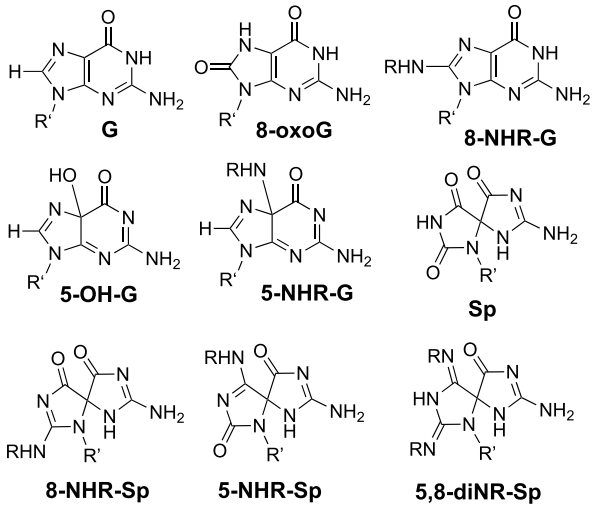
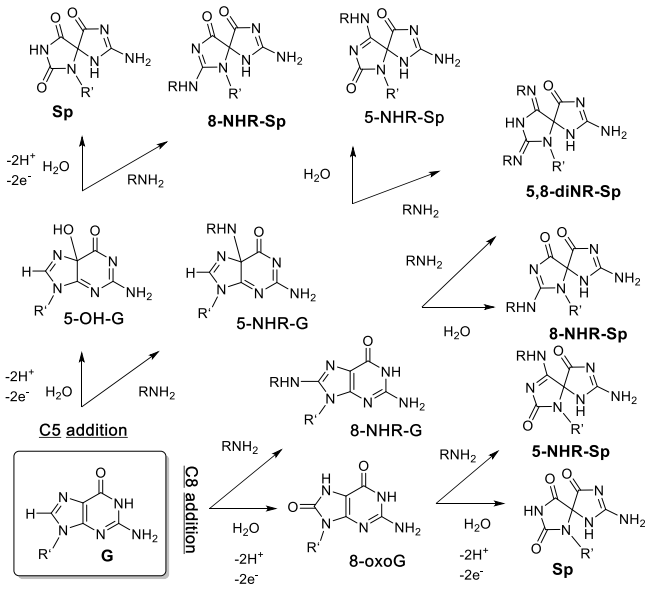
Scheme 1. Guanine, Some of Its Oxidation Products, and Mono- and Di-Substituted Adducts with Lysine or Methylamine[a]



[a]The N−R′ group represents the nitrogen bound to the sugar in guanosine.

Scheme 2. Oxidation of Guanine and Formation of Lysine/Methylamine-Substituted Spiroiminodihydantoin (Sp)



demonstrated the formation of C8 guanine:lysine cross-links in a model system with lysine tethered to the sugar of 2′-deoxyguanosine. With strong oxidizing agents such as HOCl, $ONOO^-$, and other one-electron oxidants, Burrows and co-workers found cross-links between the lysine side chains in single-stranded binding protein and C5 of **8-oxoG**.[17] Using a TGT oligonucleotide and trilysine, Perrier et al. obtained guanine:lysine cross-links at C8 and C5 via riboflavin photosensitizated oxidation.[18] Silerme et al.[19] found 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**. Burrows and co-workers[20] have used oxidizing agents such as type I photosensitizers (benzophenone and riboflavin), type II photosensitizers (Rose Bengal and methylene blue), and simple one-electron oxidants (sulfate radical anion and $Ir(Cl)_6^{2-}$) to study the formation of C5 and C8 cross-links between lysine and 2′-deoxyguanosine, and they observed that the distribution of the final cross-linked products depended on the nature of the oxidizing agents. Some of the structures associated with the formation of guanine:lysine adducts are shown in Scheme 1. In previous computational studies, we investigated the formation of guanine:lysine cross-links during oxidation mediated by type I and type II photosensitizers[26,27] and the formation of guanine−thymine cross-links resulting from oxidation by carbonate radical anion.[28] In the present study, we examine the oxidation of guanine by sulfate radical anion and the formation of guanine:lysine cross-links.

Burrows and co-workers[20] found that the major cross-link product produced with type I photosensitizer such as riboflavin and benzophenone was spiroiminodihydantoin with lysine added at the guanine C8 position, **8-Lys-Sp**. When type II photosensitizers such as Rose Bengal or methylene blue were used in the presence of lysine, the product was exclusively **5-Lys-Sp**. Singlet oxygen generated from type II photosensitizers reacts via an endoperoxide intermediate[20] to form an **8-oxoG^{ox}** intermediate. Nucleophilic addition of lysine at the C5 position produces **5-Lys-G^{ox}**, and migration of the carbonyl group forms **5-Lys-Sp**. Our computational studies of guanine:lysine cross-link formation via type I and type II photosensitizers provide a detailed description of mechanisms for cross-link formation and find product ratios in good agreement with experiment.[26,27] Strong oxidants such as sulfate radical anion (generated by continuous irradiation of $K_2S_2O_8$ for 6 h)[20] produce a mixture containing products with about 65% of **5-Lys-Sp** and smaller amounts (∼30%) of **8-Lys-Sp** and a little with lysine added at both the C5 and C8 positions, **5,8-diLys-Sp**. The present computational study examines the mechanism for guanine:lysine cross-link formation when sulfate radical anion is the oxidant.

The oxidation of guanine and subsequent reactions of guanine radical have been investigated computationally by a number of groups.[26−73] Sevilla and co-workers have studied guanine oxidation and reduction, protonation and deprotonation of guanine radical, and hydroxyl radical addition to guanine.[29−34] We have examined the reactions of guanine radical to form **8-oxoG**, **Sp**, **Gh**, and **FAPyG**[35−37] and the pK_a's and redox potentials of the intermediates in these pathways.[38−41] We have also studied the formation of guanine−thymine cross-links during oxidation by carbonate radical anion[28] and the formation of guanine−lysine cross-links resulting from oxidation mediated by type I and type II photosensitizers.[26,27] Dumont and co-workers have investigated DNA−polyamine binding modes and cross-link formation, peroxy radical addition to guanine, and singlet oxygen reactions with guanine.[42−47] Liu and co-workers have used electronic structure calculations and guided ion beam mass spectrometry to examine water addition and singlet oxygen addition to guanine and its subsequent reactions.[48−52] The formation of 8-aminoguanine and its oxidized and reduced forms have been studied both computationally[53] and experimentally.[74] Wetmore, Boyd, and co-workers have calculated OH and NO_2 radical addition to nucleobases, as well as ionization potentials and electron affinities of individual nucleobases.[54−56] Rothlisberger and co-workers have computed ionization energies and electron affinities of guanine and **8-oxoG** in two and three base pair fragments of DNA.[57] Mishra and co-workers have published numerous studies of

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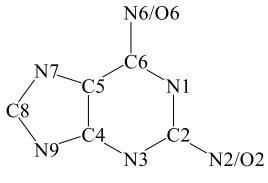
guanine oxidation by reactive oxygen species.[58−73] Nevertheless, computational studies of the formation of guanine−lysine cross-links are rather limited.[26,27,45,69]

In previous studies, we examined the formation of guanine:lysine adducts during the sequential one-electron oxidation of guanine by triplet benzophenone, a type I photosensitizer, and during oxidation by singlet oxygen, generated by type II photosensitizers.[26,27] Experimental studies find quite different ratios of guanine:lysine adducts when stronger oxidants such as sulfate radical anion are used.[20] In the present work, we use density functional theory (DFT) calculations to explore the formation of lysine-substituted spiroiminodihydantoins resulting from guanine oxidation by sulfate radical anion (Scheme 2). As in the previous studies, lysine is modeled by methylamine and additions to purine position C8, C5, and C4 are considered. Key transition states along pathways have been located, and the pK_a's and both standard state and pH 7 reduction potentials ($E°$ and E_7, respectively) of various intermediates have been calculated. The relative energies of various radical, radical cation, and neutral intermediates along each pathway are discussed and compared to the experimental results of Burrows and coworkers.[20]

COMPUTATIONAL METHODS

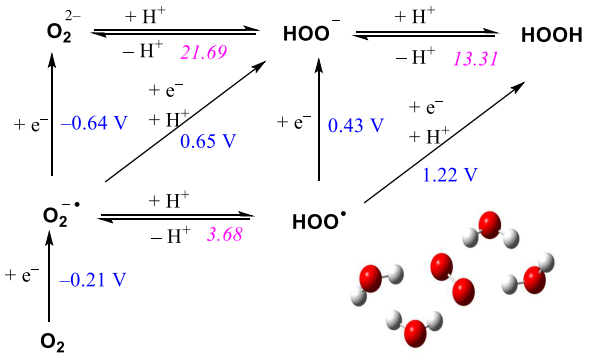
Molecular orbital calculations were carried out with the development version of the Gaussian[75] series of programs.

Scheme 3. Atom Numbering for Purine Nucleobases



Purine

Scheme 4. pK_a Values and Standard Reduction Potential ($E°$) for Oxygen Species Calculated at B3LYP/aug-cc-pVTZ with SMD Implicit Solvent and Inclusion of Four Explicit Water Molecules (the Inset Shows the Structure of Superoxide Solvated by Four Explicit Waters)[a]



[a]Numbers next to the arrow correspond to pK_a's (pink, italics) and standard redox potentials ($E°$) (blue, regular).

As in our previous studies,[26,27] optimized geometries and energies were computed with the B3LYP[76,77] density functional method using the 6-31+G(d,p)[78−80] and aug-cc-pVTZ[81] basis sets. Our earlier work with 8-hydroxy-7,8-dihydroguanyl radical (**8-OH Grad**) indicated that the potential energy profiles of adducts substituted at N9 with methyl, hydroxymethyl, and methoxyethyl were similar to those observed with hydrogen as the substituent.[36,38] Therefore, the calculations were carried out with hydrogen as the substituent at N9. There was a noticeable change in the geometries for some molecules optimized in the gas phase compared to those optimized in the solution; therefore, all geometries were optimized in aqueous solution using the B3LYP/6-31+G(d,p) level of theory with the SMD[82] solvation method. In order to achieve higher accuracy, single point energies were calculated at the B3LYP/aug-cc-pVTZ[81] level of theory using the B3LYP/6-31+G(d,p) optimized geometry in solution. Vibrational frequencies were computed in order to test that the optimized geometries are minima or transition states on the potential energy surface. Transition states were checked to verify that they had only one imaginary frequency and a suitable transition vector. Intrinsic reaction coordinate (IRC)[83,84] calculations were carried out for selected transition states to confirm that they connected the appropriate reactants and products. Test calculations showed that the barrier heights for H_2O and CH_3NH_2 addition to guanine radical were within 2 kcal/mol of those calculated with more recently developed functionals such as ωB97X-D.[85] Thermal corrections and enthalpies were calculated by standard statistical thermodynamic methods using the unscaled B3LYP frequencies and the ideal gas/rigid rotor/harmonic oscillator approximations. An acceptable accuracy of energetics in aqueous solution can be achieved without the use of thermodynamic cycles.[41,86] The energy in solution is calculated as the sum of the electronic energy calculated at SMD/B3LYP/aug-cc-pVTZ//SMD/B3LYP/6-31+G(d,p) and the zero point energy (ZPE) and thermal corrections for enthalpy at B3LYP/6-31+G(d,p). The solvent cavity was not scaled for any of the cation or neutral species and was scaled by a factor of 0.90 for anionic intermediates along the pathway. The atom numbering for purine nucleobases is shown in Scheme 3.

For computational efficiency, the side chain of lysine was modeled using methylamine (i.e., R = CH_3 in all of the schemes and figures). For the reactions involving deprotonation and protonation, imidazole and imidazolium were used as the proton acceptor and donor, respectively. Since imidazole has a pK_a of 6.9, transferring a proton to/from imidazole/imidazolium is the computational equivalent of using a pH 7 buffer solution. Transition states involving the addition of methylamine to an imine bond and proton transfer from the methylamine to the guanine subunit were modeled with one explicit molecule of water assisting the proton transfer. The transition states thus formed were six-membered rather than four-membered ring systems and therefore represent a lower energy pathway. Because of the known acidity of the HSO_4 anion (pK_a = 1.99),[87] one-electron oxidation of guanine with sulfate radical anion was modeled assuming that the reduction of sulfate radical anion produced sulfate dianion ($E°$ 2.13 V calcd with eight explicit waters and SMD vs 2.43 V exptl[88]). Wherever appropriate, the possibility of oxidation by O_2, $O_2^{-\bullet}$ or HOO$^\bullet$ has also been examined. The pK_a's and redox potential of various reactions involving the O_2 are given in Scheme 4.

Various methods for calculating pK_a have recently been reviewed by Ho and Coote.[89,90] The pK_a is obtained from the deprotonation reaction calculated in aqueous solution

$$XH_{(aq)} = X^-_{(aq)} + H^+_{(aq)} \quad (1)$$

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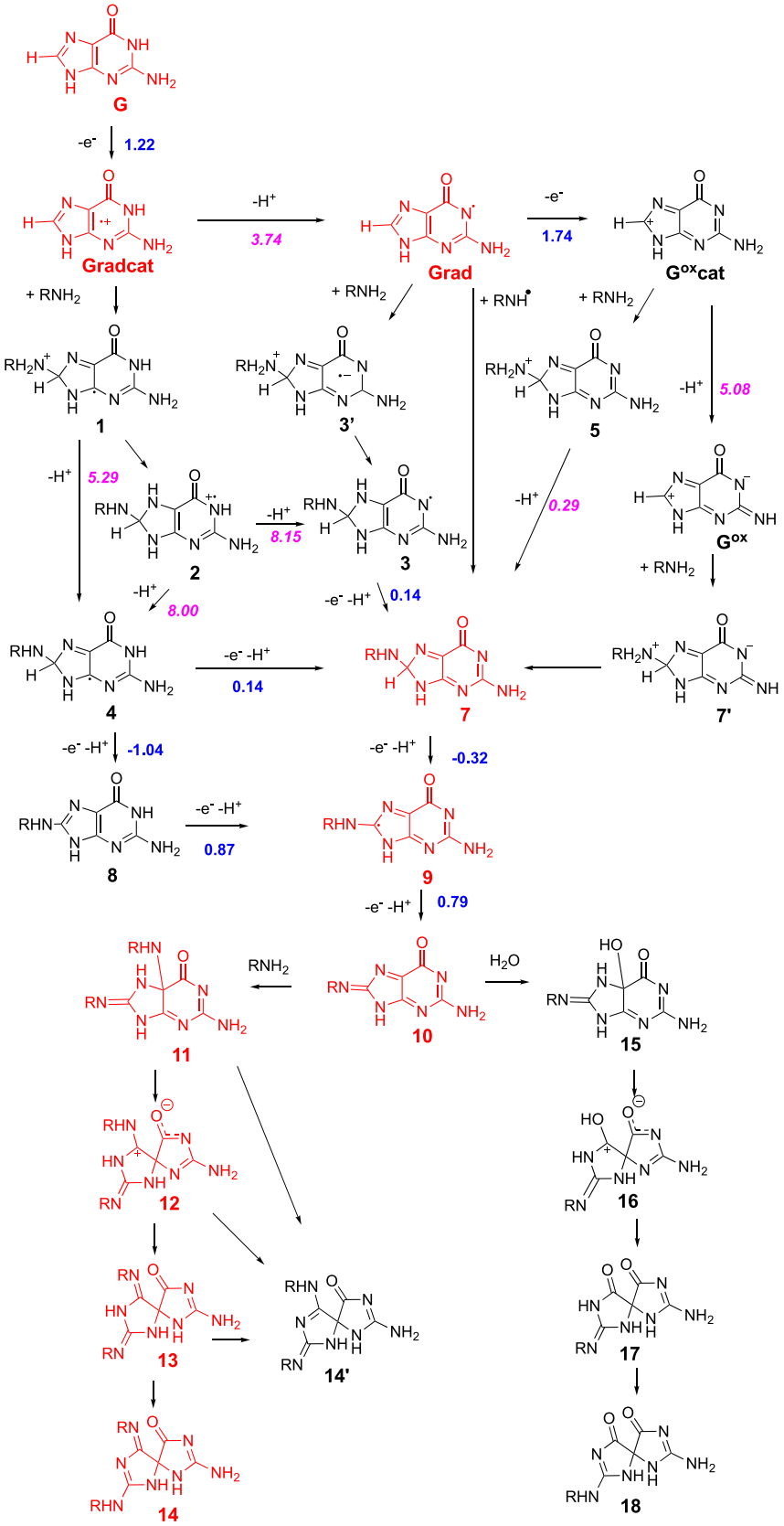
Scheme 5. Possible Pathways for the Formation of Guanine:Methylamine Adducts via Sequential One-Electron Oxidations of Guanine and Nucleophilic Attack of Methylamine (R = CH_3) at C8[a]



[a]Numbers next to the arrow correspond to pK_a's (pink, italics) and standard redox potentials ($E°$) (blue, regular). The structures shown in red represent the thermodynamically favored pathway.

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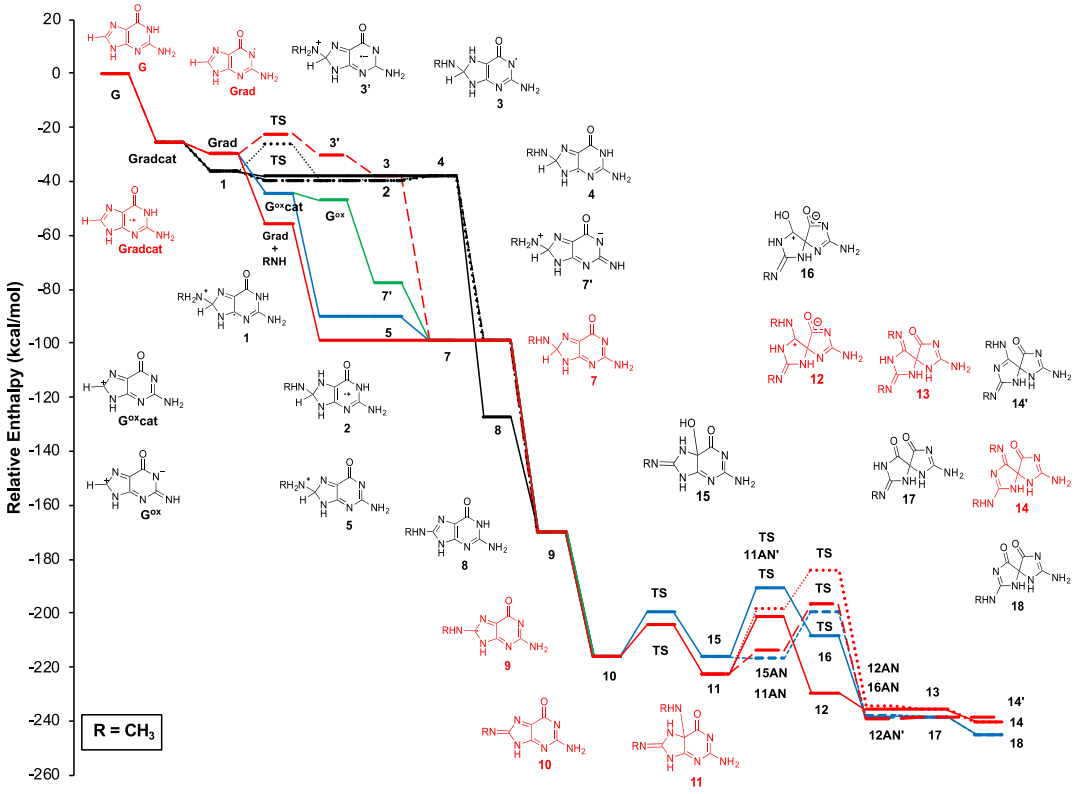

Figure 1. Comparison of the relative enthalpies (kcal/mol) of adducts resulting from the addition of methylamine at the C8 position of guanine radical cation (**Gradcat**, black), guanine radical (**Grad**, red), oxidized guanine cation (**G^{ox}cat**, blue), and neutral oxidized guanine (**G^{ox}**, green) 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 blue 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.

The pK_a is given by

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

where $\Delta G_{deprot,(aq)}$, R, and T are the aqueous solution Gibbs free energy of deprotonation, the gas constant, and the temperature, respectively. The solution phase deprotonation free energy is given by

$$\Delta G_{deprot,(aq)} = G_{X^-,(aq)} + G_{H^+,(aq)} - G_{XH,(aq)} \quad (3)$$

$G_{H^+,(aq)}$ is the solution phase Gibbs free energy of the proton, which can be expressed as

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

where $G^{\circ}_{H^+,(g)}$ is the gas phase standard free energy of a proton, $\Delta G^{1atm\rightarrow 1M}$ is the change in free energy for the change in standard states from 1 atm to 1 M, and $\Delta G^{*}_{H^+,(aq)}$ is the solvation energy of a proton in water. The superscripts $^{\circ}$ and * represent the standard state in gas and aqueous solution, respectively. For the calculations, the aqueous solvation free energy of a proton, $\Delta G^{*}_{H^+,(aq)} = -265.9$ kcal/mol, is taken from the literature.[91−94] The gas phase standard free energy of a proton, $G^{\circ}_{(g)} = -6.287$ kcal/mol at 298 K, is derived from the equation $G^{\circ}_{H^+,(g)} = H^{\circ}_{H^+,(g)} - TS^{\circ}_{(g)}$ with $H^{\circ}_{H^+,(g)} = {}^5/_2RT = 1.48$ kcal/mol and $S^{\circ}_{(g)} = 26.05$ cal/(mol K).[95,96]

The standard reduction potential for a reaction in aqueous solution

$$X^{n+}_{(aq)} + ne^{-}_{(aq)} \xrightarrow{\Delta G^{*}_{rel,(aq)}} X_{(aq)} \quad (5)$$

is given by

$$E^{\circ}_{red,(aq)} = -\frac{\Delta G^{*}_{red,(aq)}}{nF} - SHE \quad (6)$$

where $\Delta G^{*}_{red,(aq)}$, n, F, and SHE are the standard free energy change for the reduction reaction in solution, the number of electrons involved in the reaction (n = 1 for all of the cases in the present study), the Faraday constant (23.06 kcal/(mol V)), and the absolute potential of the standard hydrogen electrode (SHE = 4.281 V, obtained from the free energy of aqueous H^+).[91−94] The standard free energy change for the above reaction in solution is

$$\Delta G^{*}_{red,(aq)} = G^{*}_{X,(aq)} - G^{*}_{X^{n+},(aq)} - nG^{*}_{e^-,(g)} \quad (7)$$

where $G^{*}_{e^-,(g)} = -0.867$ kcal/mol is the free energy of an electron at 298 K, obtained on the basis of Fermi−Dirac statistics using $H^{\circ}_{H^+,(g)} = 0.752$ kcal/mol and $S^{\circ}_{(g)} = 5.434$ cal/(mol K).[95,96]

RESULTS AND DISCUSSION

The discussion of the formation of monolysine- and dilysine-substituted spiroiminodihydantoin (**Sp**) adducts can be divided into three groups on the basis of the site of initial nucleophilic addition to guanine:lysine or water adding to C8, C5, or C4 of guanine (Scheme 2; for numbering, see Scheme 3). Since the C4 lysine:guanine adducts were not reported in

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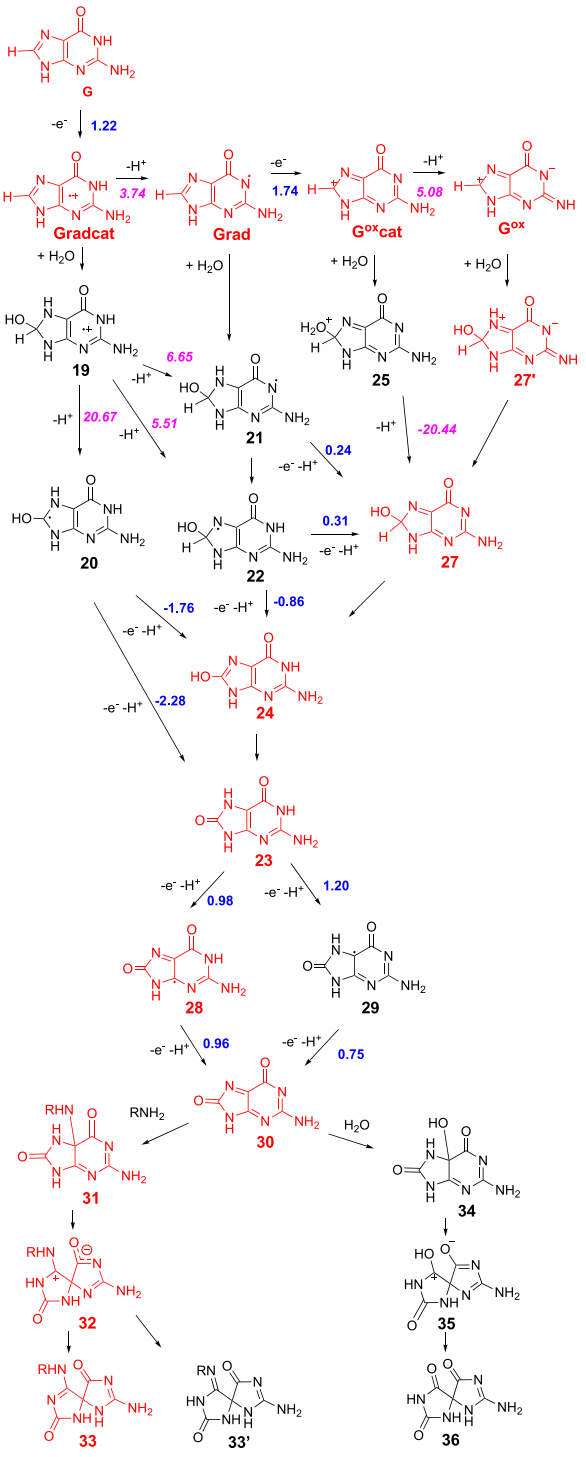
Scheme 6. Possible Pathways for the Formation of Guanine:Water Adducts via Sequential One-Electron Oxidations of Guanine and Nucleophilic Attack of Water at C8[a]



[a]The numbers next to the arrow correspond to pK_a's (pink, italics) and standard redox potentials ($E°$) (blue, regular). The structures shown in red represent the thermodynamically favored pathway.

any of the experimental studies, the barriers for key reactions along the C4 addition pathway are expected to be higher than those for C8 and C5 addition. Our calculations agree with these experimental results. Therefore, to shorten the discussion, only the C8 and C5 addition pathways are included in the text. The nucleophilic addition of lysine and water to the guanine at the C4 position is discussed in the Supporting Information.

To reduce the cost and complexity of the calculations, methylamine was used as a model for the side chain of lysine. The most probable low energy pathways for the formation of these adducts in aqueous solution are shown in Schemes 5−8 and Schemes S1 and S2. The sequential one-electron oxidation of guanine starts with the abstraction of an electron from guanine by sulfate radical anion ($E°$ = 2.43 V exptl) and forms guanine radical cation, **Gradcat** ($E°$ = 1.22 V). **Gradcat** (pK_a = 3.9 exptl, 3.74 calcd) deprotonates at physiological pH to give guanine radical, **Grad** (E_7 = 1.29 V exptl). Experimental studies by Shafirovich and co-workers[97−99] show that sulfate radical anion oxidizes guanine very rapidly (k = (2−3) × 10^9 M^{-1} s^{-1}). If carbonate radical anion is present in the reaction mixture, the sulfate radical anion also oxidizes excess bicarbonate to form carbonate radical anion, but this occurs more slowly (k = 1.5 × 10^7 M^{-1} s^{-1}). In the absence of bicarbonate, sulfate radical anion could oxidize **Grad** further to form oxidized guanine cation, **G^{ox}cat** ($E°$ = 1.74 V, E_7 = 1.63). Near physiological pH, **G^{ox}cat** (pK_a = 5.08 calcd) loses a proton from N2 to form neutral oxidized guanine, **G^{ox}**. Sulfate radical anion can also oxidize neutral methylamine to give neutral methylamine radical ($CH_3NH^•$) after deprotonation. The oxidation of water by sulfate radical anion is comparatively slow (k = 2 × 10^{-3} M^{-1} s^{-1})[100,101] and is not considered further. Thus, the first step in cross-link formation can occur by methylamine adding to C8, C5, or C4 of **Grad** or **G^{ox}** or by methylamine radical adding to C8, C5, or C4 of guanine or **Grad**. Water addition to **Grad** or **G^{ox}** must also be considered in the first step of the mechanism. Further oxidation of the initial adduct is followed by a second nucleophilic addition and rearrangement reactions to form the substituted spiroiminodihydantoin.

C8 Addition of Methylamine. Scheme 5 outlines the low energy pathways of formation of C8 guanine:methylamine cross-links, and the related thermodynamics are shown in Figure 1. One-electron oxidation of neutral guanine (**G**) produces guanine radical cation (**Gradcat**). Since methylamine (pK_a = 10.6) is protonated at physiological pH, it must be deprotonated first (ΔH = 4.1 kcal/mol at pH 7). Addition of neutral methylamine to C8 of **Gradcat** is barrier-free, forming **1**, 8-NH_2CH_3-guanine radical cation. **1** can lose a proton from the nitrogen of NH_2CH_3 (ΔH = −1.77 kcal/mol, pK_a 5.29) to form 8-$NHCH_3$-guanine radical **4** or can undergo water-assisted tautomerization with the transfer of a proton from the NH_2CH_3 group to N7 of the guanine radical cation to form **2**, 8-$NHCH_3$-guanine radical cation. This tautomerization of **1** has a barrier of 10 kcal/mol to form a slightly more stable product (ΔH = −3.20 kcal/mol). Deprotonation of **2** can occur via loss of a proton from the N1 (pK_a 8.15) or N7 (pK_a 8.00) to form two neutral radical 8-$NHCH_3$-guanine radical tautomers, **3** or **4**, respectively. Neutral methylamine can add to the neutral guanine radical (**Grad**) in two ways: via a direct addition of NH_2CH_3 to C8 of **Grad**, forming a zwitterionic radical species (barrier height 5.4 kcal/mol), **3′**, or via a water-assisted addition of neutral methylamine across the C8−N7 double bond of the **Grad** (barrier height 7.3 kcal/mol), forming neutral 8-$NHCH_3$-guanine radical intermediate, **3** (the corresponding barrier for NH_3 addition is 8.7 kcal/mol). A proton-coupled one-electron oxidation of **3** (loss of H from N7) or **4** (loss of H from N1) results in a common neutral

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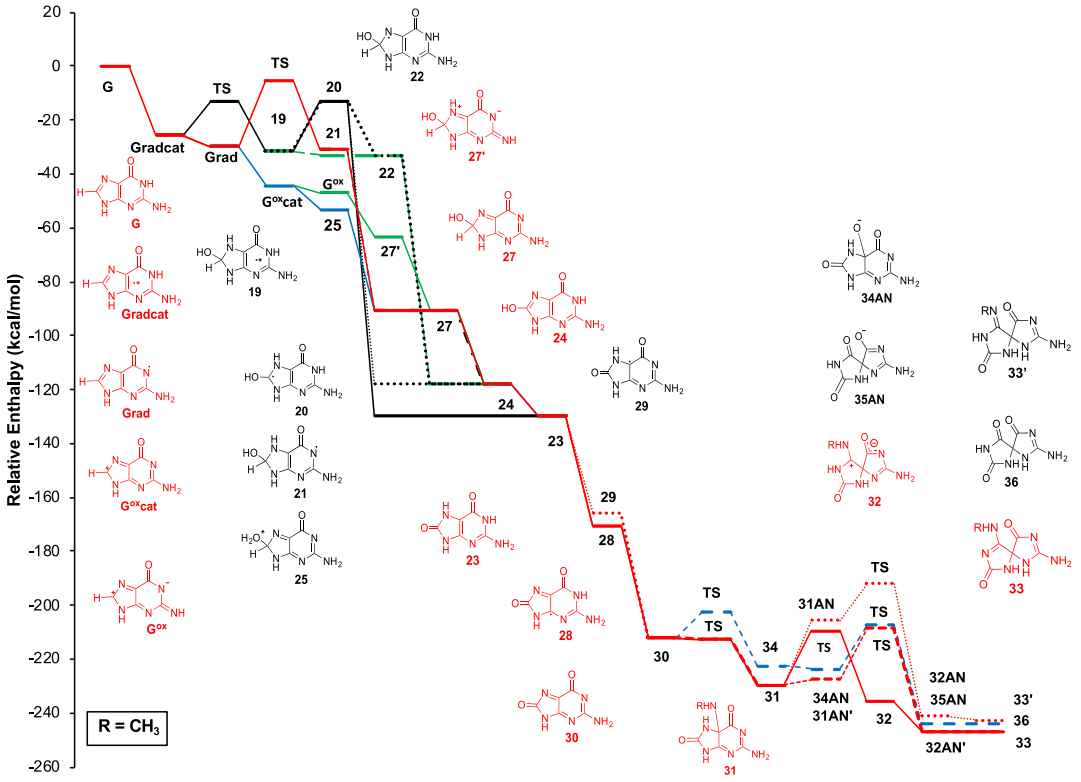

Figure 2. Comparison of the relative enthalpies (kcal/mol) of adducts resulting from the addition of water at the C8 position of guanine radical cation (**Gradcat**, black), guanine radical (**Grad**, red), oxidized guanine cation (**G^{ox}cat**, blue), and neutral oxidized guanine (**G^{ox}**, green) 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 blue line corresponds to the addition of water (pathways followed by the anion are shown in dashed or dotted lines).

intermediate, **7** ($E°$ = 0.14 V, PCET). Both of these oxidation steps are exothermic with sulfate radical anion but endothermic with $^3O_2$. Methylamine addition to the two-electron oxidized guanine intermediate **G^{ox}cat** is calculated to proceed without a barrier, producing **5** which undergoes spontaneous deprotonation to form the neutral intermediate, **7**. Structure **7** can also be formed by neutral methylamine addition to neutral oxidized guanine **G^{ox}** followed by tautomerization. Addition of methylamine radical to **Grad** also yields **7**. These three processes are all calculated to have no barrier. Proton-coupled electron transfer from **7** to **9** is calculated to be very favorable ($E°$ = −0.32 V, PCET), as it restores the planarity and aromaticity of the imidazole ring. Alternatively, **9** can also be formed when **4** undergoes two sequential one-electron oxidations and deprotonations. The first one-electron oxidation of neutral radical **4** forming neutral intermediate **8** can occur either by sulfate radical anion or by $^3O_2$ present in an aerobic environment (exothermic by 89.2 and 38.2 kcal/mol, respectively). However, the oxidation of **8** to form **9** is endothermic for $^3O_2$ and $O_2^{-\bullet}$ (by 8.6 and 5.4 kcal/mol, respectively) but is exothermic for $SO_4^{\bullet-}$ and $HOO^{\bullet}$ radical anion (42.4 and 8.6 kcal/mol, respectively). Further proton-coupled electron transfer from neutral radical intermediate **9** produces neutral intermediate **10** ($E°$ = 0.79 V, PCET).

In a second nucleophilic addition step, methylamine or water can add across the C5−N7 double bond of **10**. The barrier for water-assisted neutral methylamine addition is calculated to be 12.0 kcal/mol (16.1 kcal/mol if the energy for deprotonation is included) and forms the 5,8-methylamine disubstituted intermediate, **11**. The water addition (assisted with another water) across the C5−N7 double bond of **10** has a barrier of 27.6 kcal/mol and forms 5-OH,8-NCH$_3$-oxidized guanine, **15**. This barrier drops to 16.5 kcal/mol when assisted by two water molecules, which is comparable to the methylamine addition barrier. Intramolecular acyl group migration in **11** and **15** followed by tautomerization forms the final spiroiminodihydantoin products, **5,8-diNR-Sp** (**14**) and **8-NHR-Sp** (**18**), respectively. The barrier for the acyl group migration in **11** is lower for the neutral than for the deprotonated species, as shown in Scheme 4. As found in our earlier study,[36] acyl group migration of **15** (pK_a = 6.6) occurs only in deprotonated species.

C8 Addition of Water. Scheme 6 outlines the possible pathways for water addition at the C8 position of oxidized guanine, and the thermodynamics are shown in Figure 2. As in the case of NH_2CH_3 addition to C8, water can add to the four oxidized forms of guanine. Addition of water at the C8 position of **Gradcat** has a barrier of 12.3 kcal/mol and results in the exothermic (5 kcal/mol) formation of 8-OH-guanine radical cation intermediate, **19**. Loss of a proton from N1 (pK_a = 6.65) or N7 (pK_a = 5.51) of **19** yields **21** or **22**, respectively. Removal of the proton from the C8 position of **19** is endothermic by 18.5 kcal/mol and yields the neutral radical, **20**. A nucleophilic addition of a water molecule across the C8−N7 double bond of **Grad** also produces **21**. However, this addition reaction (assisted by one water) is calculated to have an enthalpy barrier of 24.6 kcal/mol. Loss of a proton during a proton-coupled oxidation of **20** is calculated to prefer C8-OH ($E°$ = −2.28 V for PCET) over N7−H ($E°$ = −1.76 V for

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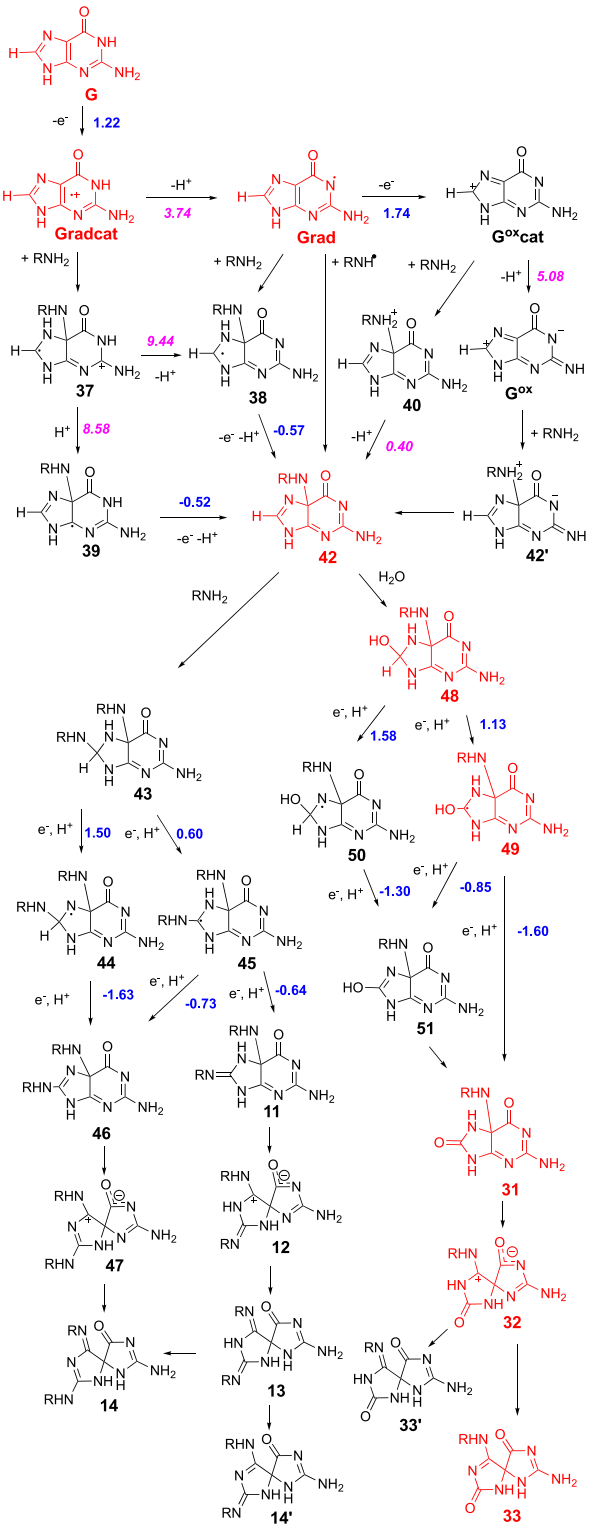
Scheme 7. Possible Pathways for the Formation of Guanine:Methylamine Adducts via Sequential One-Electron Oxidations of Guanine and Nucleophilic Attack of Methylamine (R = CH_3) at C5[a]



[a]The numbers next to the arrow correspond to pK_a's (pink, italics) and standard redox potentials ($E°$) (blue, regular). The structures shown in red represent the thermodynamically favored pathway.

PCET), forming **23** and **24**, respectively. Either SO_4 radical anion or O_2 can readily oxidize **20** to form **23** or **24** (ΔH = −96.8 and −45.8 kcal/mol, respectively). Tautomerization of 8-hydroxyguanine, **24**, results in 8-oxo-guanine, **8-oxoG** (**23**), and is exothermic by 13.7 kcal/mol. Water addition to oxidized guanine cation (**G^{ox}cat**) is calculated to be barrier-free and forms **25**, which undergoes direct deprotonation from C8−H_2O (pK_a = −20.44) to produce a neutral 8-OH-oxidized guanine, **27**. **27** can also be formed via the barrier-free C8−N7 addition of water to neutral oxidized guanine, **G^{ox}**, followed by a tautomerization reaction. Alternatively, **27** can be formed from **21** directly by PCET ($E°$ = 0.24 V). Further water-assisted tautomerization of **27** leads to the formation of the thermodynamically favored neutral products 8-hydroxyguanine (**24**) and **8-oxoG** (**23**). Oxidation of **8-oxoG**, followed by the loss of a proton from N7 ($E°$ = 0.98 V for PCET) or N1 ($E°$ = 1.20 V for PCET), forms two radicals, **28** or **29**, respectively. A further one-electron oxidation of **28** ($E°$ = 0.96 V for PCET) or **29** ($E°$ = 0.75 V for PCET) followed by loss of another proton produces oxidized 8-oxoguanine, **8-oxoG^{ox}**, **30**. Oxidations of **8-oxoG**, **28**, and **29** are exothermic with $SO_4^{\bullet-}$ and HOO$^\bullet$ but endothermic with O_2 and $O_2^{\bullet-}$.

In a second nucleophilic addition, neutral methylamine adds across the C5−N7 double bond of **30** without a barrier when the process is assisted with a water molecule. A similar process for water addition to **30** yielding **34** has a barrier of 16.2 kcal/mol assisted by one water or 9.8 kcal/mol assisted by two waters. The methylamine adduct, **31**, can undergo acyl group migration from C5 to C4 in neutral or deprotonated forms to produce the final 5-methylamine spiroiminodihydantoin products **33** and **33′**. Near physiological pH, acyl group migration in the neutral intermediate, **31**, is favored over migration in deprotonated **31**. By contrast, acyl group migration in the doubly water-substituted intermediate **34** is calculated to occur via an anionic species formed by loss of proton from 5-OH (pK_a = 6.25) or N7H (pK_a = 6.81). Attempts at acyl migration in the neutral species, **34**, lead to ring opening and formation of a very reactive isocyanate species. As found in our previous work,[36] deprotonation of **34** followed by acyl group migration and reprotonation produces the final spirocyclic product, **36**.

C5 Addition of Methylamine. The C5 site of guanine is another potential starting position for the formation of a guanine:methylamine (or lysine) cross-link. For oxidation with sulfate radical anion, Burrows and co-workers experimentally found that C5 is the most favored site for the cross-link formation.[20] Possible pathways for methylamine addition to C5 are outlined in Scheme 7, and the thermodynamics of the low energy pathways are shown in Figure 3. As in the case of C8 addition, nucleophilic addition of neutral methylamine can occur with any of the four oxidized guanine species: guanine radical cation, **Gradcat**, guanine radical, **Grad**, oxidized guanine cation, **G^{ox}cat**, or neutral oxidized guanine, **G^{ox}**. Water-assisted addition of neutral methylamine to the C5 of guanine radical cation is calculated to have an enthalpy barrier of 24.4 kcal/mol to form 5-$NHCH_3$-guanine radical cation, **37**, which can undergo deprotonation from N1 (pK_a = 9.44) or N7 (pK_a = 8.58) to form neutral radical tautomers, **38** and **39**, respectively. Further oxidation of **38** ($E°$ = −0.57 V) and **39** ($E°$ = −0.52 V) and deprotonation forms a thermodynamically favored common neutral intermediate **42**. Oxidants $SO_4^{\bullet-}$, O_2, $O_2^{\bullet-}$, or HOO$^\bullet$ can easily oxidize both **37** and **38** to produce **42**. The neutral radical species **38** can also be formed by methylamine addition to **Grad** (water-assisted barrier of 27.4 kcal/mol). Addition of methylamine to the C5 of the oxidized

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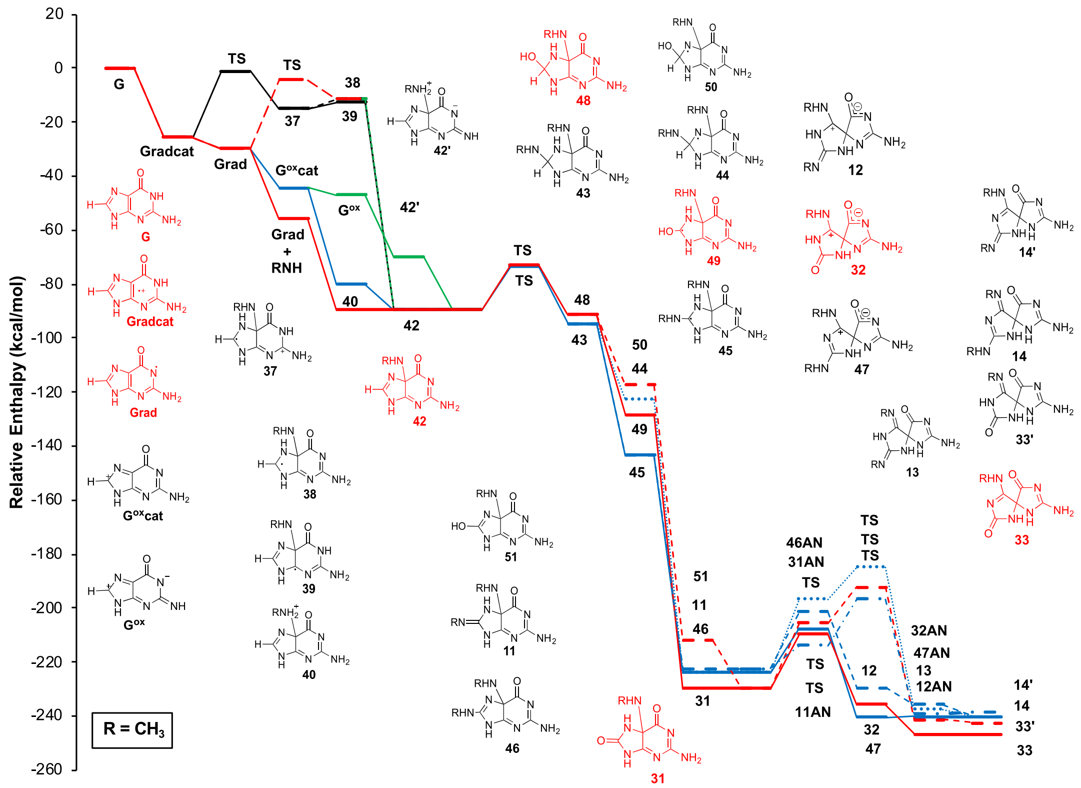

Figure 3. Comparison of the relative enthalpies (kcal/mol) of adducts resulting from the addition of methylamine at the C5 position of guanine radical cation (**Gradcat**, black), guanine radical (**Grad**, red), oxidized guanine cation (**G^{ox}cat**, blue), and neutral oxidized guanine (**G^{ox}**, green) 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 blue 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.

guanine cation (**G^{ox}cat**) is calculated to be barrierless and is exothermic by 38.5 kcal/mol. Deprotonation of the resulting adduct, **40** (pK_a = 0.40), yields **42**. The barrier-free addition of methylamine to neutral oxidized guanine **G^{ox}** followed by tautomerization produces **42** as well. Addition of neutral methylamine radical to **Grad** also yields **42** without a barrier.

The addition of water across the C8−N7 double bond of **42** is calculated to have a larger reaction barrier (22.8 kcal/mol) than neutral methylamine addition (15.8 kcal/mol). However, the water addition barrier is lowered to 16.4 kcal/mol when the transition state is assisted by two explicit water molecules. Considering the high concentration of water and the deprotonation penalty of 4.1 kcal/mol that has to be paid to obtain neutral methylamine from its stable protonated form under physiological pH, water addition is expected to be more favorable compared to the methylamine addition. The methylamine addition intermediate, **43**, can undergo two sequential one-electron oxidations and deprotonations to form **46** or **11**. While the oxidation of **43** to form **44** is only favorable with $SO_4^{\bullet-}$, either $SO_4^{\bullet-}$ or OOH$^{\bullet}$ can oxidize **43** to form **45**. Oxidation of **44** or **45** is exothermic for $SO_4^{\bullet-}$, O_2, $O_2^{\bullet-}$, and HOO$^{\bullet}$. Acyl group migration in **46** and **11** produces the final, stable **5,8-diNR-Sp** tautomers, **14** and **14′**, respectively. The addition of water across the C8−N7 double bond yields **48**. Two sequential one-electron oxidations and deprotonations of **48** produce a four-electron-oxidized, 5-methylamine-substituted, 8-oxoguanine species, **31**, which undergoes acyl group migration to form the final stable spirocyclic product, **5-NHR-Sp** (**33**). The acyl group migration is favored for neutral states of **46**, **11**, and **31** compared to their anionic states.

C5 Addition of Water. The mechanism for water addition to C5 in Scheme 8 and Figure 4 follows the same pattern as the addition of methylamine. Addition of water across the C8−N7 double bond of the guanine radical cation (**Gradcat**) has a barrier of 33.2 kcal/mol and forms **55**, which can deprotonate at N1 (pK_a = 8.37) or N7 (pK_a = 6.42) to form **56** or **57**, respectively. These neutral radicals can be oxidized by $SO_4^{\bullet-}$ or O_2 (PCET $E°$ = −0.46 V and −0.34 V, respectively) to form intermediate **60**. The neutral radical **56** can also be formed by water addition across the C8−N7 double bond of **Grad** with a barrier of 27.1 kcal/mol. Addition of water to oxidized guanine cation (**G^{ox}cat**) produces **58** (pK_a = −21.31) which deprotonates to form **60**. The barrier-free addition of water to neutral oxidized guanine, **G^{ox}**, followed by tautomerization also forms **60**.

The second nucleophilic addition of neutral methylamine (assisted by one water molecule) across the C8−N7 double bond of **60** has a barrier of 11.1 kcal/mol and forms **61** (exothermic by 10.1 kcal/mol). A similar addition of water has a barrier of 24.7 or 17.5 kcal/mol when assisted by two waters to form **67**. As shown in Scheme 7, oxidation and loss of a proton from C8 of **61** are more favorable ($E°$ = 0.60 for PCET) than loss of a proton from N7 ($E°$ = 1.63 for PCET), since C8−H loss restores partial planarity in **63**. Oxidation of **61** is exothermic for sulfate radical anion and HOO radical (ΔH = −48.2 and −14.3 kcal/mol, respectively) and nearly thermoneutral for O_2 and $O_2^{\bullet-}$ (ΔH = 2.8 and 0.1 kcal/mol, respectively). Proton-coupled electron transfers from **62** and

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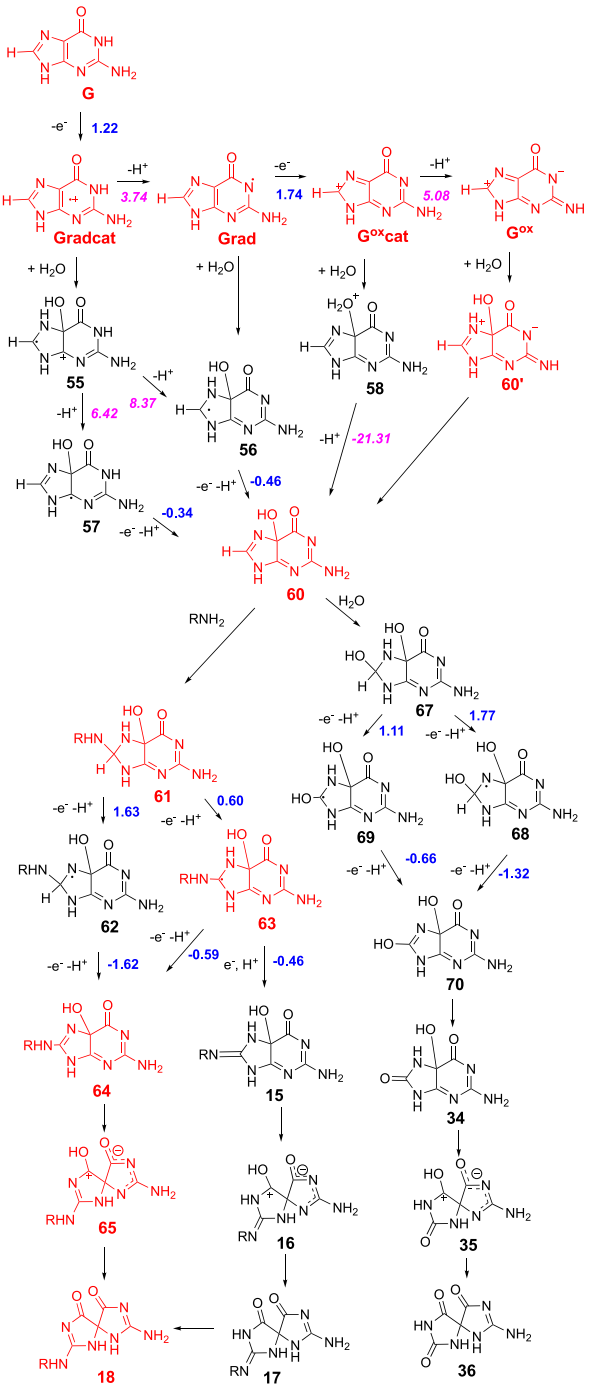
Scheme 8. Possible Pathways for the Formation of Guanine:Water Adducts via Sequential One-Electron Oxidations of Guanine and Nucleophilic Attack of Water at C5[a]



[a]The numbers next to the arrow correspond to pK_a's (pink, italics) and standard redox potentials ($E°$) (blue, regular). The structures shown in red represent the thermodynamically favored pathway.

63 produce two tautomers, **64** and **15**, respectively, with the former more stable by 2.7 kcal/mol. Acyl migration from C5 to C4 in intermediate **64** and **15** followed by tautomerization produces the final **8-NHR-Sp** products, **18** and **17**, respectively. In a similar manner, two proton-coupled oxidations of **67** produce a neutral 5,8-diOH-substituted intermediate, **70**, that can undergo hydrogen rearrangement to form 5-OH-8-oxo guanine intermediate, **34**. Acyl group migration of **34** leads to the formation of spiroiminodihydantoin, **Sp** (**36**). The neutral form is favored for acyl migration in **64** and **16**, while the deprotonated state is favored for **34**.

SUMMARY

Potential energy surfaces have been mapped for the oxidation of guanine by sulfate radical anion followed by addition of methylamine and water, leading to the formation of the monosubstituten and disubstituted spiroiminodihydantion products. One-electron oxidation of guanine leads to guanine radical cation **Gradcat** which deprotonates at physiological pH to form neutral guanine radical, **Grad**. A second oxidation by sulfate radical anion is possible and leads to **G^{ox}cat** and **G^{ox}** after deprotonation at physiological pH. Sulfate radical anion can also oxidize methylamine which deprotonates at physiological pH to give neutral CH_3NH radical. The barriers for the first and second addition steps are listed in Table 1 for singly and doubly oxidized guanine. The barriers for methylamine addition include 4.1 kcal/mol needed to deprotonate $CH_3NH_3^+$ at pH 7. The calculated results in Table 1 indicate that the barrier for methylamine addition to guanine radical at physiological pH is significantly lower at C8 than at C5 (9.5 vs 34.7 kcal/mol, respectively). Likewise, the barrier for methylamine radical addition to guanine is significantly lower at C8 than at C5 (11.3 vs 21.6 kcal/mol, respectively). The barriers for H_2O addition to C8 or C5 of guanine radical (24.6 and 27.1 kcal/mol, respectively) are significantly higher than those for methylamine addition. Following the first methylamine addition at C8, the barriers for H_2O and RNH_2 addition are comparable, yielding **8-NHR-Sp** and **5,8-diNR-Sp**. Little or no **5-NHR-Sp** should be formed by this mechanism, since the initial barriers are considerably higher, both for C8 addition of H_2O (24.6 kcal/mol) followed by C5 addition of RNH_2 and for C5 addition of RNH_2 (21.6 kcal/mol) followed by C8 addition of H_2O. This is in agreement with one-electron oxidation mediated by type I photosensitizers,[20] as discussed in our earlier paper.[26] By contrast, guanine−lysine cross-links formed by oxidation with sulfate radical anion yield about 65% **5-NHR-Sp**, 30% **8-NHR-Sp**, and ∼5% **5,8-NR-Sp**.[20] This must occur by a different pathway than type I photosensitized oxidation.

Sulfate radical anion is a strong oxidant that rapidly oxidizes guanine. When bicarbonate is present, sulfate radical anion also oxidizes bicarbonate to carbonate radical anion which, in turn, also oxidizes guanine, but more slowly than sulfate radical anion. Under the conditions used by Shafirovich and co-workers[98−100,102] (pulsed laser photolysis of $K_2S_2O_8$ with excess bicarbonate), carbonate radical anion adds to guanine radical to produce **8-oxoG** in competition with guanine−thymine cross-link formation. Our earlier calculations[28] are in agreement with this mechanism. For the conditions used by Burrows and co-workers (continuous photolysis of $K_2S_2O_8$ for 6 h, no bicarbonate), sufficient sulfate radical anions should be present to oxidize guanine and the side chain of lysine or to doubly oxidize guanine to form G^{ox}. As listed in Table 1, both RNH_2 and H_2O can add to **G^{ox}** and **G^{ox}cat** at C5 and C8 with little or no barrier, and thus could produce comparable fractions of **5-NHR-Sp** and **8-NHR-Sp**. Experimentally, however, oxidation of guanine by sulfate radical anion produces very little **8-oxoG** at pH 8 but significant amounts of **8-oxoG** at pH 2.5.[97] This would rule out the involvement of

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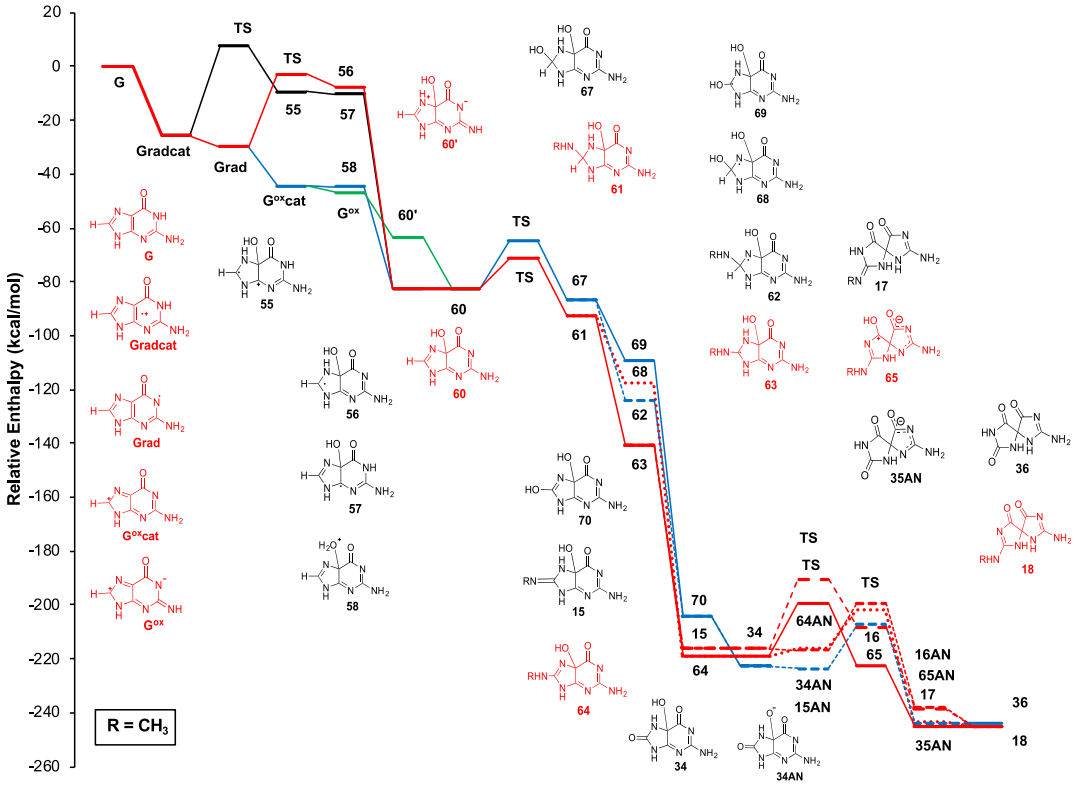

Figure 4. Comparison of the relative enthalpies (kcal/mol) of adducts resulting from the addition of water at the C5 position of guanine radical cation (**Gradcat**, black), guanine radical (**Grad**, red), oxidized guanine cation ($\mathbf{G^{ox}cat}$, blue), and neutral oxidized guanine ($\mathbf{G^{ox}}$, green) 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 blue 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.

Table 1. Summary of Enthalpy Barriers for Methylamine and Water Addition to Oxidized Guanine

first addition	barrier (kcal/mol)	intermediate	second addition	barrier (kcal/mol)	product
C8 **Grad** + RNH_2	9.5[a]	**10**	C5 RNH_2	16.1[a]	**14**, **14′** (5,8-diRN-Sp)
C8 **G** + RNH rad	11.3				
C8 $\mathbf{G^{ox}}$ + RNH_2	4.1[a]		C5 H_2O	16.5	**18** (8-RN-Sp)
C8 **Grad** + RNH rad	0.0				
C8 **Gradcat** + H_2O	12.3	**30**	C5 RNH_2	4.1[a]	**33**, **33′** (5-RN-Sp)
C8 **Grad** + H_2O	24.6				
C8 $\mathbf{G^{ox}cat}$ + H_2O	0.0		C5 H_2O	9.8	**36** (Sp)
C8 $\mathbf{G^{ox}}$ + H_2O	0.0				
C5 **Grad** + RNH_2	34.7[a]	**42**	C8 RNH_2	19.9[a]	**14**, **14′** (5,8-diRN-Sp)
C5 **G** + RNH rad	21.6				
C5 $\mathbf{G^{ox}}$ + RNH_2	4.1[a]		C8 H_2O	16.4	**33**, **33′** (5-RN-Sp)
C5 **Grad** + RNH rad	0.0				
C5 **Gradcat** + H_2O	33.2	**60**	C8 RNH_2	15.2[a]	**66** (8-RN-Sp)
C5 **Grad** + H_2O	27.1				
C5 $\mathbf{G^{ox}cat}$ + H_2O	0.0		C8 H_2O	17.5	**36** (Sp)
C5 $\mathbf{G^{ox}}$ + H_2O	0.0				

[a]Included ΔH = 4.1 kcal/mol for deprotonation of $CH_3NH_3^+$ at pH 7.

doubly oxidized guanine, since the additions of water at low pH ($\mathbf{G^{ox}cat}$ + H_2O) and high pH ($\mathbf{G^{ox}}$ + H_2O) are both calculated to be barrierless. The experimental findings can be explained by the fact that guanine radical is protonated at low pH, and the barrier for **Gradcat** + H_2O at pH 2.5 is substantially lower than the barrier for **Grad** + H_2O at pH 8. Thus, $\mathbf{G^{ox}}$ and $\mathbf{G^{ox}cat}$ are unlikely to be involved in guanine–lysine cross-link formation at physiological pH.

Because the cross-link product ratios obtained for guanine oxidation by sulfate radical anion are very different from the ratios obtained for oxidation mediated by type I photosensitizers,[20] a different mechanism must be responsible but not involving $\mathbf{G^{ox}}$ or $\mathbf{G^{ox}cat}$. The experiments of Shafirovich and co-workers[97,102] show that sulfate radical anion readily oxidizes guanine and bicarbonate and **8-oxoG** is formed by addition of carbonate radical anion to guanine radical. This indicates that sulfate radical anion can oxidize the lysine side

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chain and cross-link formation can occur by radical−radical combination, as suggested by Burrows and co-workers.[20] Methylamine radical addition to guanine radical is barrierless for C5 and C8 addition. After C5 addition, the lowest barrier is for H_2O addition at C8, leading exclusively to **5-NHR-Sp**. After C8 addition, the barriers for H_2O and methylamine addition to C5 are comparable, leading to **8-NHR-Sp** and **5,8-diNR-Sp**. Since the concentration of water is much greater than that of amine, the yield of **8-NHR-Sp** should be greater than that for **5,8-diNR-Sp**. Thus, the calculations for sulfate radical anion oxidation and cross-link formation by guanine radical−methylamine radical combination predict product yields of **5-NHR-Sp** > **8-NHR-Sp** > **5,8-NR-Sp**, in good qualitative agreement with experiment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.9b03598.

The optimized molecular geometries in Cartesian coordinates for all adducts and corresponding transition states (PDF)

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Notes

The authors declare no competing financial interest.

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