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Computational Study of the pH-Dependent Competition between Carbonate and Thymine Addition to the Guanine Radical

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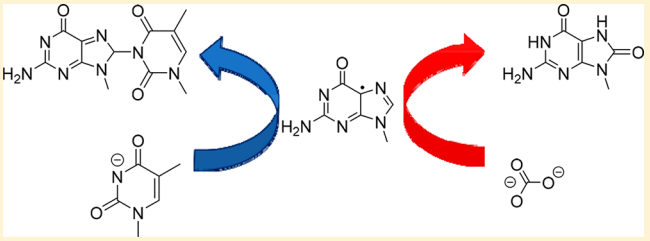
Supporting Information

ABSTRACT: When oligonucleotides are oxidized by carbonate radical, thymine and carbonate can add to guanine radical, yielding either a guanine-thymine cross-link product (G∧T) or 8-oxo-7,8-dehydroguanine (8oxoG) and its further oxidation products such as spiroiminodihydantoin (Sp) and guanidinohydantoin (Gh). The ratio of thymine addition to carbonate addition depends strongly on the pH. Details of the mechanism have been explored by density functional calculations using the ωB97XD/6-31+G(d,p) level of theory with the SMD implicit solvation method, augmented with a few explicit waters. Free energies of intermediates and transition states in aqueous solution have been calculated along the pathways for addition of thymine, CO_3^{2-}/HCO_3^- and carbonate radical to guanine radical. The pH dependence was examined by using appropriate explicit proton donors/acceptors as computational models for buffers at pH 2.5, 7, and 10. Deprotonation of thymine is required for nucleophilic addition at C8 of guanine radical, and thus is favored at higher pH. The barrier for carbonate radical addition is lower than for bicarbonate or carbonate dianion addition; however, for low concentrations of carbonate radical, the reaction may proceed by addition of bicarbonate/carbonate dianion to guanine radical. Thymine and bicarbonate/carbonate dianion addition are followed by oxidation by O_2, loss of a proton from C8 and decarboxylation of the carbonate adduct. At pH 2.5, guanine radical cation can be formed by oxidization with sulfate radical. Water addition to guanine radical cation is the preferred path for forming 8oxoG at pH 2.5.



1. INTRODUCTION

DNA oxidative damage is known to be associated with various degenerative conditions, including aging, Alzheimer's disease, and cancer.[1] Of the nucleobases, guanine has the lowest oxidation potential[2] and gives rise to a wide variety of post-oxidation reaction products. Scheme 1 illustrates some of the guanine oxidation products, including oxygen or nitrogen incorporation from sources such as $O_2^{-\bullet}$, $NO_2\bullet$, and $CO_3^{-\bullet}$ radicals to form 8-oxo-7,8-dehydroguanine (8oxoG) and 8-nitroguanine (8nitroG), cross-links with amino acids and other nucleobases, and intramolecular rearrangement to products such as 5-carboxamido-5-formamido-2-iminohydantoin (2Ih), spiroiminodihydantoin (Sp), and 5-guanidino-4-nitroimidazole (NIm). Many oxidation pathways involve one or more decomposition steps yielding end products such as guanidinohydantoin (Gh) and oxidized guanidinohydantoin (Gh_{ox}), iminoallantoin (Ia) and oxidized iminoallantoin (Ia_{ox}), and imidazolone (Iz) and its hydrolysis product, oxazolone (Z).[3−8]

The formation and reaction pathways of guanine radical have been widely studied experimentally.[3−38] Reactive oxygen species (ROS) can readily oxidize guanine and/or react with guanine radical under biological conditions. Superoxide and other ROS occur naturally in cellular metabolism and can react with NO radicals, leading to cytotoxicity.[39,40] Hydroxide radicals are produced by ionizing radiation and homolytic dissociation of peroxides, such as protonated peroxynitrite (HOONO, pK_a = 6.8) formed from superoxide combining with NO radical.[41] Nitrosoperoxycarbonate, which results from anionic peroxynitrite reacting with CO_2, dissociates into carbonate and nitrite radicals.[42−44] While the nitrite radical cannot readily oxidize guanine, it reacts rapidly with guanine radical to produce NIm and 8nitroG.[9−11,37,38] Carbonate radical is able to selectively oxidize guanine, but not other nucleobases, making it a very useful reagent for probing guanine oxidation reactions.[11,13−23]

Oxidation of guanine radical leads to 8oxoG, Sp, Gh, 2Ih, and related products.[8,11−25,27,28,31−36,45] In competition with these oxidation pathways, guanine radical can form cross-links with amino acids and nucleobases.[11,13,19−22,26,29,46] The formation of guanine-thymine cross-links has been studied largely using carbonate and sulfate radicals to selectively oxidize guanine.[11,13,19−22] Carbonate radicals can be generated by photolysis of $S_2O_8^{2-}$ to produce sulfate radicals which rapidly oxidize HCO_3^-/CO_3^{2-} in solution. Carbonate radicals can also be generated by photolysis of $[Co(NH_3)_4CO_3]^+$,[21] or by decomposition of nitrosoperoxycarbonate.[11,44] Reactions of guanine with carbonate radical anions produce varying amounts of 2Ih, 8oxoG, and guanine-thymine cross-links,[11,13,19,21] while reactions of guanine with sulfate radical

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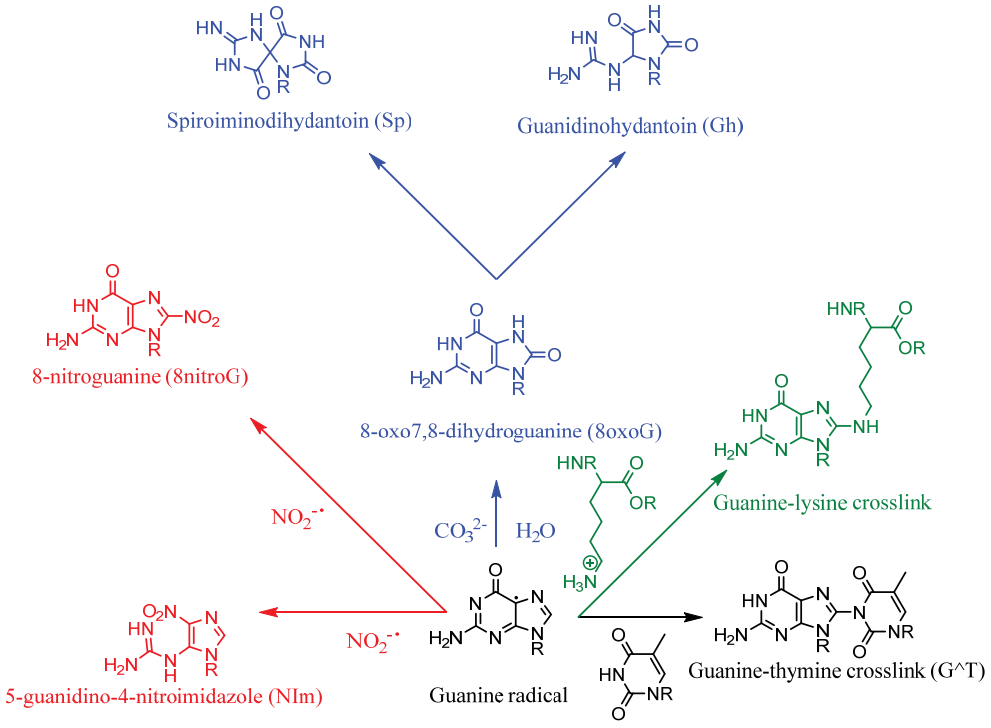
Scheme 1. Possible Reaction Products of Guanine Radical and Relevant Nucleophiles



anions at neutral pH produce only the cross-link.[13] $H_2{}^{18}O$ radiolabeling experiments determined that oxygen incorporated at the C8 position in the formation of 8oxoG and Sp from neutral guanine radical at pH 7.5 does not arise from solvent water, but instead originates from carbonate.[27,28] On the other hand, ESR studies find that the oxygen incorporated in 8oxoG produced from guanine radical cation does come from solvent water.[47] Thymine addition to guanine radical competes with carbonate addition and shows a significant pH dependence.[11,13,19,21] Acidic pH environments favor formation of 8oxoG and basic pH favors formation of guanine-thymine cross-links.[13,21] At basic pH, a greater concentration of thymine is deprotonated, facilitating nucleophilic addition, whereas thymine is protonated at acidic pH, hampering nucleophilic addition. Based on the yield of Sp and Gh (oxidation products of 8oxoG), the ratio of carbonate addition to thymine addition is about 1.5−1.8 to 1 at pH 7,[21] while thymine addition is the only product seen at pH 10,[21] even though carbonate/bicarbonate is present in much greater concentration. Another study finds increasing yields of guanine-thymine cross-link as the pH is increased from 7.5 to 9, but nearly steady yield of 8oxoG over the same pH range.[13] Both the thymine addition and the bicarbonate/carbonate dianion addition pathways require a second oxidation step in order to form the observed products.[20,21] Experiments show that molecular oxygen, or another sufficiently strong oxidant, is needed to produce thymine cross-link products but not to produce 8oxoG or Sp/Gh from guanine reacting with carbonate radical.[19,20] For an analogous system, guanine-uracil cross-link formation was absent under deoxygenated conditions, while benzoquinone was found to restore cross-link formation.[20] 8oxoG was still formed without oxygen, which can be explained by the combination of guanine radical and carbonate radical circumventing the need for a second oxidation step.[19,20]

A number of computational studies have addressed the reactions of guanine radical.[33,34,45,47−85] Sevilla and co-workers have investigated guanine oxidation and reduction, protonation and deprotonation of guanine radical, and hydroxyl radical adding to guanine.[47−52] We have used DFT calculations to examine the mechanisms for the formation of 8oxoG, Sp, Gh, and FAPyG from guanine radical,[53,54] the formation of guanine-lysine cross-links during oxidation mediated by type I and type II photosensitizers,[55,56] and the pK_a's and redox potentials of nucleobases and intermediates in the pathway from guanine radical to Sp and Gh.[57−60] Dumont and co-workers have investigated the addition of singlet oxygen to guanine to form 8oxoG and Sp.[61−63] Liu and co-workers have used guided ion beam mass spectrometry and density functional calculations to examine gas-phase singlet oxygen reactions with guanine and its oxidation products.[32−35] Wetmore, Boyd, and co-workers have calculated ionization potentials and electron affinities of nucleobases,[64] NO_2 radical addition to guanine radical,[65] and hydroxyl radical addition to nucleobases.[66] Mishra and co-workers have published numerous studies of guanine oxidation by reactive oxygen species,[67−82] including the oxidation of guanine and 8oxoG with carbonate radical,[67] addition of carbonate radical to guanine radical,[68,69] as well as NO_2 and OH radical reactions with guanine.[70,71] Ding and co-workers have used molecular dynamics to investigate the structure and stability of guanine-thymine cross-link products in double strand DNA.[83] They find the deformation of the double helix is greater when guanine and thymine are separated by a cytosine than when they are adjacent.

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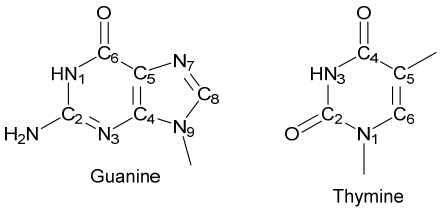
In this computational paper, we focus on the competition between addition of thymine and carbonate to guanine radical. In the next study, we will investigate the formation of 2Ih, which is also produced in the reaction of carbonate radical and other reactive oxygen species with guanine.[13,24,25,86] In the present study, we start with an examination of the oxidation of guanine by carbonate radical. Then we investigate the addition of thymine to C8 of guanine radical to form a guanine-thymine cross-link product, and the addition of bicarbonate/carbonate dianion and carbonate radical to C8 of guanine radical leading to 8oxoG. In each case, the C8 hydrogen must be removed in order to form products. After considering a number of pathways, we find that loss of the C8 hydrogen from guanine in the thymine and bicarbonate/carbonate dianion addition intermediates can occur by oxidation with $^3O_2$, followed by deprotonation. The formation of 8oxoG from carbonate addition also involves decarboxylation, which can occur before or after removal of the C8 hydrogen. The reactions are calculated at pH 2.5, 7, and 10 to determine the effect of pH on the competition between the pathways leading to guanine oxidation products and cross-link formation.

2. METHODS

Calculations were performed using the Gaussian series of programs[87] and the ωB97XD density functional[88] with the 6-31+G(d,p) basis set.[89−93] Additional single point calculations at the ωB97XD/aug-cc-pVTZ[94] level of theory were carried out for the thymine and carbonate addition steps. Reaction energies and barrier heights changed by only a few kcal/mol, but the difference in barrier heights and reaction energies was less than 1 kcal/mol (Table S3). SMD implicit water solvation[95] was used to model aqueous conditions. Explicit waters were additionally used for the thymine and carbonate addition pathways. Nucleobases were capped with methyl groups in place of the sugar moiety. The numbering of the atoms in guanine and thymine are shown in Scheme 2.

Scheme 2. Atom Numbering for Guanine and Thymine



The pK_a's for reactants and intermediates are calculated from the free energy differences for deprotonation in solution

$$pK_a = \frac{G_{deprotonated} + G_{H^+_{(aq)}} - G_{protonated}}{2.303RT} \quad (1)$$

where R is the gas constant (1.987 cal K^{-1} mol^{-1}), T is the temperature (298.15 K). The free energy of the proton in water, $G^+_{H(aq)}$ = −270.3 kcal/mol,[96−99] is obtained from the gas phase free energy, $G^+_{H(g)}$ = −6.287 kcal/mol, the conversion from 1 atm to 1 mol/L, $\Delta G^{1atm\rightarrow 1M}$ = 1.89 kcal/mol,[100] and free energy of solvation of a proton, $\Delta G^+_{H(solv.)}$ = −265.9 kcal/mol.[96−99]

$$G_{H^+_{(aq)}} = G_{H^+_{(g)}} + \Delta G^{1atm\rightarrow 1M} + \Delta G_{H^+_{(solv)}}$$
$$= -6.287\ \text{kcal/mol} + 1.89\ \text{kcal/mol} + -265.9\ \text{kcal/mol}$$
$$= -270.3\ \text{kcal/mol} \quad (2)$$

The free energy of H$^+$ is not needed for any of the protonation/deprotonation steps in the reaction mechanism, since these steps are calculated by transferring a proton to/from the appropriate buffer species (fluoroacetic acid for pH 2.5, imidazole for pH 7, and guanine for pH 10). The conversion from 1 atm to 1 mol/L was added to the free energy of molecules in solution, $G_{A_{(aq)}} = G_{A_{(g)}} + \Delta G^{1atm\rightarrow 1M} + \Delta G_{A_{(solv.)}}$.

The standard state reduction potentials E_0 are calculated by

$$E_0 = -\frac{G_{reduced} - G^\circ_{e^-}(g) - G_{oxidized}}{nF} - \text{SHE} \quad (3)$$

where SHE is the absolute potential of the standard hydrogen electrode (4.281 V[97,98,101]), F is the Faraday constant (23.06 kcal/mol V), n is the number of electrons (n = 1 in all cases in the present study), and $G^\circ_{e^-}(g)$ = −0.867 kcal/mol[102,103] is the gas phase energy of the electron at 298 K.

Experimentally, the reactions are carried out in buffered solutions. Computationally, the relative free energies of different protonation states of a compound in a buffer can be determined by calculating the free energy for the transfer of a proton to/from a proton acceptor/donor that has a pK_a equal to the pH of the buffer. The relative concentrations of the different protonation states can be determined from the Boltzmann distribution and the relative free energies. Fluoroacetic acid (pK_a = 3.0 calc., 2.6 exp.[104,105]), imidazole (pK_a = 7.7 calc., 6.95 exp.[106]), and guanine (pK_a = 9.5 calc., 9.5 exp.[58,107]) were used to model pH 2.5, 7, and 10 buffers, respectively. Calculations of guanine anion were found to give good pK_a values with scaling of the solvent cavity of the anion (α = 0.90) and no explicit waters.[58] The pK_a for protonation of neutral guanine at N7 is 2.7 calc., 3.1[58,107] (no scaling). The pK_a for deprotonation of thymine is 9.9 calculated with 7 explicit waters, compared to the experimental value of 9.7[108] Carbonate radical remains deprotonated across the pH range of 2.5 to 10.[109,110] The calculated pK_a values of various intermediates are collected in Table S1 of the Supporting Information.

3. RESULTS AND DISCUSSION

In this study, we consider the oxidation of guanine by carbonate radical and the competition between thymine, bicarbonate/carbonate dianion, and carbonate radical anion addition to the guanine radical in aqueous solution at different pH's. The carbonate radical anion, $CO_3^{-\bullet}$, is known to selectively oxidize guanine, as the redox potentials of other nucleotides are too high to be oxidized by the carbonate radical.[14] Carbonate radicals can be generated by oxidation of carbonate dianion, CO_3^{2-} by sulfate radical, $SO_4^{-\bullet}$,[13,14,18,21] which is produced by photolysis of $S_2O_8^{2-}$ at neutral or basic pH or by oxidation of the $CoNH_4(CO_3)^+$ complex[21] under neutral or acidic conditions. At neutral pH, both methods were found to give similar product distributions.[21] Carbonate radicals can also be produced from the dissociation of nitrosoperoxycarbonate, which can be formed from nitric oxide, superoxide, and carbon dioxide ($NO\cdot + O_2^{-\bullet} + CO_2 \rightarrow ONOO^- + CO_2 \rightarrow ONOOCO_2^-$).[11,17,21] While carbonate radical is not protonated for the range of pH's considered, guanine, guanine radical, thymine, and carbonate dianion may be protonated or deprotonated, depending on the pH. As described in the Methods section, the relative energies of the protonated/deprotonated structures at a given pH are obtained by calculating the energy for transferring a proton to/from a suitable proton acceptor/donor with a pK_a close to the pH of interest. The calculation of pK_a's and reduction potentials requires a reliable treatment of solvation energies. As solvation energies are more difficult to calculate for anions and dianions than for cations and neutral systems,[58] we first examine approaches for calculating the pK_a of carbonate/bicarbonate. The most suitable solvation method is then used to explore guanine oxidation, carbonate, and thymine addition to the

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guanine radical, and subsequent steps to produce the final products.

3.1. Solvation Treatment. The SMD implicit solvation method has been found to perform well for pK_a's and reduction potentials involving neutrals and cations.[58,59] For anions, cavity scaling was needed in many cases to get agreement with experiment.[58] Better agreement with experiment can also be obtained by using explicit water molecules in addition to implicit solvation.[111−113] Water positioned around carbonate dianion has been considered in a number of recent studies.[114] Figure 1 shows examples of the placement of waters around bicarbonate/carbonate used in the present work. The positions of explicit waters for other structures considered in this study are included in the Cartesian coordinate provided in the Supporting Information.

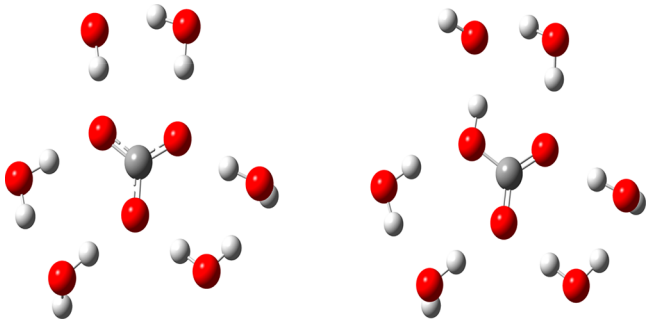

Figure 1. Orientations of explicit water molecules around carbonate dianion and bicarbonate.

Figure 2 compares the effect of cavity scaling and different numbers of explict waters on the calculated pK_a values for bicarbonate/carbonate. Implicit solvation with SMD produced a pK_a of about 18, compared to the experimental value of 10.3.[115] Scaling the solvent cavity of the dianion by 0.90 brought the calculated pK_a closer to experiment by only about 1 pK_a unit. Two, four, and six explicit waters systematically improved the calculated pK_a's. The best results were obtained with six explicit waters (as shown in Figure 1), giving a calculated pK_a of 10.4, compared to 10.3 from experiment. The E_0 for the reduction of carbonate radical anion to carbonate dianion was calculated to be 0.75 V with only SMD implicit solvation, significantly lower than experimental value of 1.59 V.[116] With four and six explicit waters, the E_0 was calculated to be 1.29 and 1.43 V, respectively. This is in good agreement with recent calculations on carbonate radical reduction potentials.[117] Triplet oxygen was used to oxidize the guanine addition intermediates. Depending on the pH, the products of this step can be either superoxide or hydroperoxyl radical. With only implicit solvation, the E_0 for oxygen was caluclated to be −0.38 V, with the pK_a for protonation calculated to be 6.8, compared to the experimental values of −0.16 V[118] and 5.0, respectively.[119,120] Addition of four explicit waters changed the calculated E_0 and pK_a values to −0.17 V and 4.2, respectively, giving much better agreement with experiment. For thymine, calculations gave a pK_a of 15.2 without scaling or explicit waters and 12.0 with scaling, compared to the experimental pK_a of 9.7.[108] Calculations for thymine with three, five, and seven explicit waters yielded pK_a's of 13.5, 11.6, and 9.9, respectively. The present study used three and six explicit waters and SMD implicit solvation for calculations of reactions involving thymine and carbonate, respectively, with deprotonation of thymine calculated with seven explicit waters.

3.2. Reaction Path Overview. Overviews of the thymine and carbonate addition pathways are given in Schemes 3 and 4. The first step is oxidation of guanine by carbonate radical, and is common to both pathways. Depending on the pH, oxidation can be followed by deprotonation of guanine radical cation and/or protonation of carbonate dianion. These steps are discussed in section 3.3. Thymine must be deprotonated before it can add to guanine radical. Proton equilibration of the addition adduct leads to a stable intermediate. The next step involves removal of the C8 hydrogen to form the cross-link product, G∧T. This can occur by oxidation and deprotonation or by hydrogen atom abstraction by $^3O_2$. Details of thymine addition and loss of the C8 hydrogen to form the guanine-thymine cross-link product at various pH's are discussed in section 3.4. Carbonate could add as the radical or the ion depending on the relative rates and concentrations, and decarboxylation could occur before or after loss of the C8 hydrogen. The final product is 8oxoG. Details of the carbonate addition pathway are described in section 3.5. The calculations are compared to experiment in section 3.6. As described in the Methods section, fluoroacetic acid (exp pK_a = 2.6[104,105]), imidazole (exp. pK_a 6.95[106]), and guanine (exp pK_a 9.5[58,107])

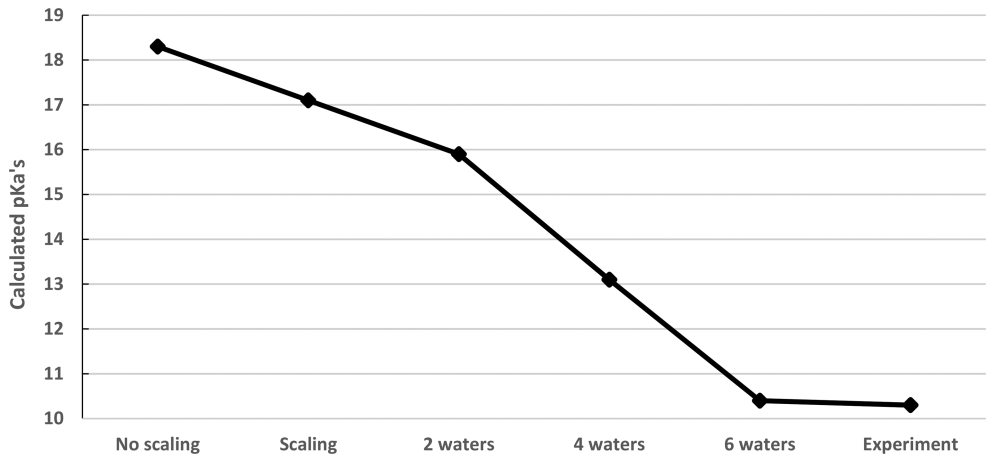

Figure 2. Comparison of the calculated pK_a's for bicarbonate; α = 0.90 was used to scale the solvent cavity of the dianion. Scaling was not used with explicit waters. See Figure 1 for the structures with 6 waters.

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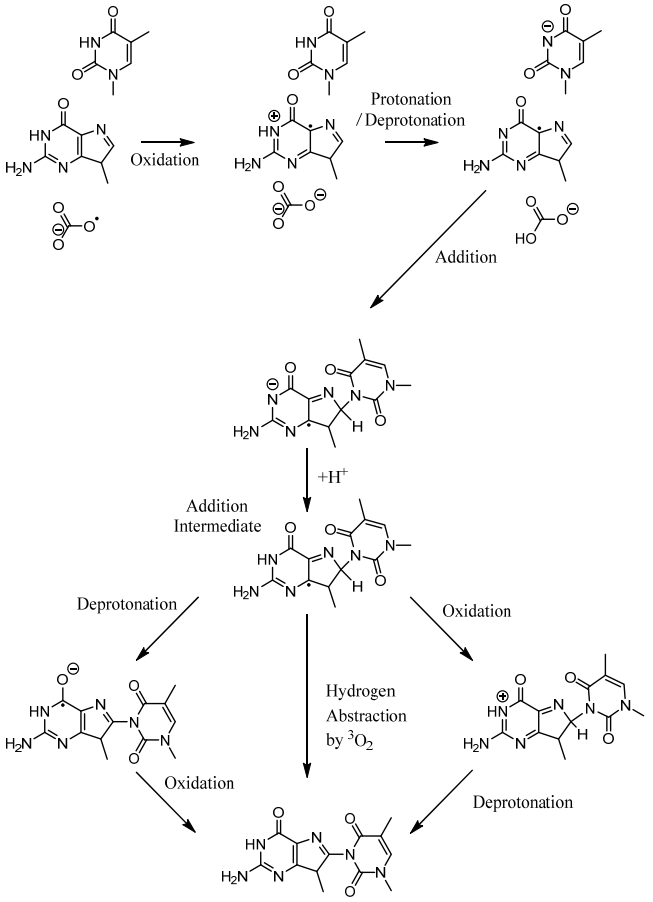
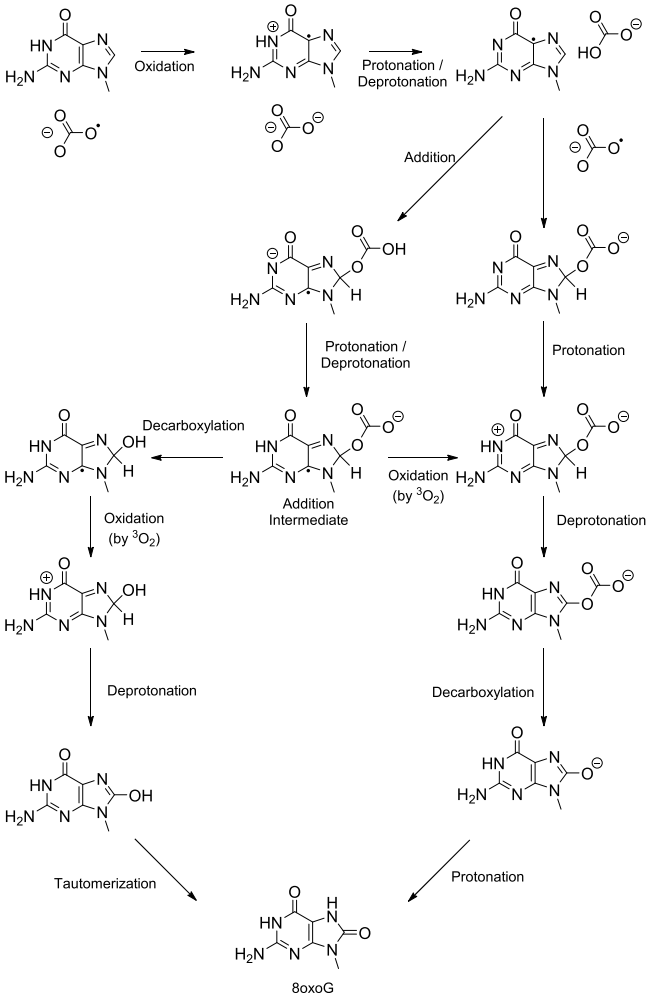
Scheme 3. Overview of the Addition of Thymine to Guanine Radical to Form the Guanine-Thymine Cross-Link Showing Various Pathways for Removal of the C8 Hydrogen



Scheme 4. Overview of the Addition of Carbonate to Guanine Radical to Form 8oxoG Showing Relevant Pathways for Decarboxylation and C8 Hydrogen Abstraction



are used to calculate the relative free energies for protonation/deprotonation at pH 2.5, 7, and 10, respectively.

One of the challenges in modeling the addition of thymine and carbonate to guanine is the numerous tautomers and protonation states that need to be considered for each intermediate and transition state at the various pH's. To further complicate the situation, the lowest energy transition state for a particular reaction step may be a different tautomer or protonation form than the corresponding lowest energy reactant. For example, thymine is neutral at pH 7 but must be deprotonated before it can add to guanine radical. The overall barrier for this step is therefore calculated as the sum of the free energy for the deprotonation plus the free energy barrier for addition. Assuming a prior equilibrium, the corresponding reaction rate is the product of the equilibrium constant for deprotonation and the rate for addition of thymine anion. For the purpose of the discussion, we assume that each of the intermediates is sufficiently long-lived to be at equilibrium (or nearly so) with regard to protonation and tautomerization. The rates for various steps in the mechanism can then be compared by looking at the free energy difference between the lowest energy intermediate and the appropriate transition state for the step. All guanine N1, N7, and C8 protonation states of the reaction intermediates were considered. A full list of structures and their structure numbers can be found in Figure S1 in the Supporting Information. Only the structures along the lowest energy pathways have been included in schemes and figures in the text; the rest may be found in Table S1 of the Supporting Information. Red, purple, and blue are used to designate pH 2.5, 7, and 10 pathways, respectively (i.e., the colors of litmus pH indicator).

3.3. Oxidation of Guanine by Carbonate Radical. The first step in both addition pathways is the oxidation of guanine by carbonate radical. Carbonate radical is a monoanion throughout this pH range.[109] Since the experimental pK_a's of guanine are 3.1 and 9.5,[107] the neutral form is the predominant species at pH 7, and there is a significant population of the cation at pH 2.5 and the anion at pH 10. The oxidation step at pH 2.5 involves an initial deprotonation to form neutral guanine since the cation is much more difficult to oxidize (E_0 = 2.02 V calc. for guanine cation compared to 1.31 V for the neutral). Oxidation of neutral guanine by carbonate radical produces carbonate dianion, **1**, and guanine radical cation, **6**. Carbonate dianion picks up a proton at pH 7 to form bicarbonate, **2** (and a second proton at pH 2.5 to decompose to $CO_2 + H_2O$). The experimental pK_a's for guanine radical are 3.9 and 10.9.[12] Thus, guanine radical cation loses a proton at pH 7 to form neutral guanine radical, **7**. The structures and relative energies are shown in Scheme 5. These calculations

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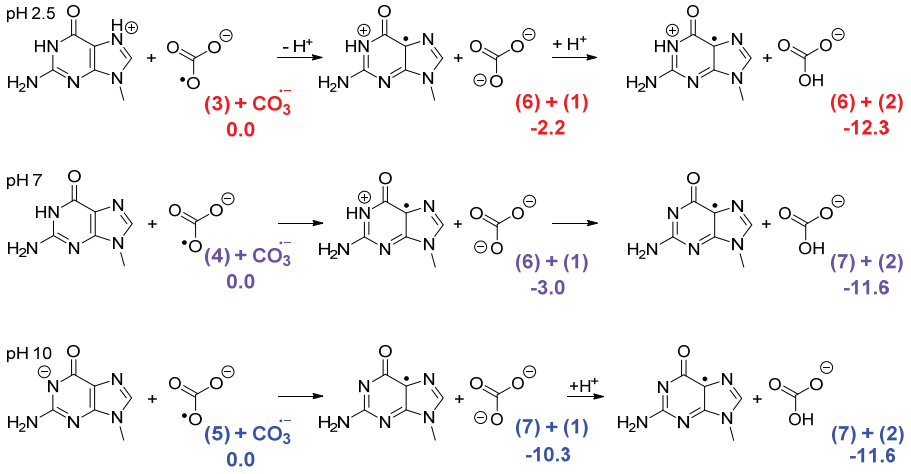
Scheme 5. Oxidation of Guanine by Carbonate Radical at pH 2.5, 7, and 10 (Relative Free Energies in kcal/mol)[a]



[a]Energies were calculated as the sum of separate species.

included SMD solvation and 6 explicit waters on carbonate (as shown in Figure 1).

3.4. Reaction of Thymine with Guanine Radical. The rate of thymine addition to guanine radical depends on the pH.[13,21] Since the experimental pK_a of thymine is 9.7,[108] thymine is in its neutral form, **8**, at pH 2.5 and pH 7, but a significant amount of thymine anion, **9**, is present at pH 10. The unaided, direct addition of neutral thymine to guanine radical does not produce a stable product. Even when imidazole is used as a proton acceptor during the addition process, the calculation of thymine addition to the C8 of guanine radical leads to a barrier of about 26.6 kcal/mol. This indicates that thymine must be deprotonated before addition can occur. Using fluoroacetate and imidazole as proton acceptors to model pH 2.5 and 7 buffered conditions, the free energy for the deprotonation of thymine with 7 waters was calculated to be endothermic by 9.3 and 2.9 kcal/mol, respectively. Thymine anion can add to either guanine radical neutral or cation. The pK_a of guanine radical cation was calculated to be 4.1, with the cation radical calculated to be 4.8 and 7.3 kcal/mol higher than the neutral at pH 7 and 10, respectively. The barrier for the addition of thymine anion, **9**, to guanine radical cation, **6**, is 11.1 kcal/mol and to neutral guanine radical is16.8 kcal/mol (calculated with 7 waters). The barrier for addition of O6 of thymine (with 3 waters) to C8 of neutral guanine radical, **7**, is 3−4 kcal/mol higher than addition of N3 of thymine to C8 of guanine (with 3 waters) and is not considered further. Since thymine is neutral at pH 2.5 and 7, and N1 of guanine radical is protonated in the lowest energy transition state, the energy of guanine radical protonation/thymine deprotonation needs to be added to the barrier for addition, resulting in overall barriers of 20.4 and 18.9 kcal/mol at pH 2.5 and 7, respectively. At pH 10, the lowest barrier is 16.8 kcal/mol for the addition of thymine anion to neutral guanine radical. Scheme 6 and Figure 3 summarize the thymine addition pathway. The thymine addition step was calculated with 7 explicit waters to appropriately treat the anionic character of the respective states. The G∧T radical addition intermediate was calculated to have an N1 pK_a of between 12.5 and 13.6 using 0, 3, and 7 explicit waters. This insensitivity of the pK_a compared to free thymine suggests the radical anion intermediate does not require the same extent of explicit solvation to be appropriately stabilized. Calculations related to the addition intermediate were computed with 3 waters to reduce system size and complexity.

As the concentration of deprotonated thymine increases with pH, the possibility of oxidation of the thymine anion and subsequent combination with guanine radical becomes relevant. The reduction potential of thymine anion was calculated to be 1.31 V with 3 explicit waters and 1.54 V with 7 explicit waters, suggesting oxidation of deprotonated thymine is a possibility. However, calculations of the thymine radical showed that it is a π radical with the spin delocalized across the N1−C6−C5 portion of the ring (spin density of 0.857) and not a σ radical localized at the N3 position that would be conducive to forming a guanine-thymine adduct.

The free energy barrier for thymine adding to guanine radical includes the entropy contribution for bringing the reactants together. In most of the experiments, thymine is in the same DNA strand and separated from the guanine by a cytosine. An estimate of the barrier for the addition of a tethered thymine can be obtained by starting from the guanine radical−thymine anion complex rather than separated reactants. This lowers the free energy barrier by 2−3 kcal/mol.

Addition of thymine anion to guanine radical cation produces a neutral intermediate, **14**, while addition to neutral guanine yields an anionic intermediate, **15**. Protonation of the anionic intermediate at N1 is exothermic by 13.3, 7.0, and 4.5 kcal/mol for pH 2.5, 7, and 10, respectively, resulting in the neutral radical, **14**. To produce the final guanine-thymine cross-link product, the hydrogen at C8 must be removed. The thymine addition intermediate cannot be readily deprotonated at the C8 position (pK_a's greater than 20; for relevant intermediates considered, ΔG = +15.2 kcal/mol is the lowest value calculated for deprotonation with imidazole). Hydrogen atom abstraction by O_2 is an alternative route to the G∧T cross-link product **22** and has barriers of 21.5, 14.5, and 13.7 kcal/mol at pH 2.5, 7 and 10, respectively. A tautomer of **14** with N7 protonated instead of N1 (**13**) is nearly equal in

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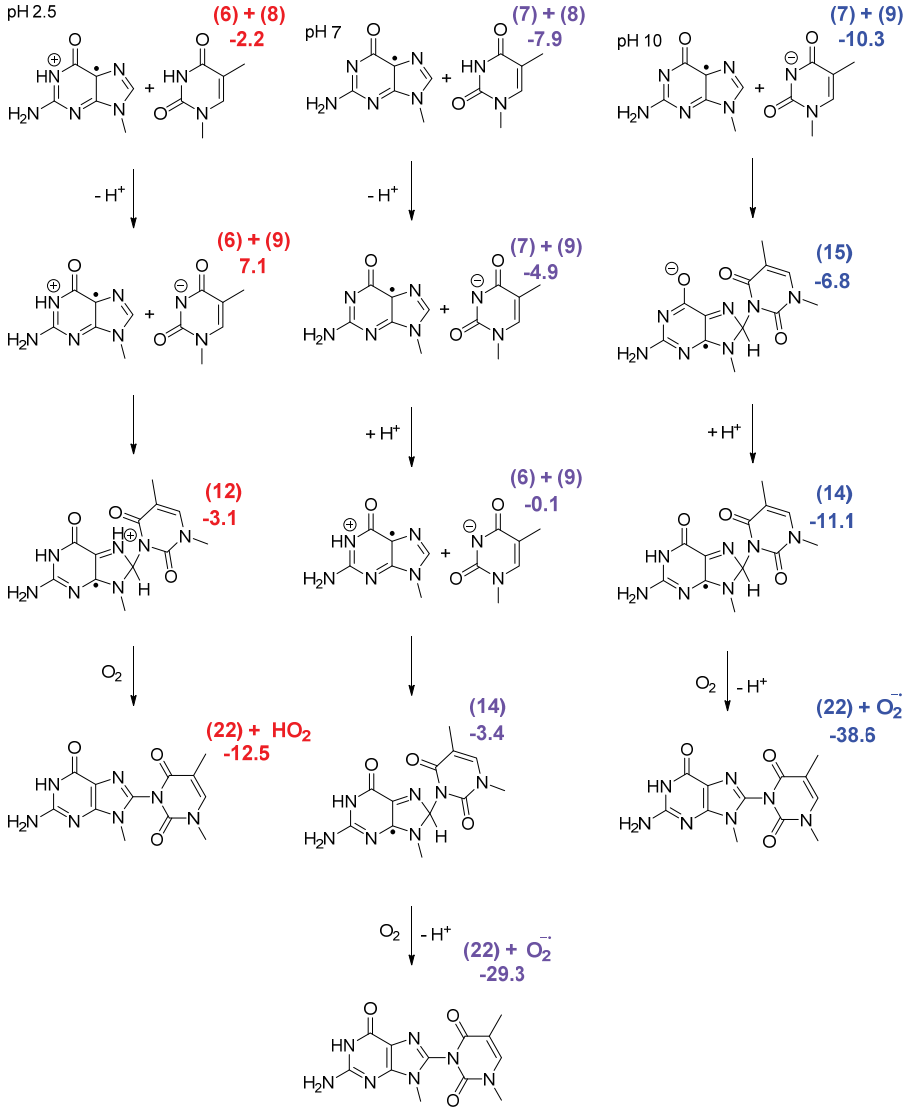
Scheme 6. Addition of Thymine to Guanine Radical at Various pH's to Form the Guanine-Thymine Cross-Link[a]



[a]Free energies of species indicated plus CO_3^{2-} in kcal/mol relative to guanine and carbonate radical. Energies given are for 7 water solvation of the thymine-guanine complex. Refer to text for details related to explicit waters. Reactant energies were calculated as the sum of separate species.

energy, but the barrier for hydrogen abstraction is 10 kcal/mol higher due to the diradical character in the transition state (see Figure S2 in the Supporting Information). At pH 7 and 10, direct electron transfer from **14** to molecular oxygen (E_0 = −0.04 V for **14**, ΔG = +3.0 kcal/mol, calculated with 4 waters around O_2) followed by deprotonation (pK_a = −13.7, ΔG = −29.0 kcal/mol for imidazole) is the lowest energy and most viable pathway for the removal of the C8 hydrogen from **14**. At pH 2.5, protonation of N7 of **14** is exothermic by 6.9 kcal/mol, producing a cation radical, **12**, with both N1 and N7 protonated. However, the energy for electron loss from **12**, via C8 hydrogen abstraction or electron transfer, is high. C8 hydrogen abstraction from **12** has a barrier of 26.6 kcal/mol and oxidation of **12** is difficult (E_0 = 0.67 V for **12**, ΔG = +22.5 kcal/mol for $^3O_2$) unless it is deprotonated first. Consequently, the lower energy pathway at pH 2.5 involves N7 deprotonation of the cation radical **12**, followed by electron transfer from neutral radical **14**. The overall barriers for oxidation by O_2 and loss of the C8 proton to produce the guanine-thymine cross-link product, **22**, is 8.5 kcal/mol from **12** at pH 2.5, and 2.9 kcal/mol from **14** at pH 7 and 10.

Figure 3 summarizes the lowest energy pathways for addition of thymine to guanine radical at pH 2.5, 7, and 10. The addition barrier at pH 2.5 is higher than at pH 7 or 10, due to the significant energetic penalty of deprotonating thymine under acidic conditions. The lowest energy path for C8 hydrogen loss proceeds by electron transfer from the neutral addition intermediate, **14**, to triplet oxygen. The variation of the energy of the final G∧T cross-link product, **22**, with pH is due to the protonation/deprotonation of thymine and $O_2^{\cdot-}/HO_2$ with changes in pH. The pK_a of hydroperoxyl radical is about 5[119,120] (calculated pK_a 4.2 with 4 explicit waters) and is therefore preferred deprotonated above acidic pH, leading to lower energy of the system at pH 10.

3.5. Reaction of Carbonate with Guanine Radical.

Three scenarios can be considered for carbonate addition to

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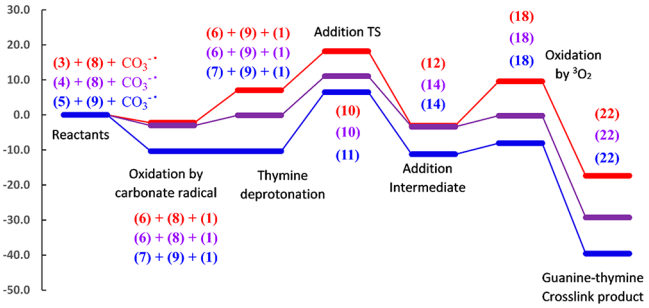


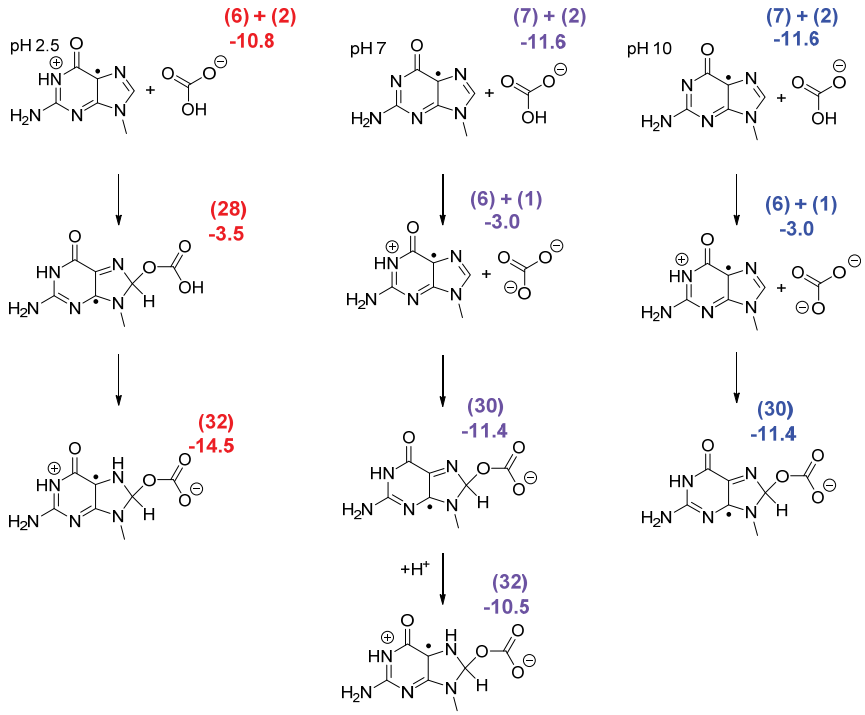
Figure 3. Gibbs free energies for the pathway for thymine addition to guanine radical. Free energies were calculated using ωB97XD/6-31+G(d,p) and are given in kcal/mol. The positions of explicit waters are included in the Cartesian coordinate provided in the Supporting Information. Acidic pH 2.5 (red), neutral pH 7 (purple), and basic pH 10 (blue) are modeled using fluoroacetic acid, imidazole, and guanine anion as the proton donor/acceptor for protonation/deprotonation reactions. Refer to text for details related to explicit waters.

guanine radical. For very low concentrations of carbonate radical, oxidation of guanine would consume all of the carbonate radical. If significant amounts of bicarbonate or carbonate dianion are present, they could add to guanine radical to produce 8oxoG. This scenario would be the most relevant under biological conditions. Alternatively, if oxidation of guanine proceeds by an inner sphere mechanism, either carbonate radical may add directly to guanine, or electron transfer from guanine to carbonate radical forming guanine radical cation and carbonate dianion may lead to rapid reaction without separation of the complex. In a third alternative, carbonate radical could add to guanine radical, if not all of it is consumed in the initial oxidation of guanine.

Scheme 7 shows the pathways for bicarbonate/carbonate dianion addition to guanine radical. At pH 2.5, both carbonate and the N1 position of guanine radical are protonated, while at pH 7, only carbonate is protonated at equillibrium. At pH 10, carbonate and both the N1 and N7 positions of guanine radical are deprotonated. At pH 2.5, addition of bicarbonate to guanine radical cation has a barrier of 11.9 kcal/mol, while carbonic acid would decompose before addition could occur. At pH 7, the barrier for the addition of bicarbonate to neutral guanine radical is 18.9 kcal/mol. The lowest energy pathway at this pH, however, involves protonation of guanine radical and deprotonation of bicarbonate before addition, yielding an overall barrier of 15.3 kcal/mol for carbonate addition. At pH 10, addition of carbonate to guanine neutral radical has a barrier of 16.3 kcal/mol yielding **31**. Protonation at N1 of **31** is exothermic by 6 kcal/mol and produces the stable intermediate **30**. A lower energy pathway involves deprotonation of bicarbonate and protonation of guanine radical, leading to carbonate dianion addition to guanine radical cation to produce **30**. This process has an overall barrier of 15.3 kcal/mol at pH 10.

In principle, carbonate radical could add directly to guanine. However, when carbonate radical addition is calculated with 6 explicit waters, electron transfer occurs before the transition state as the carbonate radical approaches guanine. The subsequent transition state is the same as for carbonate dianion addition to guanine radical cation and is 5.3 kcal/mol above separated guanine and carbonate radical. This process involves a nonequilibrated guanine radical cation and

Scheme 7. Addition of Carbonate to Guanine Radical to Form the Guanine-Carbonate Addition Intermediates at Various pH's[a]



[a]Relative free energies in kcal/mol. Reactant energies were calculated as the sum of separate species.

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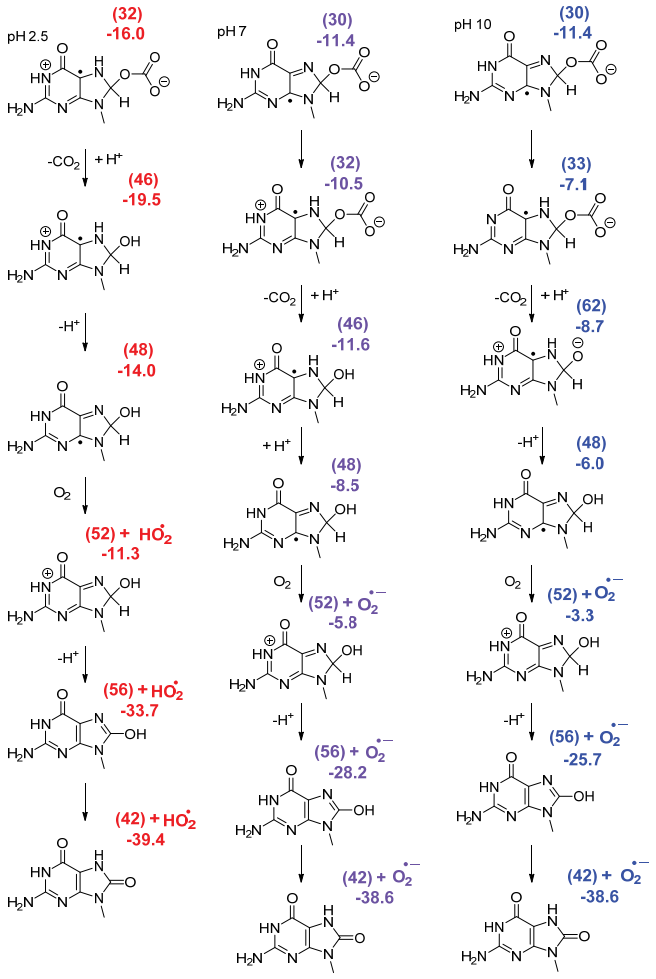
carbonate dianion and therefore may bypass the pH dependence of the system, so long as reaction of the two species is faster than protonation of carbonate or deprotonation of guanine radical cation. This may be a viable pathway at low pH, such as with $[Co(NH_3)_4CO_3]^+$,[21] which would avoid the protonation of carbonate dianion and decomposition of carbonic acid.

If all of the carbonate radical is not consumed in oxidizing guanine, carbonate radical could add to guanine radical, yielding either structure **36** or **37** directly (see Scheme 9). This appears to be the case for the reaction conditions employed by Shafirovich and co-workers.[21] This pathway has been invesigated by Mishra and co-workers,[68] and occurs without an enthalpy barrier, as expected for radical−radical reactions. In the present calculations, we find the oxidation of guanine radical by carbonate radical is slightly endothermic (ΔH = 2.8 kcal/mol) at infinite separation. At separations of 6 Å or less, the oxidation of guanine radical is exothermic. This suggests the first step of the reaction occurs by outer sphere electron transfer with a rate governed by Marcus theory. This is followed by the addition of carbonate dianion to the cation formed by oxidizing guanine radical. While the strong Coulombic attraction between the ions assures that this second step has no enthalpy barrier, there may be a small free energy barrier resulting from the loss of rotational and translational degrees of freedom. Whether the reaction occurs in a single step radical−radical combination or in a two step electron transfer/addition reaction, the reaction will be very facile. Mishra and co-workers obtain 75 kcal/mol for the exothermicity of the guanine radical/carbonate radical reaction,[68] whereas the present calculations yield 35 kcal/mol. The difference is likely due to the treatment of solvation combined with differences in the respective computational methodologies. As discussed in section 3.1, the redox potential of carbonate radical is too low by 0.65 eV in the absence of explicit waters, understabilizing the radical and overstabilizing the combined product. Nevertheless, the overall rate of the reaction between guanine radical and carbonate radical may be small because of the low concentrations of both radicals.

After the addition of carbonate radical to guanine radical, deprotonation of C8 and decarboxylation occur readily. However, for bicarbonate/carbonate dianion addition to guanine radical, proton equillibration and oxidation must occur before deprotonation of C8. The carbonate group in the intermediate formed by bicarbonate addition to neutral guanine radical has a calculated pK_a of 1.76, similar to methyl carbonate (calculated pK_a 1.37). Consequently at pH 2.5, the bicarbonate addition intermediate tautomerizes by deprotonation of the bicarbonate moiety and protonation of the N7 position (**32**). The diprotonated form, **32**, is the most stable at pH 2.5, whereas the N1 monoprotonated form, **30**, is the most stable carbonate addition intermediate at pH 10; both mono- and diprotonated forms may be present in significant amounts at pH 7. The pK_a's of the N1 and N7 positions in **32** were calculated from the carboxylate-solvated minima to be 7.9 and 6.0, respectively, while pK_a's of N1 and N7 in monoprotonated intermediates **30** and **33** were calculated to be 14.1 and 13.0, respectively.

Scheme 8 shows the formation of 8oxoG from the bicarbonate/carbonate dianion addition intermediate, which requires both decarboxylation and the removal of the C8 hydrogen. Calculation of the decarboxylation barrier is complicated by the strong interaction of the explicit waters

Scheme 8. Formation of 8oxoG from the Carbonate Addition Intermediate at Various pH's by Decarboxylation, Oxidation and C8 Deprotonation (Relative Free Energies in kcal/mol)



with the negatively charged carbonate moiety. In the reactants, the negative charge is delocalized in the carboxylate group, leading to more favorable solvation of the CO_2 group. As the CO_2 group leaves in the transition state, the charge begins to localize on the remaining C8-bound oxygen. Solvation of this bridging oxygen stabilizes the TS due to the shift in charge density. When the explicit waters are around the CO_2 group, the N1 protonated structure, **30**, is 2.5 kcal/mol lower than the N7 protonated structure, **33**. Shifting the water orientation in **30** to solvate the C8-bound oxygen was calculated to be 4 kcal/mol higher. Solvation of the C8 bound oxygen in **33** was calculated to be 1.5 kcal/mol higher in energy. The N1,N7 protonated cation, **32**, is 1 kcal/mol higher than **30** at pH 7 and 6 kcal/mol higher at pH 10. While these effects may seem trifling, the difference in the barriers is quite substantial: the decarboxylation barrier for the N7 protonated structure, **33**, is about 10 kcal/mol lower than for the N1 protonated form, **30**. Specifically, the barriers for decarboxylation are 13.3, 21.8, and 11.7 for **32**, **30**, and **33**, respectively. Because **33** was calculated to have the lowest barrier for decarboxylation at pH 10, the lowest energy pathway for decarboxylation proceeds from **30** to **33** to the decarboxylation TS, with an overall barrier of 15.9 kcal/mol. At pH 2.5 and 7, the lowest energy pathways for

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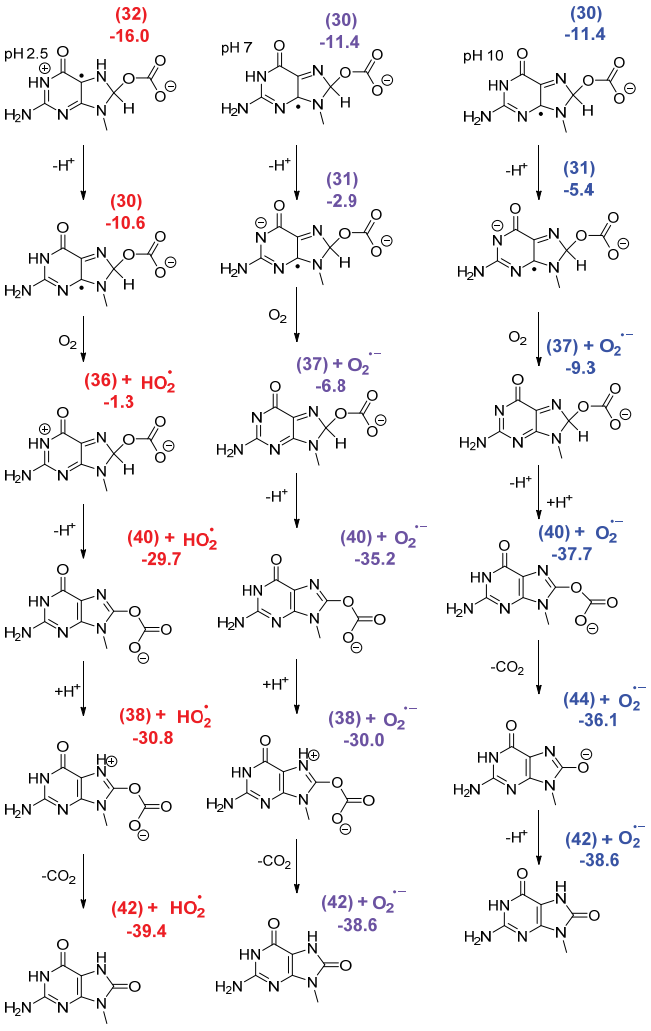
decarboxylation have overall barriers of 13.3 and 14.2 kcal/mol, respectively. Decarboxylation is followed by protonation to the C8 alcohol, leading to **46** at pH 2.5, and **47** subsequently tautomerizing to **48** at pH 7. At pH 10, decarboxylation from **33** is followed by N1 protonation to the zwitterion rather than alcohol protonation, which is favored by 2.5 kcal/mol.

Scheme 9 shows the pathway for the formation of 8oxoG (**42**) from the bicarbonate/carbonate dianion addition

Scheme 9. Formation of 8oxoG by Oxidation, C8 Deprotonation and Subsequent Decarboxylation of the Carbonate Addition Intermediate at Various pH's (Relative Free Energies in kcal/mol)



intermediate starting with removal of the C8 hydrogen. Similar to the thymine addition pathway, the calculations confirm that C8 hydrogen loss occurs by $^3O_2$ oxidation followed by C8 deprotonation rather than by hydrogen atom abstraction or C8−H deprotonation followed by oxidation. At pH 2.5, oxidation of the cationic intermediate **32** was calculated to be high in energy (E_0 = 0.79 V for **32**, ΔG = +22.0 kcal/mol for $^3O_2$). Deprotonation of **32** to **30** is uphill by 5.4 kcal/mol, but oxidation of **30** is 12.8 kcal/mol lower than **32** (E_0 = 0.23 V for **30**, ΔG = +9.2 kcal/mol for $^3O_2$). At pH 7 and 10, deprotonation of N1 from **30** to **31** leads to favorable oxidation, with an overall energy for oxidation of 4.5 and 2.1 kcal/mol, respectively.

Structures **36** and **37** are obtained by oxidation of the intermediates for bicarbonate/carbonate dianion addition to guanine radical, or directly from carbonate radical addition to guanine radical. Deprotonation of C8 of **36** and **37** is very facile and strongly exothermic (C8 pK_a = −16.7 for **36**, ΔG = −19.7, −24.4, and −26.2 kcal/mol for pH 2.5, 7, and 10, respectively), yielding structures **38** and **40** after proton equilibration. Decarboxylation of these structures has a low barrier (1.3, 6.4, and 8.2 kcal/mol at pH 2.5, 7, and 10, respectively) and produces the final product, 8oxoG, **42**.

For bicarbonate/carbonate dianion addition, the initial step could be decarboxylation (Scheme 8) or oxidation and deprotonation (Scheme 9). In both cases, the barriers for the initial step are higher than the subsequent steps in the conversion to 8oxoG. If decarboxylation occurs before oxidation and C8 deprotonation, the first step is nearly thermoneutral (2.7 to −3.4 kcal/mol) and the second step has barrier of only 7.2, 5.6, and 5.3 kcal/mol at pH 2.5, 7, and 10, respectively. If oxidation and C8 deprotonation occur before decarboxylation, the first step is strongly exothermic (−32.9 to −20.2 kcal/mol) and the second step has barriers of 1.3, 6.5, and 8.2 kcal/mol at pH 2.5, 7, and 10, respectively. Thus, for bicarbonate/carbonate dianion addition, decarboxylation → oxidation → C8 deprotonation (Scheme 8) is slightly favored at pH 2.5, and oxidation → C8 deprotonation → decarboxylation (Scheme 9) is strongly preferred at pH 7 and 10.

Figure 4 summarizes the lowest energy pathways for bicarbonate/carbonate dianion addition at pH 2.5, 7, and 10. Oxidation by carbonate radical is the initial step for all pH's. After proton equilibration, this results in guanine radical and bicarbonate at pH 7 and 10. At pH 2.5, bicarbonate adds to guanine radical cation directly with a 11.9 kcal/mol barrier. At pH 7 and 10, carbonate dianion adds to guanine cation radical with a barrier of 15.3 kcal/mol in both cases. The lowest energy addition intermediate was found to be **32** at pH 2.5 and **30** at pH 7 and 10, with relative energies of −16.0, −11.4, and −11.4, respectively. Oxidation of **32** at pH 2.5 occurs more readily after deprotonation of N7 and is overall uphill by 14.7 kcal/mol. Decarboxylation is slightly lower in energy than oxidation at pH 2.5, with a barrier of 13.3 kcal/mol. At pH 7 and 10, deprotonation on N1 of **30** leads to facile oxidation of the anion. At pH 2.5, decarboxylation leads to **46**, which has an overall barrier of 8.2 kcal/mol for deprotonation and oxidation to form **52**. Deprotonation of the C8 is downhill by more than 25 kcal/mol at all pH's. After C8 deprotonation, the barriers for decarboxylation are low (1.3, 6.4, and 8.2 kcal/mol at pH 2.5, 7, and 10 respectively).

At low pH, carbonate addition may be prevented by the formation of carbonic acid, and its subsequent decomposition. Alternatively, 8oxoG can also be formed by the addition of water to guanine radical. Water addition to the neutral guanine radical is calculated to have a relatively high barrier, 24.6 kcal/mol,[55] but a significantly lower barrier of 12.3 kcal/mol for addition to guanine radical cation.[55] This is likely the dominant pathway to produce 8oxoG at low pH.

3.6. Comparison with Experimental Studies. A number of papers have reported experimental investigations of the oxidation of guanine by carbonate radicals.[13−22] Carbonate radicals are typically produced by oxidation of carbonate/bicarbonate ions by sulfate radicals. Sulfate radicals are usually

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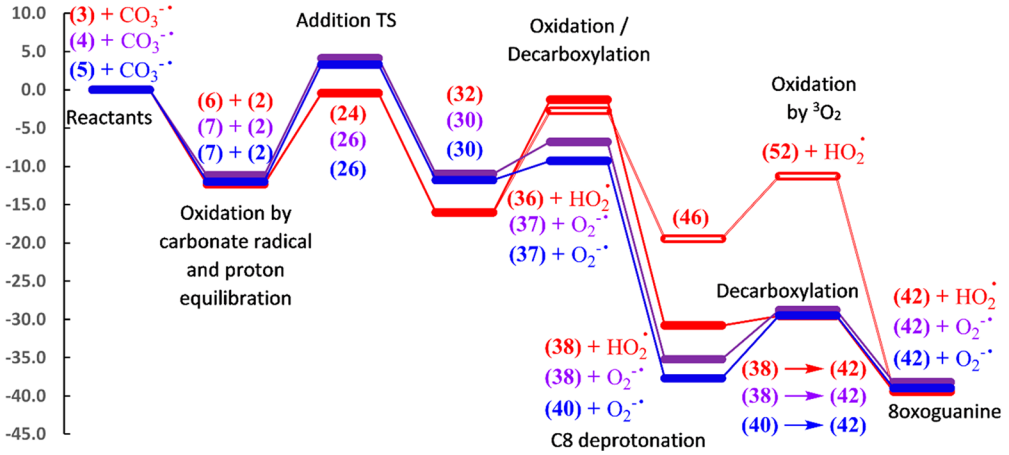

Figure 4. Gibbs free energies for carbonate addition to guanine radical followed by oxidation C8 deprotonation and decarboxylation at various pH's. Acidic pH 2.5 (red, closed) is modeled using fluoroacetic acid, neutral pH 7 (purple) is modeled using imidazole, and basic pH 10 (blue) is modeled using guanine anion for the purpose of calculating energies associated with protonation and deprotonation from the solvent. Decarboxylation has a low barrier at pH 2.5, and decarboxylation followed by oxidation (red, open) is competitive with oxidation followed by decarboxylation (red, solid). Free energies were calculated using ωB97XD/6-31+G(d,p) and are given in kcal/mol.

generated by photolysis of persulfate with laser pulses or continuous illumination by a xenon arc lamp. The recent study by Rokhlenko, Geacintov, and Shafirovich[13] provides a detailed investigation of the kinetics of the formation of the guanine oxidation products and guanine thymine cross-links. Sulfate radicals were produced by single nanosecond laser pulses and reacted rapidly with the bicarbonate/carbonate buffer to produce carbonate radicals, which in turn reacted with guanine to produce guanine radicals. Since the concentration of bicarbonate in these studies was rather high (to facilitate the formation of carbonate radical), guanine radical could react with bicarbonate or carbonate dianion to form a carbonate addition product leading to 8oxoG. However, the calculated barrier for addition of bicarbonate or carbonate dianion to guanine radical or radical cation is rather high (15−19 kcal/mol), so these reactions would be much slower than the observed rate of formation of 8oxoG and further oxidation products. Sulfate radical also rapidly oxidizes guanine, and under the conditions used by Rokhlenko et al., produced initial concentrations of ca. 1.25 μM guanine radical as well as 3.60 μM carbonate radical. Despite the low concentrations of the two radicals, the addition of carbonate radical to guanine radical would occur more rapidly than the addition of bicarbonate, because of the much higher rate constant for the radical−radical reaction (ca. 10^8 M^{-1} s^{-1}). However, it should be noted that under biological conditions, oxidation of guanine by a reactive oxygen species (ROS) may be an isolated event, and the reaction of guanine radical with a second radical may have a low probability. In this case, reactions of guanine radical with other nucleophiles such as bicarbonate may predominate.

For lower pH values, a more suitable method for generating carbonate radicals is by photolysis of $[Co(NH_3)_4CO_3]^+$. With continuous illumination by a xenon arc lamp, sufficient amounts of carbonate radical should be generated so that the guanine radical−carbonate radical would still be the dominant pathway. For pH 2.5, sulfate radicals can be used to oxidize guanine yielding guanine radical cation. The calculated barrier for the addition of water to guanine radical cation is much lower than the barrier for addition to neutral guanine radical (12.3 vs 24.6 kcal/mol,[55] respectively). Further oxidation of the water addition product leads to 8oxoguanine.

Near neutral pH, the yield of the guanine-thymine cross-link product is comparable to the yield of guanine oxidation products. This might give the impression that the barriers for the two processes are similar. However, the formation of a guanine-thymine cross-link within an oligonucleotide is a unimolecular reaction with a small rate constant (15 s^{-1}) while carbonate radical addition to guanine radical is a bimolecular reaction with a large rate constant (ca. 10^8 M^{-1} s^{-1}) but with low concentration of the reactants (ca. 10^{-6}).[13] The calculated barrier for thymine addition (16.8−20.4 kcal/mol) is in accord with the small rate constant measured for this reaction. The yield of guanine-thymine cross-links is observed to increase with increasing pH, indicating that thymine must be deprotonated before it can add to guanine radical. The calculations do not yield a stable product for the addition of protonated thymine to guanine radical but find a barrier of 16.8 kcal/mol and stable intermediate for thymine anion adding to neutral guanine radical. Since the pK_a of thymine is 9.7, changing the pH from 7.5 to 8.5 should increase the amount of thymine anion by a factor of 10, but experiments show only a factor of 3 increase in the yield of the guanine-thymine cross-link. The calculations reveal that this is due to a switch in the preferred pathway. With the free energy required for protonation/deprotonation of guanine radical (pK_a = 3.9) and thymine (pK_a = 9.7) taken into account, the lowest free energy pathway at pH 7 is thymine anion adding to guanine radical cation, but at pH 10 the lowest energy pathway is thymine anion adding to neutral guanine radical.

The present calculations model the formation of guanine-thymine cross-links starting from free thymine in solution. The experimental studies find that formation of cross-links between guanine and thymine in oligonucleotides is optimal when they are separated by a single cytosine.[19] The yield diminishes rapidly if they are adjacent or separated by more than one base. This suggests that separation by one base results in a favorable orientation between the guanine and thymine, thereby lowering the free energy barrier. Starting the calculations for thymine addition from the reactant complex between thymine and guanine radical would approximate a favorable arrange-

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ment for cross-link formation in an oligonucleotide. This results in free energy barriers that are 2−3 kcal/mol lower than for addition of free thymine. The molecular dynamics calculations by Ding et al.[83] show that the cross-link products stack favorably with the other bases in double-stranded DNA, but the interactions were not as strong as without cross-links. They found the distortions and destabilizing effects were greater when the cross-linked guanine and thymine were separated by a cytosine.

In the study by Rokhlenko et al.,[13] the yield of guanine-thymine cross-link increased steadily from pH 7 to 9. If the data is extrapolated, the yield of the cross-link product increases by a factor of 6 on going from pH 7.5 to pH 10, while the yield of the oxidized product (8oxoG) remains steady. Earlier work by Crean et al.[21] found a similar change in the yield of the cross-link product, increasing by a factor of 4.5 on going from pH 7.5 to pH 10. They saw similar amounts of oxidized products at pH 7.5 (in this case Sp and Gh) but did not detect any oxidized products at pH 10. This may be due to the different conditions used to generate the radicals: single nanosecond laser pulse in the study by Rokhlenko et al.[13] compared to continuous illumination by a xenon arc lamp in the study by Crean et al.[21]

4. CONCLUSIONS

When oligonucleotides are oxidized by carbonate radical, thymine and carbonate addition to guanine are competitive. Experimental studies find that thymine addition is favored at high pH, while the formation of oxidation products 8oxoG, Sp, and Gh is not diminished by pH.[13,21] In the present computational study, we have used density functional methods to explore the mechanisms for these reactions. The pH dependence of the reactions has been treated by using appropriate explicit proton donors/acceptors as computational models for buffers when calculating the free energies of the compounds in their various states of protonation/deprotonation. In addition to accounting for different tautomers and protonation states of reactants, intermediates, and products, we needed to consider that transitions structures may have protonation/deprotonation states that differ from their reactants and products at equillibrium. For example, thymine is neutral at pH 2.5 and 7, but must be deprotonated before it can add to guanine radical.

Anions in aqueous solution are challenging for current implicit solvation models. To overcome these difficulties, we have used 3−7 explicit water molecules in combination with the SMD implicit solvation model. For oxidation of guanine by carbonate radical anion, electron transfer/oxidation occurs before addition when six explicit waters are used. Another key step in the mechanism is loss of the C8 hydrogen. Oxidation by O_2 followed by deprotonation is the lowest energy path, but requires four explicit waters placed around oxygen to stabilize the $O_2^{-\cdot}$ product. Decarboxylation is also difficult to model, because the CO_2 group in R−O−CO_2^- is strongly solvated in the reactants, but the R−O moiety needs to be solvated in the TS and R−O^- product.

For the formation of guanine-thymine cross-links, the present calculations show that the addition of thymine anion to guanine radical is the rate-determining step. Loss of the C8 hydrogen occurs by O_2 oxidation followed by spontaneous C8 deprotonation to form the final guanine-thymine cross-link product. Cross-link formation is in competition with guanine oxidation to form 8oxoG. For the conditions used by Shafirovich and co-workers, the calculations confirm that the dominant pathway at pH 7 and 10 is the addition of carbonate radical to guanine radical followed by deprotonation at C8. If the concentration of carbonate radical is too low for the radical−radical pathway to be viable, the formation of 8oxoG at pH 7 and 10 can proceed by the addition of bicarbonate/carbonate dianion to guanine radical. This addition step is followed by oxidation, loss of the C8 proton and decarboxylation, yielding 8oxoG. At pH 2.5, oxidation of guanine by carbonate radical is not practical, but guanine radical cation can be formed by oxidation with sulfate radical. Addition of water to guanine radical cation results in 8oxoG after a second oxidation and C8 deprotonation.

The present work considers guanine and thymine as free nucleobases. This is directly relevant to oxidative damage by carbonate radical to single stranded DNA, where the bases are readily accessible. In double-stranded DNA, the reaction energies and barriers will be different because the bases are less accessible and their motions are more constrained. Carbonate oxidation of guanine also yields other products, such as 2Ih.[13] Pathways for the formations of these products will be examined in a separate study.[86]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.8b00302.

> Optimized molecular geometries in Cartesian coordinates and free energies (in hartree) for all the reactants, intermediates, transition states, and products in PDF format. Calculated pK_a and redox potential values for relevant intermediates and numbering for structures considered in this study. (PDF)

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Notes

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■ ABBREVIATIONS

8oxoG,8-oxo-7,8-dihydroguanine; Sp,spiroiminodihydantoin; Gh,guanidinohydantoin; G∧T,guanine-thymine cross-link product; 8nitroG,8-nitroguanine; NIm,5-guanidino-4-nitroimidazole; FapyG,2,6-diamino-4-hydroxy-5-formamidopyrimidine

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