

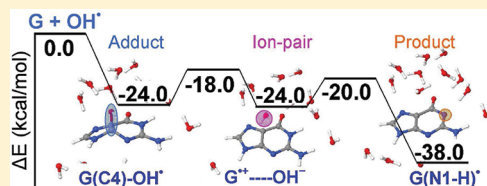
# Hydroxyl Radical ( $\text{OH}^\bullet$ ) Reaction with Guanine in an Aqueous Environment: A DFT Study

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Supporting Information

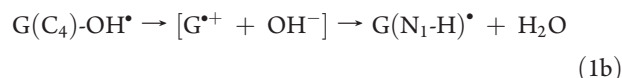
**ABSTRACT:** The reaction of hydroxyl radical ( $\text{OH}^\bullet$ ) with DNA accounts for about half of radiation-induced DNA damage in living systems. Previous literature reports point out that the reaction of  $\text{OH}^\bullet$  with DNA proceeds mainly through the addition of  $\text{OH}^\bullet$  to the  $\text{C}=\text{C}$  bonds of the DNA bases. However, recently it has been reported that the principal reaction of  $\text{OH}^\bullet$  with dGuo (deoxyguanosine) is the direct hydrogen atom abstraction from its exocyclic amine group rather than addition of  $\text{OH}^\bullet$  to the  $\text{C}=\text{C}$  bonds. In the present work, these two reaction pathways of  $\text{OH}^\bullet$  attack on guanine (G) in the presence of water molecules (aqueous environment) are investigated using the density functional theory (DFT) B3LYP method with 6-31G\* and 6-31++G\*\* basis sets. The calculations show that the initial addition of the  $\text{OH}^\bullet$  at  $\text{C}_4=\text{C}_5$  double bond of guanine is barrier free and the adduct radical ( $\text{G}-\text{OH}^\bullet$ ) has only a small activation barrier of ca. 1–6 kcal/mol leading to the formation of a metastable ion-pair intermediate ( $\text{G}^{++}\cdots\text{OH}^-$ ). The formation of ion-pair is a result of the highly oxidizing nature of the  $\text{OH}^\bullet$  in aqueous media. The resulting ion-pair ( $\text{G}^{++}\cdots\text{OH}^-$ ) deprotonates to form  $\text{H}_2\text{O}$  and neutral G radicals favoring  $\text{G}(\text{N}_1-\text{H})^\bullet$  with an activation barrier of ca. 5 kcal/mol. The overall process from the  $\text{G}(\text{C}_4)-\text{OH}^\bullet$  (adduct) to  $\text{G}(\text{N}_1-\text{H})^\bullet$  and water is found to be exothermic in nature by more than 13 kcal/mol. ( $\text{G}-\text{OH}^\bullet$ ), ( $\text{G}^{++}\cdots\text{OH}^-$ ), and  $\text{G}(\text{N}_1-\text{H})^\bullet$  were further characterized by the CAM-B3LYP calculations of their UV–vis spectra and good agreement between theory and experiment is achieved. Our calculations for the direct hydrogen abstraction pathway from  $\text{N}_1$  and  $\text{N}_2$  sites of guanine by the  $\text{OH}^\bullet$  show that this is also a competitive route to produce  $\text{G}(\text{N}_2-\text{H})^\bullet$ ,  $\text{G}(\text{N}_1-\text{H})^\bullet$  and  $\text{H}_2\text{O}$ .



## INTRODUCTION

Exposure of living systems to ionizing radiation generates damage by the ionization of DNA (direct effect), ionization of the first hydration shell that transfers damage to DNA (quasi direct effect) and by ionization of water (indirect effect) which produces  $\text{OH}^\bullet$ ,  $\text{H}^\bullet$  and aqueous electron ( $e^-_{\text{aq}}$ ).<sup>1,2</sup> Of the water radical species, it is the reaction of  $\text{OH}^\bullet$  with DNA has been found to be the most damaging as it accounts for over half of radiation damage to cellular systems by low LET (linear energy transfer) radiation.<sup>1,2</sup> For this reason, the reactions of  $\text{OH}^\bullet$  with DNA components including DNA bases, nucleosides and nucleotides have been extensively studied with a variety of experimental techniques.<sup>3–8</sup> O'Neill and co-workers<sup>8</sup> studied the reaction of  $\text{OH}^\bullet$  with guanine and adenine in Guo and in Ado using pulse radiolysis and the reaction of  $\text{OH}^\bullet$  was reported to be addition reactions at  $\text{C}_4$ ,  $\text{C}_5$ , and  $\text{C}_8$  atoms of adenine and guanine.<sup>3–5,8</sup> Later, using pulse radiolysis with optical and conductance detection the reaction of  $\text{OH}^\bullet$  with guanine and adenine were extensively explored by Steenken, Vieira, and Candeias.<sup>5,9</sup> They reported that addition of  $\text{OH}^\bullet$  at  $\text{C}_4$  or  $\text{C}_5$  in adenine or guanine leads to neutral radical formation ( $(\text{A}-\text{H})^\bullet$  and  $\text{G}(\text{N}_1-\text{H})^\bullet$ ) through dehydration reaction while addition at  $\text{C}_8$  results in the formation of a highly reducing radical, i.e., 8-hydroxy-7,8-dihydroguanine-7-yl-radical, that on further oxidation leads to 8-oxo-G or on reduction to Fapy-G.<sup>3,4</sup> They, also, proposed that in monomers, the addition reaction of  $\text{OH}^\bullet$  with guanine at its  $\text{C}_4=\text{C}_5$  dominates ( $\sim 60$ – $70\%$ ) over addition to

its  $\text{C}_8$  ( $\sim 17\%$ ).<sup>9g</sup> We note here that this relatively small extent of addition of  $\text{OH}^\bullet$  to the  $\text{C}_8$  of guanine in monomers has been further supported by the small yield ( $\sim 10\%$ ) of  $\text{OH}^\bullet$  induced formation of 8-oxo-G and Fapy-G in  $\gamma$ -irradiated  $\text{N}_2\text{O}$ -saturated solution of dGuo.<sup>4b</sup> They further proposed that the addition of  $\text{OH}^\bullet$  to either  $\text{C}_4$  or  $\text{C}_5$  atoms of guanine ( $\text{G}-\text{OH}^\bullet$ ) lead to the radical cation and hydroxyl anion ( $\text{G}^{++} + \text{OH}^-$ ) and/or the formation of the neutral deprotonated guanine radical ( $\text{G}(\text{N}_1-\text{H})^\bullet$ ) and water (see scheme 3 in ref 9g), as given in eqs 1a, 1b



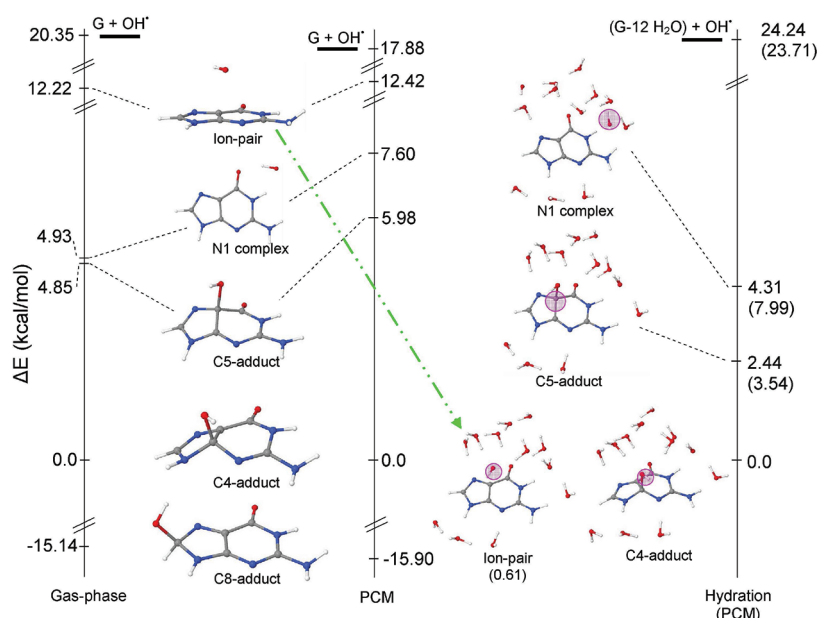
Using theory, addition reactions of  $\text{OH}^\bullet$  with guanine and adenine were reported by Colson and Sevilla,<sup>10a–c</sup> Eriksson and co-workers,<sup>10d,e</sup> Mishra and co-workers,<sup>10f–h</sup> Naumov and Sonntag,<sup>10i</sup> and Mundy and co-workers.<sup>13</sup> Very recently, the hydrogen abstraction reaction by  $\text{OH}^\bullet$  from sugar moiety in hydrated DNA was studied using molecular dynamics simulation.<sup>14</sup>

Recently, using pulse radiolysis with optical detection, the reaction of  $\text{OH}^\bullet$  with guanine has been reevaluated by Chatgililoglu and co-workers.<sup>11</sup> It was proposed that the main reaction of  $\text{OH}^\bullet$

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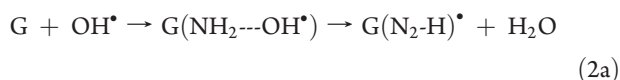
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**Figure 1.** Relative stabilities of  $C_4$ ,  $C_5$ , and  $C_8$  adducts and  $N_1$ -H complex formed on the interaction of guanine and OH radical in gas phase (column 1), PCM model (column 2) and in the presence of 12 explicit water molecules and in PCM (single point calculation) (column 3). B3LYP/6-31G\* method was used for the calculation. Pink circle shows the location of OH in the hydrated guanine.

with guanine is the formation of  $G(N_2-H)^{\bullet}$  via direct hydrogen abstraction from the exocyclic  $NH_2$  group of guanine moiety by  $OH^{\bullet}$  which after tautomerization forms  $G(N_1-H)^{\bullet}$  as shown in eqs 2a, 2b



In keeping with Chatgililoglu's interpretation that H-atom abstraction processes dominate and using the B3LYP/DZP++ level of theory, Cheng et al.<sup>12</sup> recently studied the  $OH^{\bullet}$ -induced hydrogen atom abstraction reaction from the different sites of adenine. Direct hydrogen atom abstraction reaction from guanine's exocyclic  $NH_2$  group by  $OH^{\bullet}$  in the gas-phase using Car–Parinello molecular dynamics (MD) was also reported by Mundy et al.<sup>13a</sup> However, in a later study from this group, the dehydrogenation reaction from four sites ( $N_1$ ,  $N_2$ ,  $N_9$ , and  $C_8$ ) of guanine by  $OH^{\bullet}$  was carried out in an aqueous phase of many explicit water molecules.<sup>13b</sup> For the reaction of  $OH^{\bullet}$  at  $NH_2$  of guanine, they suggested a possible transient ion-pair ( $G^{\bullet+} \cdots OH^-$ ) intermediate that subsequently underwent deprotonation from the  $NH_2$  group of transiently formed guanine radical cation within a few picoseconds.<sup>13b</sup> It is interesting to note that Steenken also suggested a polar ion-pair type transition state for the dehydration of the  $C_4$ -OH adduct of adenine.<sup>5</sup> In this work, we perform DFT calculations which also predict such a metastable ion-pair formation after  $OH^{\bullet}$  addition to the  $C_4=C_5$  double bond in the guanine ring (eq 1a).

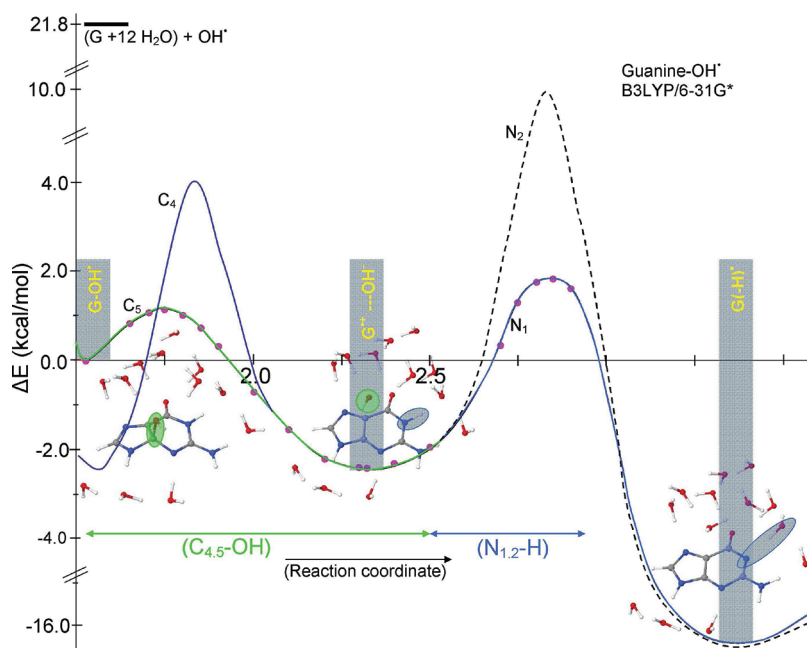
From the review of the literature described above, the mechanism of action of OH radical with DNA, which has been a focus of study for 30 years, has become somewhat controversial. The fundamental importance of the reactions of aqueous OH radical with DNA in living systems suggests that this issue needs careful consideration by theory. The earlier studies employing theory; treated

systems in the gas-phase or in aqueous medium through the use of the solvation models that did not include discrete water molecules and none considered the full reaction pathway of addition/elimination vs abstraction. In the present study, the reaction of  $OH^{\bullet}$  with guanine is modeled in the presence of explicit water molecules and both addition/elimination and hydrogen abstraction reactions by  $OH^{\bullet}$  as given in eqs (1) and (2) are considered. Our results show that both pathways, addition/elimination, and abstraction reactions of  $OH^{\bullet}$ , are likely with the relative yields from each pathway dependent on kinetic considerations.

## CALCULATIONS

The interaction of OH radical with guanine at different sites has been considered in the gas phase and in the full solvation through the use of IEF-PCM model (integral equation formalism-polarized continuum model) using the B3LYP/6-31G\* method. Our calculations show that the reaction of  $OH^{\bullet}$  with guanine depends on the mode of approach of  $OH^{\bullet}$  with guanine. Interaction of  $OH^{\bullet}$  above the plane of the guanine ring at  $C_4=C_5$  bond shows the possibility of ion-pair formation in addition to  $C_4$  and  $C_5$  adducts formation, see the Results and Discussion section. The relative stabilities of the different complexes formed due to OH radical interaction with guanine are shown in Figure 1. This preliminary result sets the stage to explore the reaction of  $OH^{\bullet}$  with guanine in the presence of explicit water molecules.

The addition reaction of  $OH^{\bullet}$  at  $C_4=C_5$  double bond with guanine solvated by twelve explicit water molecules is explored using the B3LYP method and 6-31G\* and 6-31++G\*\* basis sets. The structures of the adduct formation ( $G-OH^{\bullet} + 12H_2O$ ) (reactant), ion-pair ( $G^{\bullet+} \cdots OH^- + 12H_2O$ ) (intermediate) and the radicals ( $G(N_1-H)^{\bullet} + 13H_2O$  and ( $G(N_2-H)^{\bullet} + 13H_2O$ )) (product) were fully optimized using the B3LYP method and 6-31G\* and 6-31++G\*\* basis sets. The reaction profile of the formation of neutral radical ( $G(N_1-H)^{\bullet}$  or  $G(N_2-H)^{\bullet}$ ) from the adduct ( $G-OH^{\bullet} + 12H_2O$ ) proceeds on two surfaces which



**Figure 2.** B3LYP/6-31G\* calculated potential energy surfaces (PES) for the reaction of OH radical with hydrated guanine ( $G + 12H_2O$ ). Reaction proceeds on each surface are depicted as  $C_4$ ,  $C_5$ ,  $N_1$ , and  $N_2$ .  $G-OH^*$  =  $C_4$ ,  $C_5$  adduct;  $G^+ \cdots OH^-$  = ion-pair;  $G(-H)^* = G(N_1-H)^*$  or  $G(N_2-H)^*$ . For clarity only the structures of  $G(C_4-OH)^*$  adduct and  $G(N_1-H)^*$  are shown. The optimized structures of  $G(C_5-OH)^*$  adduct and  $G(N_2-H)^*$  are given in the Supporting Information.

are investigated as follows: (i) The potential energy surface (PES) of the formation of ion-pair from the adduct ( $G-OH^* + 12H_2O$ ) is calculated by stretching the  $C_4-OH$  and  $C_5-OH$  bonds from its equilibrium bond lengths up to 2.5 Å, and (ii) the PES for the radical formation ( $G(N_1-H)^*$  or  $G(N_2-H)^*$ ) is calculated by stretching the  $N_1-H$  and  $N_2-H$  bonds of guanine from the ion-pair. In steps (i) and (ii) the bond dissociation was carried out in the step size of 0.05 Å and at each chosen point on the PES the structures were fully optimized by constraining only the corresponding bond length, see Figure 2. The maxima or the highest points on the PES (Figure 2), which connect the reactant and product or intermediate, are estimates of the barrier heights; however, frequency calculations were not performed to verify a true transition states as this is not practical for such large systems. Relative energy ( $\Delta E$  in kcal/mol) involved in the reaction along with the optimized structures of reactant (adduct), intermediate (ion-pair) and product (radical) is shown in Figure 2. The direct hydrogen abstraction reaction (eq (2)) was also considered by placing the OH radical in the plane of the guanine ring near the  $N_1-H$  and  $N_2-H$  sites in the presence of twelve water molecules and PES for hydrogen abstraction from both the sites ( $N_1$  and  $N_2$ ) was calculated, as described above, using the B3LYP/6-31G\* method. In the text,  $N_1$  refers to the N1 atom of guanine.  $N_2$  and  $NH_2$  refer to the exocyclic amine nitrogen and amine group of guanine

The B3LYP method is widely used in several studies and found to be quite accurate in producing ground state molecular properties of DNA bases and base pairs in their neutral and radical ion states.<sup>10e-j,12,13</sup> Considering the optimized hydrated structures of adduct, ion-pair and radical, single point energy calculations were done to take into account the effect of full solvation through the use of IEF-PCM model, see Table 1. Our calculations show that the B3LYP/6-31G\* and B3LYP/6-31++G\*\* give similar

results, see Table 1, thus in the text, we discuss our results derived from B3LYP/6-31G\* calculations unless otherwise stated.

The presence of adduct, ion-pair and radical during the reaction were also characterized by their calculated vertical excited state UV-vis spectra using the time-dependent (TD) CAM-B3LYP method incorporating the effect of full solvation through the IEF-PCM model using the B3LYP optimized geometries. We used TD-CAM-B3LYP-PCM method for excited state calculations because the B3LYP method is not appropriate for ion-pair or species involving charge transfer excited states.<sup>15</sup> The transition energies for charge transfer states are greatly underestimated by the B3LYP method especially as the distance increases.<sup>15,16</sup> The CAM-B3LYP method is a long-range corrected hybrid functional and found to be very successful to study the excited states of systems involving charge transfer states.<sup>16</sup>

These calculations were done using Gaussian 09 suite of programs.<sup>17</sup> GaussView<sup>18a</sup> molecular modeling software was used to plot the molecular orbitals and draw the molecular structure. The Gabedit molecular modeling package was used to plot UV-vis spectra.<sup>18b</sup>

## RESULTS AND DISCUSSION

**Guanine and  $OH^*$  Interaction and Ion-Pair Formation.** *Adduct Formation.* To get information about the relative stabilities of binding of OH radical with guanine at different sites, we added OH radical at  $C_4$ ,  $C_5$ , and  $C_8$  atoms of guanine and placed OH radical near the  $N_1-H$  site in the plane of the guanine ring. The geometries thus generated were fully optimized in the gas phase as well as in the solution phase using the IEF-PCM model at the B3LYP/6-31G\* level of theory. From the calculations, we found that  $C_8$  adduct is the most stable and it is stabilized by more than 34 kcal/mol relative to the reactant ( $G + OH^*$ ) in the gas phase and in solution (PCM). The gas phase and solution



**Table 1.** Relative Reaction Energies ( $\Delta E$ ) in kcal/mol for OH Radical Addition/Elimination Processes with Guanine Solvated with 12 Water Molecules<sup>a</sup>

species <sup>b</sup>	$\Delta E$ (kcal/mol)					
	B3LYP					
	dist. (Å)		6-31G*		6-31++G**	
	6-31G*	6-31++G**	gas phase	PCM	gas phase	PCM
(G + 12H <sub>2</sub> O) + OH <sup>•</sup>			21.8	20.17	24.38 <sup>g</sup>	21.11 <sup>g</sup>
G(C <sub>5</sub> )–OH <sup>•</sup>	1.52	1.48 <sup>c</sup>	0.0	0.0 <sup>d</sup>	0.0 <sup>c</sup>	0.0 <sup>f</sup>
Barrier Height <sup>e</sup>	1.75 (G---OH)	1.75 <sup>c</sup>	1.12		2.38 <sup>c</sup>	
G <sup>•+</sup> ---OH <sup>−</sup>	2.32	2.39 <sup>c</sup>	−2.43	−2.94 <sup>d</sup>	−2.11 <sup>c</sup>	−2.70 <sup>f</sup>
Barrier Height <sup>e</sup>	1.35 (N <sub>1</sub> ---H)	1.30 <sup>c</sup>	1.85		2.44 <sup>c</sup>	
G(C <sub>4</sub> )–OH <sup>•</sup>	1.48		−2.44	−3.55 <sup>d</sup>	−4.04 <sup>g</sup>	−4.90 <sup>g</sup>
G(N <sub>1</sub> –H) <sup>•</sup>			−16.40	−18.43 <sup>d</sup>	−19.38 <sup>g</sup> (−18.09) <sup>c</sup>	−21.42 <sup>g</sup> (−19.90) <sup>f</sup>
G(N <sub>2</sub> –H) <sup>•</sup>			−16.51	−16.42 <sup>d</sup>	−20.22 <sup>g</sup>	−20.13 <sup>g</sup>

<sup>a</sup> Calculations were done using the B3LYP method and 6-31G\* and 6-31++G\*\* basis sets. <sup>b</sup> See Figures S2 and S3 of the SI. <sup>c</sup> Values obtained from B3LYP/6-31++G\*\* optimization. <sup>d</sup> Values obtained from single point calculations using PCM model. <sup>e</sup> Highest point on the PES. <sup>f</sup> Geometries optimized at B3LYP/6-31++G\*\*. <sup>g</sup> Values obtained from single point calculations using PCM model. <sup>h</sup> Single point calculations using B3LYP/6-31G\* optimized geometries.

phase (PCM) calculations gave almost similar stabilization energies and has a maximum difference of ca. 2.5 kcal/mol, see Figure 1. The relative stabilities of C<sub>4</sub>, C<sub>5</sub> (adducts) and N<sub>1</sub>–H---OH<sup>•</sup> (complex) in solution using PCM are −17.9, −11.9, and −10.3 kcal/mol, respectively, with respect to the separate G + OH<sup>•</sup> (reactant), see Figure 1. The addition of OH<sup>•</sup> at C<sub>2</sub>, C<sub>4</sub>, C<sub>5</sub>, and C<sub>8</sub> atoms of guanine and 8-oxoguanine has been studied by Jena and Mishra<sup>10f,g</sup> using the B3LYP method and found the relative stability of the OH<sup>•</sup> adducts formation with guanine in the order C<sub>8</sub> > C<sub>2</sub> > C<sub>4</sub> > C<sub>5</sub>.

**Ion-Pair Formation.** Apart from the adduct formation, we also found a new structure which is an ion-pair complex, (G<sup>•+</sup>---OH<sup>−</sup>), formed after OH radical attack on guanine. In the gas phase or in solution using the PCM model only, the ion-pair is found to be more stable than the separate G + OH<sup>•</sup> but less than the OH adducts, see Figure 1. In this ion-pair complex, the OH is situated above the C<sub>4</sub>=C<sub>5</sub> bond at a distance of 2.74 Å and 2.37 Å from C<sub>4</sub> and C<sub>5</sub> atoms of guanine in solution (PCM) and 2.70 Å and 2.38 Å in gas phase, respectively. To make sure that it is not the transition state structure to form either C<sub>4</sub> or C<sub>5</sub> adduct, we calculated the vibrational frequencies of this complex using the B3LYP/6-31G\* method in the gas phase and in solution (PCM) and found all the frequencies to be positive, which confirms the formation of metastable complex between guanine and OH radical. To understand the nature of this complex, we plotted the spin density distribution of this complex, shown in Figure S1 of the Supporting Information, SI. From Figure S1 of the SI, we found that the unpaired spin is largely localized on the guanine, which characterizes this complex as G<sup>•+</sup>---OH<sup>−</sup> (ion-pair). From stability point of view, this ion-pair lies ca. 12 kcal/mol above the C<sub>4</sub> adduct and 6 kcal/mol above the C<sub>5</sub> adduct, see Figure 1.

The higher energy for the complex (ion-pair; G<sup>•+</sup>---OH<sup>−</sup>) compared to the adducts raised an important question, as to whether this ion-pair becomes more stable in the presence of explicit water molecules. To answer this question, we optimized the complex structure in the presence of 12 water molecules at the B3LYP/6-31G\* level of theory and calculated the relative stabilities of the formation of ion-pair, C<sub>4</sub> and C<sub>5</sub> adducts and in

plane N<sub>1</sub>---OH complex. The B3LYP/6-31G\* calculation shows that ion-pair is stabilized by more than 2 kcal/mol with respect to the C<sub>5</sub> adduct and it is isoenergetic to C<sub>4</sub> adduct, see Figure 1. In the presence of 16 water molecules, ion-pair is found to be more stable than C<sub>4</sub> or C<sub>5</sub> adducts by 1.4 and 5 kcal/mol, respectively, see Figure S2 in the SI. Thus, these calculations suggest that in an aqueous environment the formation of ion-pair is a likely pathway. To save computational time we used the 12 water complex in our study to investigate the energetics of deprotonation reaction from N<sub>1</sub> and N<sub>2</sub> sites of guanine, which is discussed in the next section.

**Ion-Pair Formation on OH<sup>•</sup> Reaction with Hydrated Guanine.** The addition reaction of OH radical with hydrated guanine (G + 12H<sub>2</sub>O) and subsequent processes were calculated using the B3LYP method with the 6-31G\* and 6-31++G\*\* basis sets. These results are summarized in Table 1 and discussed below.

Figure 2 shows the potential energy surface (PES) for reaction of OH radical with hydrated guanine (G + 12H<sub>2</sub>O) using the B3LYP/6-31G\* method. The figure shows the relative energies of initial reactants, i.e., G + 12H<sub>2</sub>O and OH radical, the C<sub>4</sub> and C<sub>5</sub> adducts (G(C<sub>4</sub>)–OH<sup>•</sup>; G(C<sub>5</sub>)–OH<sup>•</sup>), the ion-pair (G<sup>•+</sup>---OH<sup>−</sup>) and finally the radicals formed by hydrogen abstraction from N<sub>1</sub> and N<sub>2</sub> sites, i.e., G(N<sub>1</sub>–H)<sup>•</sup> and G(N<sub>2</sub>–H)<sup>•</sup>, respectively. The optimized geometries of G–OH<sup>•</sup> + 12H<sub>2</sub>O, G<sup>•+</sup>---OH<sup>−</sup> + 12H<sub>2</sub>O, and G(–H)<sup>•</sup> + 13H<sub>2</sub>O are shown in Figure S3 of the SI.

The addition reaction of OH radical at C<sub>4</sub> or C<sub>5</sub> atom of hydrated guanine proceeds without a barrier to G(C<sub>4</sub>)–OH<sup>•</sup> or G(C<sub>5</sub>)–OH<sup>•</sup> adduct formation and the process is exothermic by ca. 22 to 24 kcal/mol, see Figures 1 and 2. In the adduct, OH radical bonds either to C<sub>4</sub> or C<sub>5</sub> atom of guanine with bond lengths 1.48 and 1.52 Å, respectively, see Figure S3 of the SI. Addition of OH<sup>•</sup> at C<sub>4</sub> is slightly energetically favored by −2.44 kcal/mol over C<sub>5</sub>, and the subsequent reaction mechanism proceeds through a transition state, see Figure 2. From the C<sub>5</sub> adduct (G(C<sub>5</sub>)–OH<sup>•</sup> + 12H<sub>2</sub>O), the PES was scanned by detaching the OH moiety from C<sub>5</sub> atom, shown in Figure 2. We note that after surpassing the barrier of ca. 1 kcal/mol, ion-pair formation takes place and it is more stable than the adduct

(G(C<sub>5</sub>)-OH<sup>•</sup> + 12 H<sub>2</sub>O) by 2.44 kcal/mol. The B3LYP/6-31G\* calculated PES for the detachment of the OH from C<sub>4</sub> adduct (G(C<sub>4</sub>)-OH<sup>•</sup> + 12 H<sub>2</sub>O) to form ion-pair (G<sup>•+</sup>---OH<sup>-</sup> + 12H<sub>2</sub>O) has a barrier of 6.45 kcal/mol and the formation of ion-pair was found to be isoenergetic with respect to the adduct. From the ion-pair, the PES for deprotonation from N<sub>1</sub> and N<sub>2</sub> sites of guanine and the formation of G(N<sub>1</sub>-H)<sup>•</sup> and G(N<sub>2</sub>-H)<sup>•</sup> and water (G<sup>•+</sup>---OH<sup>-</sup> → G(-H)<sup>•</sup> + H<sub>2</sub>O (reaction 1b)) was calculated by gradually stretching the N<sub>1</sub>-H and N<sub>2</sub>-H bonds of guanine with optimization the remaining structure. The B3LYP/6-31G\* calculated barrier height for deprotonation from the N<sub>1</sub> site of guanine was calculated to be 4.28 kcal/mol with respect to the ion-pair (G<sup>•+</sup>---OH<sup>-</sup> + 12H<sub>2</sub>O). The formation of N<sub>1</sub> radical (G(N<sub>1</sub>-H)<sup>•</sup>) was found to be highly exothermic by -16.40 kcal/mol relative to G(C<sub>5</sub>)-OH<sup>•</sup> calculated using the B3LYP/6-31G\* method, see Figure 2. Using the B3LYP-PCM/6-31+G(d,p) level of theory Naumov and Sonntag<sup>10i</sup> also studied the water elimination reaction from G(C<sub>4</sub>)-OH<sup>•</sup> and G(C<sub>5</sub>)-OH<sup>•</sup> adducts to form G(N<sub>1</sub>-H)<sup>•</sup> and also found this reaction to be exothermic in nature.

The barrier for the N<sub>2</sub>-H deprotonation of guanine to form G(N<sub>2</sub>-H)<sup>•</sup> is ca. 12 kcal/mol relative to the ion-pair (G<sup>•+</sup>---H<sup>-</sup> + 12H<sub>2</sub>O) calculated at the B3LYP/6-31G\* level of theory, see Figure 2. The formation of (G(N<sub>2</sub>-H)<sup>•</sup>) is found to be exothermic by 16.5 kcal/mol relative to G(C<sub>5</sub>)-OH<sup>•</sup> which is identical with G(N<sub>1</sub>-H)<sup>•</sup>. However, calculations employing the PCM model for full solvation of the 12H<sub>2</sub>O system show that G(N<sub>1</sub>-H)<sup>•</sup> is found to be more stable than G(N<sub>2</sub>-H)<sup>•</sup> by 2 kcal/mol at the B3LYP-PCM/6-31G\* and 4 kcal/mol calculated at the B3LYP-PCM/6-31++G\*\* level of theory. The preferred stability of G(N<sub>1</sub>-H)<sup>•</sup> over G(N<sub>2</sub>-H)<sup>•</sup> has also been found in earlier theoretical and experimental studies.<sup>9a,19,20</sup>

At the B3LYP/6-31++G\*\* level of theory, the barrier height for ion-pair formation from G(C<sub>5</sub>)-OH<sup>•</sup> + 12H<sub>2</sub>O was calculated to be 2.38 kcal/mol and the ion-pair was found to be more stable than the adduct by 2.11 kcal/mol, see Table 1. The B3LYP/6-31++G\*\* calculated barrier height for N<sub>1</sub> deprotonation was found to be 2.44 kcal/mol while the formation of G(N<sub>1</sub>-H)<sup>•</sup> was found to be -18 kcal/mol with respect to G(C<sub>5</sub>)-OH<sup>•</sup> + 12H<sub>2</sub>O.

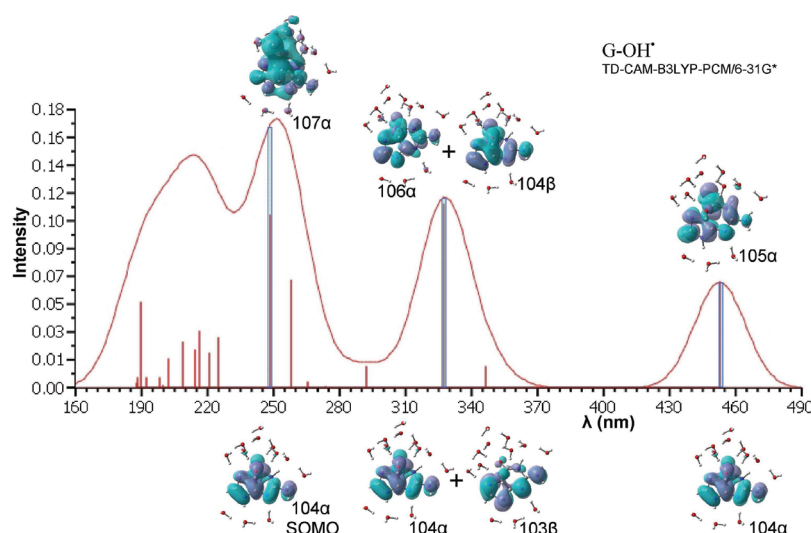
The C<sub>4</sub> or C<sub>5</sub> adduct structure induces appreciable nonplanarity in the guanine structure similar to butterfly shape as reported in earlier studies.<sup>10c,h,j</sup> As OH group leaves the guanine base to form ion-pair (G<sup>•+</sup>---OH<sup>-</sup> + 12H<sub>2</sub>O), the guanine base becomes near planar, see Figure S3(b) of the SI. The spin density distributions found for G-OH<sup>•</sup> + 12H<sub>2</sub>O, G<sup>•+</sup>---OH<sup>-</sup> + 12H<sub>2</sub>O and G(-H)<sup>•</sup> + 13H<sub>2</sub>O are shown in Figure S4 of the SI. From Figure S4 of the SI, we see that the spin density distribution of the adduct (G-OH<sup>•</sup> + 12H<sub>2</sub>O) is quite different than the spin density distribution of the G<sup>•+</sup>---OH<sup>-</sup> + 12H<sub>2</sub>O (ion-pair). The plots of spin density distribution in G<sup>•+</sup> and G<sup>•+</sup> + 12H<sub>2</sub>O calculated in the gas-phase and in PCM model are shown in Figure S4(d)-(f) of the SI. From Figure S4 of the SI, it is evident that the spin density distribution in ion-pair (G<sup>•+</sup>---OH<sup>-</sup>) (Figure S4(b) of the SI) is similar to the spin density distribution in G<sup>•+</sup> (see Figure S4(d)-(f) of the SI which is calculated using the B3LYP-PCM/6-31G\* and B3LYP-PCM/6-31++G\*\* methods). In addition to spin density distributions, the B3LYP/6-31++G\*\* calculated Mulliken net charges residing on OH, G and 12H<sub>2</sub>O in (G(C<sub>5</sub>)-OH<sup>•</sup> + 12H<sub>2</sub>O) adduct in gas-phase (PCM) are as follows: 0.014e (0.023e) for OH, -0.227e (-0.210e) for G, and 0.213e (0.187e), for 12H<sub>2</sub>O. In the ion-pair the corresponding

net charges are -0.364e (-0.390e), 0.213e (0.296e), and 0.151e (0.094e) (e = electronic charge), respectively. Thus, in ion-pair, the OH moiety gains a substantial charge -0.390e and becomes anionic and guanine loses a large 0.296e charge and becomes cationic in nature. These results clearly support the formation of a metastable ion-pair (G<sup>•+</sup>---OH<sup>-</sup>) after OH addition to C<sub>4</sub>/C<sub>5</sub> of guanine as was suggested by Mundy and co-workers for OH attack on the NH<sub>2</sub> portion of guanine.<sup>13b</sup> Such reactions are well-known for planar conjugated systems; for example, using time-resolved resonance Raman spectroscopy, Tripathi studied the addition reaction of OH<sup>•</sup> with p-dimethoxybenzene and found the cation radical formation after the loss of OH<sup>-</sup>.<sup>21</sup>

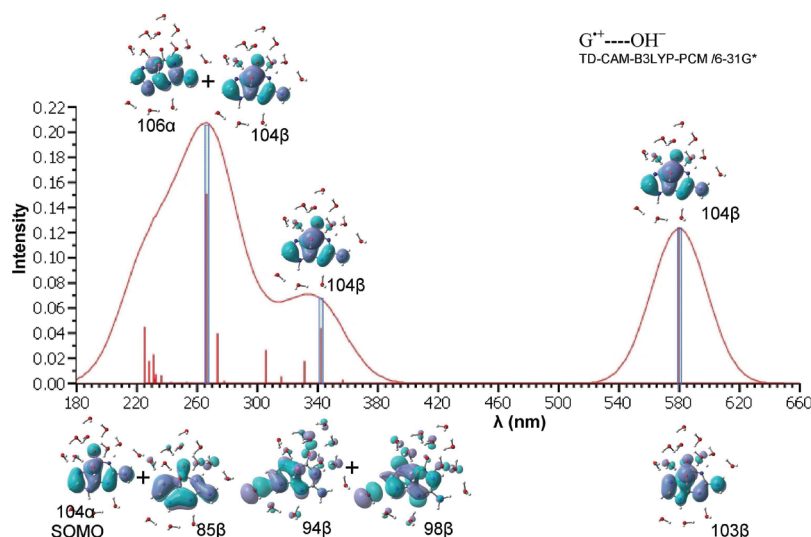
In summary, our calculations show that the reaction of OH radical with guanine has no barrier to C<sub>4</sub> or C<sub>5</sub> adduct formation as reported in earlier studies<sup>10f,g,i</sup> and with hydration it subsequently transformed to the more stable ion-pair. From the ion-pair (G<sup>•+</sup>---OH<sup>-</sup> + 12H<sub>2</sub>O) deprotonation reaction takes place either from N<sub>1</sub> or N<sub>2</sub> sites of guanine analogous to the guanine radical cation which also prefers these sites for deprotonation. The present calculation shows that the lowest barrier path for deprotonation is from N<sub>1</sub> site of guanine (Figure 2). It is well-known that G<sup>•+</sup> becomes acidic (pK<sub>a</sub> = 3.9)<sup>9a,g</sup> and quickly deprotonates in the presence of aqueous media from N<sub>1</sub> site as found in other studies.<sup>20,23,24</sup> We are also aware that in a hydrated environment, a number of dynamic solvation structures are available which will affect the presently calculated PESs and the barrier heights of the reaction. Our calculations represent one of the possible local minimum structures present during solvent dynamics and can not be considered as the expected experimental barriers.

*Direct Hydrogen Abstraction from N<sub>1</sub>H and NH<sub>2</sub> Group of Guanine.* The direct hydrogen abstraction reaction (eq 2) by OH<sup>•</sup> from N<sub>1</sub> and N<sub>2</sub> sites of guanine to form neutral radical was also calculated using the B3LYP/6-31G\* method in gas phase as well as in aqueous phase using PCM model. From our calculations, we found that the in-plane reaction of OH radical depends on the direction of approach of the OH radical toward guanine. Our calculations show that when OH radical approaches between N<sub>1</sub> and NH<sub>2</sub> region, it preferentially abstracts a hydrogen from NH<sub>2</sub> group (in gas phase) and in the presence of aqueous solution modeled through PCM the OH radical preferentially abstracts a hydrogen atom from N<sub>1</sub> site, see Figure S8 of the SI. These reactions proceed with negligible barriers to form radicals, i.e., G(N<sub>2</sub>-H)<sup>•</sup> (in gas phase) or G(N<sub>1</sub>-H)<sup>•</sup> (in PCM). If the in plane approaching OH radical is in between the O<sub>6</sub> and N<sub>1</sub>H region of guanine, then a hydrogen-bonded complex is formed as shown in Figure 1 and in Figure S8 of the SI.

Another picture arises for the reactivity of the OH radical in aqueous environment with explicit water molecules. The reaction critically depends on the conformation and arrangement of the interacting water molecules and this is well documented in the literature.<sup>22</sup> Depending on the water conformation the barrier for the hydrogen abstraction from CH<sub>4</sub> by OH radical with various levels of solvation (0-3H<sub>2</sub>O) ranged from 2.37 to 5.25 kcal/mol as calculated using the CCSD(T)/aug-cc-pVDZ level of theory.<sup>22</sup> In our case, we also found that for guanine solvated by 12 waters (G + 12H<sub>2</sub>O) the energy involved for direct hydrogen abstraction from the N<sub>1</sub>H or NH<sub>2</sub> site by the OH radical increases from the gas phase results which had negligible barriers to 7 kcal/mol for N<sub>2</sub> and 10 kcal/mol for N<sub>1</sub> hydrogen abstraction, see Figure S9 of the SI. In this calculation, we placed OH radical near N<sub>1</sub> and N<sub>2</sub> sites of hydrated guanine (G + 12H<sub>2</sub>O) and scanned the PES



**Figure 3.** TD-CAM-B3LYP-PCM/6-31G\*//B3LYP/6-31G\* calculated excited states of  $G(C_5)OH^\bullet + 12H_2O$ . MOs dominantly involved in the transitions are also shown.



**Figure 4.** TD-CAM-B3LYP-PCM/6-31G\*//B3LYP/6-31G\* calculated excited states of  $G^{\bullet+}---OH^- + 12H_2O$ . MOs dominantly involved in the transitions are also shown.

for hydrogen abstraction from  $N_1$  and  $N_2$  sites, see Figure S9 of the SI. As stated above, this barrier likely depends on the arrangement of the specific water molecules and would change if dynamics were considered. The calculation also showed that in these arrangements, the spin density is fully localized on OH until abstraction takes place (see Figure S9 of the SI) which is different than the addition reaction in which the spin density is transferred to the guanine immediately on interaction, see Figures 2 and S4 of the SI.

**Excited States of Hydrated  $G(C_5)OH^\bullet$ ,  $G^{\bullet+}---OH^-$ , and  $G(-H)^\bullet$ .** In Figures 3, 4, 5, we show the UV–vis spectra of  $G-OH^\bullet + 12H_2O$ ,  $G^{\bullet+}---OH^- + 12H_2O$ , and  $G(N_1-H)^\bullet + 13H_2O$  calculated using the TD-CAM-B3LYP-PCM/6-31G\* method incorporating the full solvent through the IEF-PCM model. The corresponding spectra computed using the TD-CAM-B3LYP-PCM/6-31++G\*\* method are shown in the SI as Figures S5–S7, respectively. In Table S1 of the SI, we provided transition

energies along with the oscillator strengths of  $G^{\bullet+} + 12H_2O$ ,  $G(C_5)OH^\bullet + 12H_2O$ ,  $G^{\bullet+}---OH^- + 12H_2O$ ,  $G(N_1-H)^\bullet + 13H_2O$  and  $G(N_2-H)^\bullet + 13H_2O$  calculated using the TD-CAM-B3LYP-PCM and TD-B3LYP-PCM methods using 6-31G\* and 6-31++G\*\* basis sets. From Table S1 of the SI, a good agreement between theory and experiment where available is evident.

Excited state spectra of  $G(C_5)OH^\bullet + 12H_2O$  (adduct), shown in Figure 3, shows three intense peaks located at 249, 328, and 453 nm having oscillator strengths 0.1122, 0.1197, and 0.0682, respectively, and these transitions are  $\pi\pi^*$  in nature. The transition at 249 nm is due to excitation from SOMO (singly occupied molecular orbital)  $(104\alpha) \rightarrow 107\alpha$ ; the transition at 328 nm involves  $(104\alpha + 103\beta) \rightarrow (106\alpha + 104\beta)$  and the transition at 453 nm involves  $104\alpha$  (SOMO)  $\rightarrow 105\alpha$ , respectively, see Figure 3. The spectra of  $G(C_5)OH^\bullet + 12H_2O$ , calculated using the CAM-B3LYP-PCM/6-31++G\*\* method, are similar to those calculated at the CAM-B3LYP-PCM/6-31G\* level of theory





These previous results strongly suggest that for OH<sup>•</sup> attack on guanine both abstraction and addition elimination mechanisms are in effect.

## CONCLUSIONS

The present DFT investigation of OH radical reaction with guanine in aqueous environment confirms that OH<sup>•</sup> can react with guanine through adduct formation at C<sub>4</sub>, C<sub>5</sub>, or C<sub>8</sub> atom or by direct abstraction of a hydrogen atom from N<sub>1</sub> or N<sub>2</sub> sites. Our calculations show that the thermodynamic energy of initial binding of OH<sup>•</sup> with guanine in a hydrated environment (or in the gas phase) is in this order C<sub>8</sub>OH<sup>•</sup> > C<sub>4</sub>OH<sup>•</sup> > C<sub>5</sub>OH<sup>•</sup> > (H-bonded to N<sub>1</sub>). The binding is found to be exothermic in nature and lie in the range ca. −35 to −15 kcal/mol, see Figure 1. Since the C<sub>8</sub> adduct and its chemistry are well understood,<sup>2–5,9</sup> we focused in this work on the relation between C<sub>4</sub>/C<sub>5</sub> addition and N<sub>1</sub>/N<sub>2</sub> abstraction pathways. Under the influence of the hydration the initially formed OH adducts at C<sub>4</sub> or C<sub>5</sub> that have no barrier for their formation on further reaction have only a small activation barrier of ca. 1–6 kcal/mol to the formation of the more stable ion-pair (G<sup>+</sup>...OH<sup>−</sup>). The resulting ion-pair (G<sup>+</sup>...OH<sup>−</sup>) undergoes proton transfer reaction either from N<sub>1</sub> or N<sub>2</sub> site of guanine to form neutral radical, i.e., (G(N<sub>1</sub>–H)<sup>•</sup> or G(N<sub>2</sub>–H)<sup>•</sup>) and H<sub>2</sub>O. The present study shows that N<sub>1</sub> deprotonation is favored over N<sub>2</sub> deprotonation because of lower barrier height, see Figure 2. Our CAM-B3LYP calculations predict absorption between 580 and 670 nm for ion-pair which brackets the 620 nm absorption spectra found in pulse radiolysis studies of the OH radical reaction with 2'-deoxyguanosine.<sup>8a,9g,11</sup> However, we note that the lifetime of such an ion-pair transient would be expected to be quite short as predicted by Mundy and co-workers<sup>13</sup> from MD simulations. Thus, considering the relatively low barrier inherent to OH abstraction reactions it is clear that there must be a contribution to radical production from OH radical direct hydrogen abstraction from the NH<sub>2</sub> group as proposed by Chatgililoglu et al.<sup>11</sup> However, the present calculations show that in the presence of hydration, the barrier for direct hydrogen abstraction from N<sub>1</sub> or N<sub>2</sub> site may be significant (see Figure S9 of the SI), whereas, for addition barrierless formation of the addition complex is predicted which then proceeds with a small barrier to form the ion-pair and products (Figure 2). As pointed out above, the reaction of OH radical with similar structures such as aniline shows a predominance of the addition/elimination reaction over the abstraction reaction from the NH<sub>2</sub> group.<sup>11,25,26</sup> Thus, for OH attack on guanine from both our and other theoretical treatments<sup>10,13</sup> and experiments on similar structures, it would appear that addition/elimination should be a very competitive route with direct H-atom abstraction. Further, G(N<sub>1</sub>–H)<sup>•</sup> and G(N<sub>2</sub>–H)<sup>•</sup> can be products of both addition/elimination and abstraction which makes the distinction of the pathways of formation of G(N<sub>1</sub>–H) and G(N<sub>2</sub>–H) radicals quite difficult unless the C<sub>4</sub>/C<sub>5</sub> adducts or ion-pair species are observed by experiments at short times. Thus, experiments that look at shorter time scales are strongly encouraged. In dsDNA, the picture would, of course, be altered by base stacking, base pairing, and solvent accessibility.<sup>7,14</sup> Stacking would hinder addition reactions by lowering the accessibility and H-bonding between the DNA bases would inhibit H-abstraction reactions. These effects need to be investigated in future theoretical studies. Our study, also, shows that the CAM-B3LYP functional in conjunction with B3LYP optimized geometries provide transition

energies that favorably compare to experiment for the radical species considered in this work (Table S1 of the SI).

## ASSOCIATED CONTENT

**S Supporting Information.** B3LYP/6-31G\* optimized geometries of C<sub>4</sub>, C<sub>5</sub> (adducts), ion-pair, G(N<sub>1</sub>–H)<sup>•</sup> and G(N<sub>2</sub>–H)<sup>•</sup> due to reaction of guanine and OH radical in the presence of explicit water molecules and their spin density plots. Excited state spectra of hydrated adduct, ion-pair and G(N<sub>1</sub>–H)<sup>•</sup> calculated using the CAM-B3LYP-PCM/6-31++G\*\* method. PES for direct hydrogen abstraction from N<sub>1</sub> and N<sub>2</sub> of guanine by OH radical in the presence of water molecules. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## NOTE ADDED IN PROOF

A recent related work has just appeared entitled “Spectral Characterization of Guanine C<sub>4</sub>–OH Adduct: A Radiation and Quantum Chemical Study” by Phadatare et al. *J. Phys. Chem. B* **2011**, *115*, 13650–13658. Using pulse radiolysis experiments and theory, the authors characterize the initial formation of a G(C<sub>4</sub>)–OH radical adduct which is found to undergo dehydration to produce the G(N<sub>1</sub>–H) radical. Our present study supports the addition-dehydration reaction as a major pathway because it involves lower initial barriers than abstraction. Our work predicts that in aqueous solutions an ion-pair intermediate in the addition-dehydration pathway is likely.

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