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Molecular Structures and Energetics Associated with Hydrogen Atom Addition to the Guanine−Cytosine Base Pair

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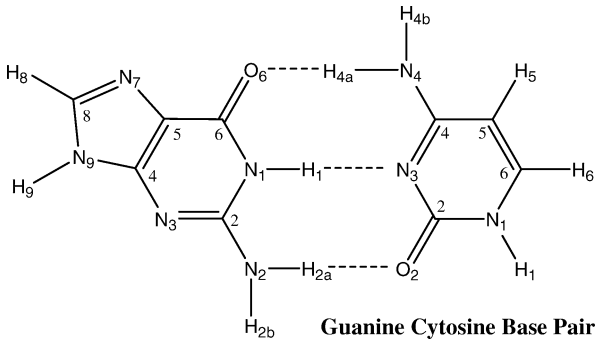
Abstract: The radicals generated by hydrogen-atom addition to the Watson−Crick guanine−cytosine (G−C) DNA base pair were studied theoretically using an approach that has proved effective in predicting molecular structures and energetics. All optimized structures were confirmed to be minima via vibrational frequency analysis. The dissociation energies of the base-pair radicals are predicted and compared with that of the neutral G−C base pair. The lowest-energy base-pair radical is that with the hydrogen atom attached to the C8 position of guanine, resulting in the nitrogen radical designated G(C8)−C. In this, the most favorable radical, the G−C pair C8═N7 distance of 1.310 Å increases to 1.453 Å when the π bond is broken upon hydrogen-atom addition. This radical has a dissociation energy of 28 kcal/mol, which may be compared with 27 kcal/mol for neutral G−C. The other (GC + H)• radical dissociation energies range downward to 8 kcal/mol. Significant structural changes were observed when the hydrogen was added to the sites where the interstrand hydrogen bonds are formed. For example, "butterfly"-shape structures were found when the hydrogen atom was added to the C4 or C5 sites of guanine. The formation of radical G(C2)−C may cause a single-strand break because of significant strain in the closely stacked base pairs. Radical G(C8)−C is of biological importance because it may be an intermediate in the formation of 8-oxo guanine.

Introduction

With the obvious exception of normal cellular DNA metabolism, lesions resulting from external agents such as ionizing radiation are believed to be the most important source of DNA damage with respect to the preservation of genetic integrity.[1] To gain insight concerning the processes and mechanisms of radiation-induced damage to DNA, two underlying chemical pathways, the direct type and indirect type, have been introduced.[2] The direct-type damage involves energy which mainly comes from monoenergetic photons[3−6] or electrons,[4,7−10] being deposited directly on the nucleotide of the DNA strand.[11] For example, as a primary product of direct damage, the base radical cation $B^{\bullet+}$ often deprotonates to yield the different radicals $B^{\bullet}(-H^{+})$.[12] A distinctly different effect involves indirect damage pathways related to the radiosensitive environment. As a tightly surrounding medium, water can be irradiated to form free radicals such as atomic hydrogen and reactive oxygen species (ROS) like $O_2^{\bullet-}$, $OH^{\bullet}$, and H_2O_2.[13] These radicals may then react with nucleotides

Chart 1. Numbering Scheme for the Guanine−Cytosine Base Pair



Guanine Cytosine Base Pair

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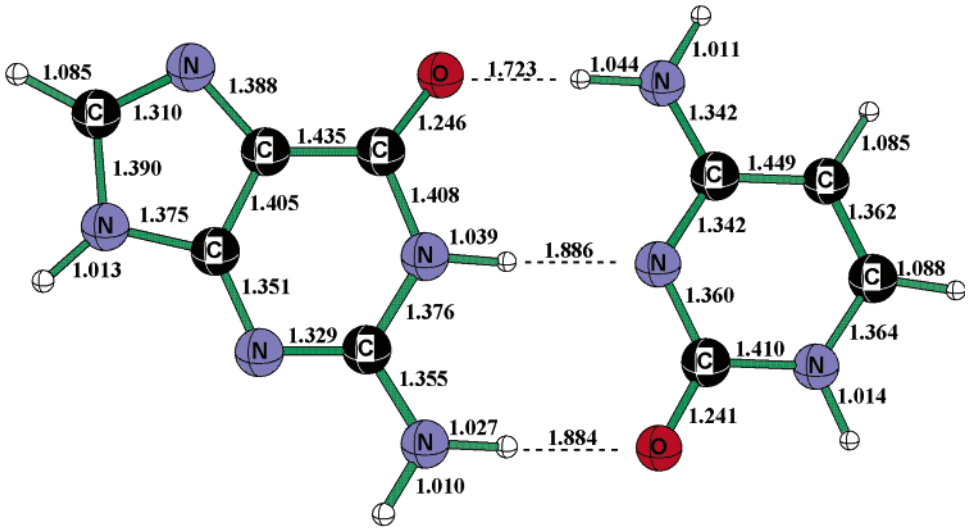

Figure 1. Optimized geometry of the neutral guanine−cytosine (G−C) base pair at the B3LYP/DZP++ level of theory. The unit of bond length in this figure and the following figures is the angstrom (Å).

Table 1. Total and Relative Energies of (G + H)•−C and G−(C + H)• Base-Pair Radicals (See Chart 1 for Atom Numbering)

	non-ZPVE		ZPVE-corrected	
radicals	total energy (Eh)	relative energy (kcal/mol)	total energy (Eh)	relative energy (kcal/mol)
G(C8)−C	−938.28079	0.0	−938.05436	0.0
G−C(N3)	−938.27585	3.1	−938.04959	3.0
G−C(C5)	−938.27503	3.6	−938.04835	3.8
G−C(C6)	−938.27152	5.8	−938.04528	5.7
G(N7)−C	−938.26148	12.1	−938.03528	12.0
G(C4)−C	−938.25296	17.5	−938.02651	17.5
G(C5)−C	−938.24890	20.0	−938.02309	19.6
G(C2)−C	−938.24606	21.8	−938.01945	21.9
G(O6)−C	−938.24108	24.9	−938.01452	25.0
G−C(O2)	−938.23572	28.3	−938.01054	27.5
G(N3)−C	−938.23540	28.5	−938.00920	28.3
G−C(C4)	−938.22578	34.5	−937.99867	34.9

to induce structural damage such as single-strand breaks (SSBs)[14] and double-strand breaks,[15] as well as genetic information changes like pairing mismatches and mutations.[16] There are extensive studies both theoretical[17−19] and experimental[20−23] of OH• radicals reacting with purines and pyrimidines by removing one hydrogen atom[12a] or directly attacking double bonds.[24,25] However, the role played by non-ROS such as hydrogen atoms in DNA radiation damage is less clear.

Some earlier studies of isolated hydrogenated nucleic acid base (NAB) radicals must be noted. In 1998, Wetmore et al.[26] showed H-atom addition to guanine to be preferable at position C8 (See Chart 1 for the standard atom numbering in guanine) from electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) experiments, as well as density functional theory (DFT). Hydrogenated cytosine has also been explored at the DFT level theoretically[27,28] and by EPR/ENDOR[29,30] experiments. As transient intermediates, the resultant NAB radicals either (a) undergo

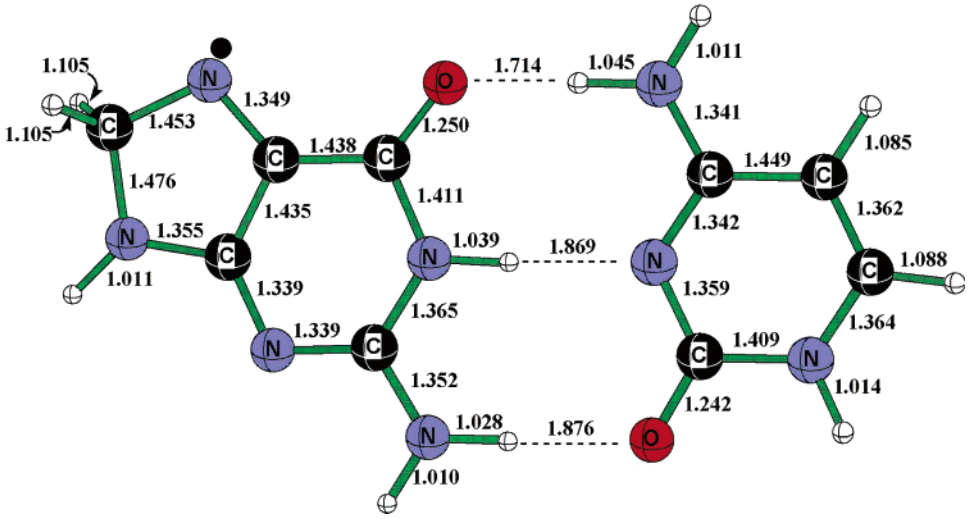

Figure 2. Optimized geometry of the G(C8)−C base-pair radical at the B3LYP/DZP++ level of theory. Relative to neutral G−C, there is a hydrogen atom attached to guanine C8. The dot (•) shows the formal radical center position in the qualitative valence structure.

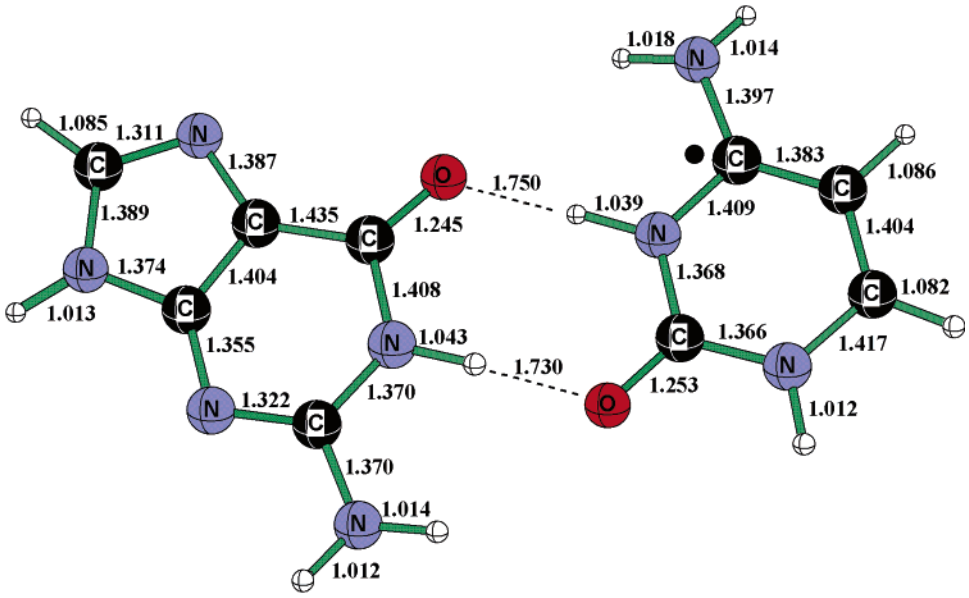

Figure 3. Optimized geometry of the G−C(N3) base-pair radical at the B3LYP/DZP++ level of theory. A hydrogen atom is attached to cytosine N3 when compared to the neutral G−C pair. The dot (•) shows the formal radical center position in the qualitative valence structure.

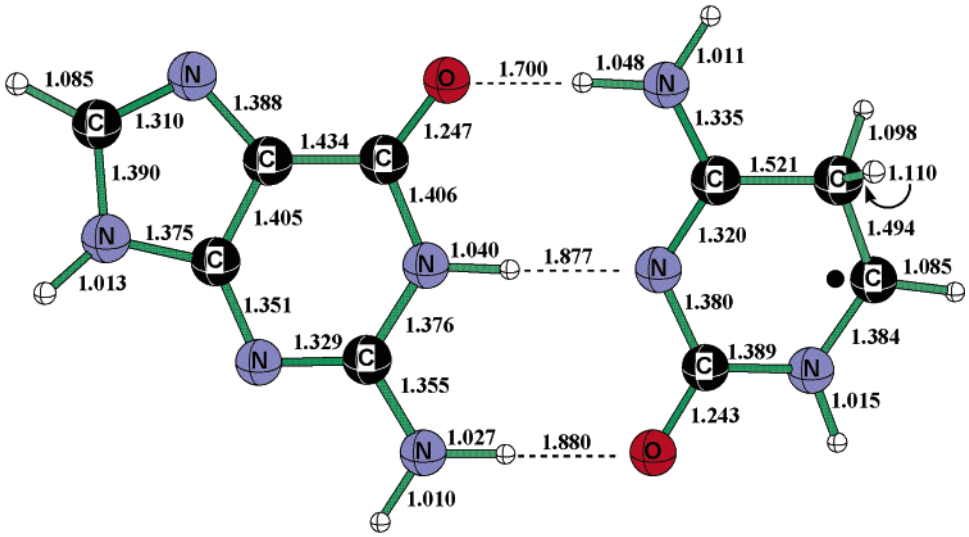

Figure 4. Optimized geometry of the G−C(C5) base-pair radical at the B3LYP/DZP++ level of theory. There is a hydrogen atom attached to cytosine C5 when compared to neutral G−C pair.

fast proton rearrangement reactions to form more thermodynamically stable species that will induce DNA tandem lesions[31a] and interstrand cross-links[31b] after trapping electrons or (b) are repaired[24,32] by thiols in biological systems.

Further investigations of damage in DNA have been extended to the Watson−Crick base pairs and the stacked double helix. Mechanisms of radical formation and further reaction channels have been discussed.[33−41] The formation of radicals sometimes involves excited triplet states of deoxyribose, which are expected to dissociate efficiently into radicals, leading to subsequent strand breaks.[35] Electron transfer through a π-stacked helix increases the chance of exciting closed-shell neutrals,[36,37] and captured electrons may cause strand breaks and mutations.[38−40] Recent research has also shown that the electron capturing probability scales with the number of guanines in the single-strand DNA oligomer.[41] Proton transfer also plays a crucial role in DNA biological processes.[42−46] However, studies of hydrogen addition to base *pairs*, the structures and energies of the different hydrogenated base-pair radicals, and how the deformed single NABs affect the pairing pattern as well as the stacked helix have not been reported.

Hydrogen bonds in the neutral guanine cytosine (G−C) base pair have been discussed extensively. Various theoretical methods have been used to determine DNA base-pair structural features.[47−51] Complex environments, including the interactions between the NAB pair and metal cations[47,48] and water molecules,[48] are required to explain the discrepancies between X-ray crystal structures and the structure of the gas-phase base pairs.[51] Combining Monte Carlo and DFT [B3LYP/6-31+G(d)] methods, Coutinho et al.[49] concluded in 2004 that the G−C interaction in an aqueous environment is weakened to about 70% of the value obtained for the isolated G−C complex. Recently, Sponer et al.[48] examined

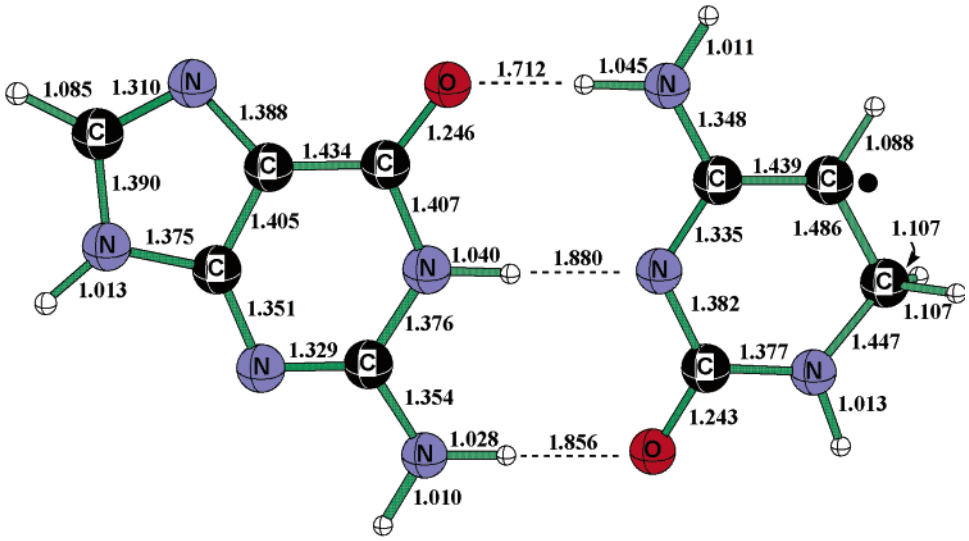

Figure 5. Optimized geometry of the G−C(C6) base-pair radical at the B3LYP/DZP++ level of theory. Relative to neutral G−C, there is a hydrogen atom attached to cytosine C6.

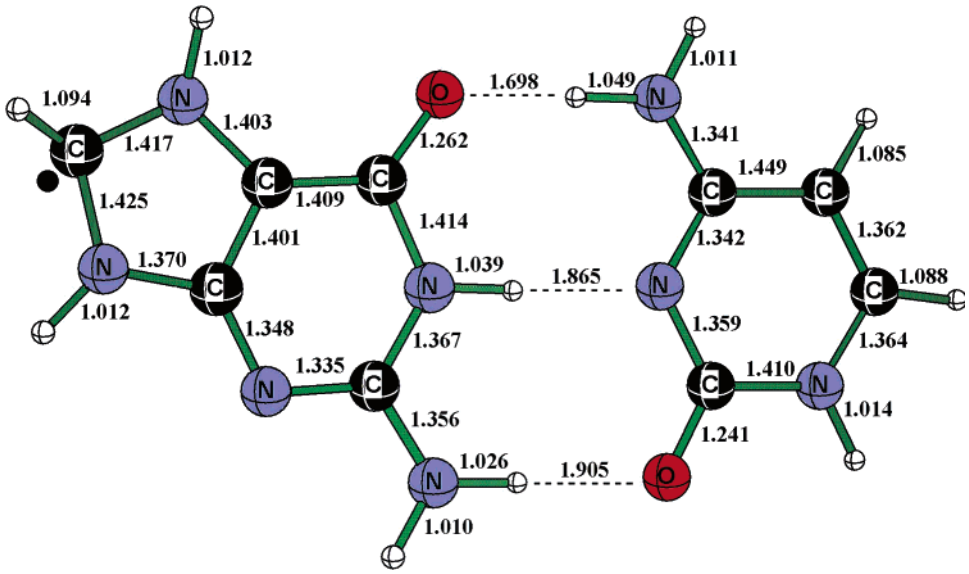

Figure 6. Optimized geometry of the G(N7)−C base-pair radical at the B3LYP/DZP++ level of theory. There is a hydrogen atom attached to guanine N7 position.

the different nucleic acid base pairs using resolution of identity (RI) MP2 methods and coupled-cluster corrections with the inclusion of noniterative triple contributions [CCSD-(T)], combined with complete basis set extrapolations.

In this research, we focus on the neutral radical isomers generated by adding one hydrogen atom to the G−C base pair, the latter depicted in Figure 1. The hydrogen atom may break C═C, C═N, or C═O double bonds on the G−C pair to yield radicals. By using the B3LYP functional combined with DZP++ basis sets, we predict the geometrical structures of 12 possible (G + H)•−C and G−(C + H)• radical isomers, as well as the dissociation energies of each pair. Vibrational frequencies were employed to characterize all optimized geometrical structures as stationary points on the potential energy surfaces. Upon attachment of one hydrogen atom to the G−C pair, the original planarity, demonstrated by gas-phase theoretical methods,[50] is destroyed. For example, a "butterfly" conformation[52] is adopted for the guanine base rings when a hydrogen atom is added at the C4 or C5 positions.

Theoretical Methods

A carefully calibrated DFT approach[28,33,50,53] has been used in this research to optimize geometries and predict vibrational frequencies. The B3LYP method is a combination of the Becke's three-parameter exchange functional (B3),[54] and the dynamic correlation functional of Lee, Yang, and Parr (LYP).[55] The Gaussian 94 system of DFT programs[56] was used for all computations.

This research was carried out using double-ζ-quality basis sets with polarization and diffuse functions, denoted as DZP++. The DZP++ basis sets were constructed by augmenting the Huzinaga−Dunning[57,58] set of constructed double-ζ Gaussian functions with one set of p-type polarization functions for each H atom and one set of five d-type

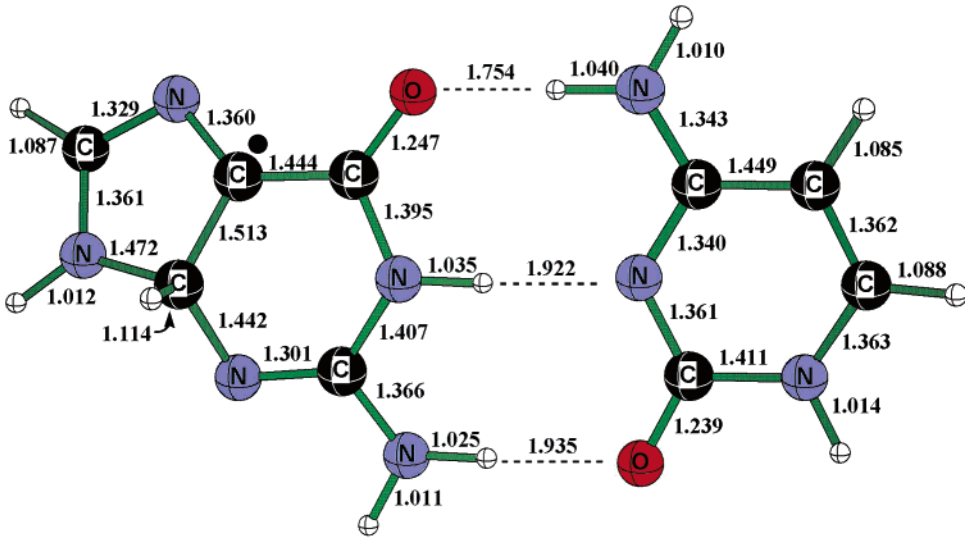

Figure 7. Optimized geometry of the G(C4)−C base-pair radical at the B3LYP/DZP++ level of theory. Relative to neutral G−C, there is a hydrogen atom attached to guanine C4.

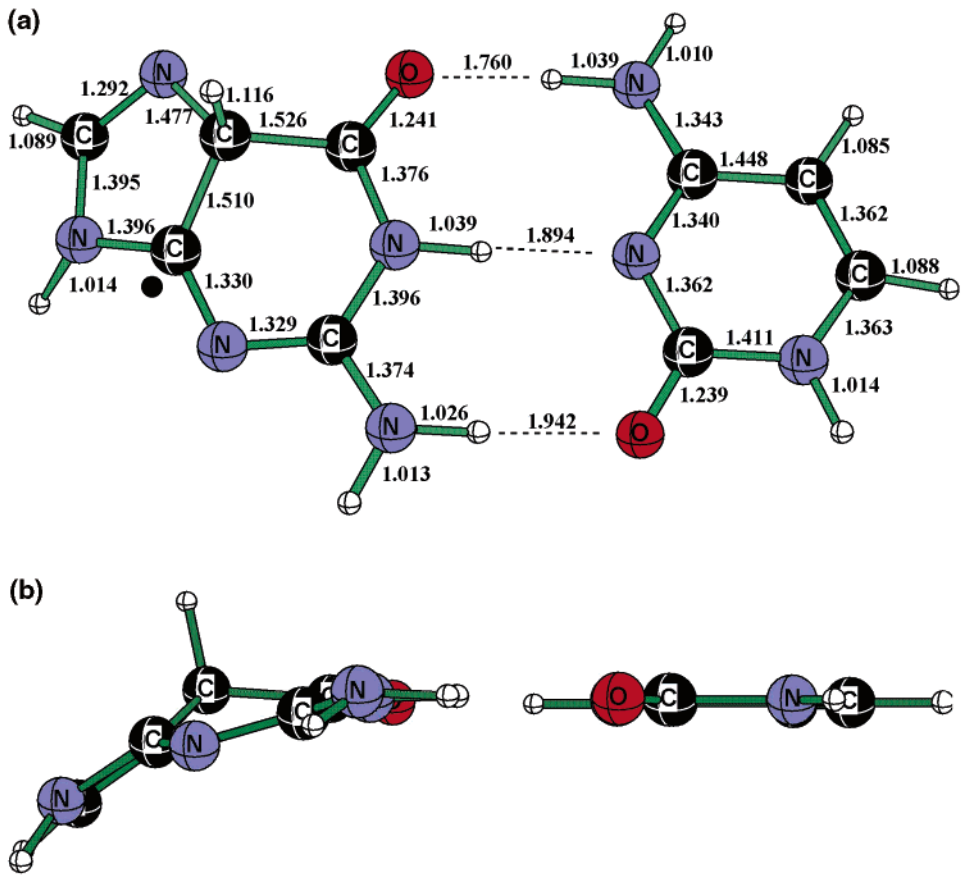

Figure 8. (a) Optimized geometry of the G(C5)−C base-pair radical at the B3LYP/DZP++ level of theory. Relative to the neutral G−C, there is a hydrogen atom attached to guanine C5. (b) Side view of the G(C5)−C radical, showing that the planarity of isolated guanine−cytosine is lost.

polarization functions for each C, N, and O atom [$\alpha_p(H) = 0.75$, $\alpha_d(C) = 0.75$, $\alpha_d(N) = 0.80$, and $\alpha_d(O) = 0.85$]. To complete the DZP++ basis, one even-tempered diffuse s function was added to each H atom while sets of even-tempered diffuse s and p functions were centered on each heavy atom. The even-tempered orbital exponents were determined by the following convention:[59]

$$\alpha_{\text{diffuse}} = \frac{1}{2}\left(\frac{\alpha_1}{\alpha_2} + \frac{\alpha_2}{\alpha_3}\right)\alpha_1$$

in which α_1, α_2 and α_3 are the three smallest primitive Gaussian orbital exponents for a given atom ($\alpha_1 < \alpha_2 < \alpha_3$). The final DZP++ set contains six functions per H atom (5s1p/3s1p) and 19 functions per C, N, or O atom (10s6p1d/5s3p1d), yielding a total of 427 contracted Gaussian functions

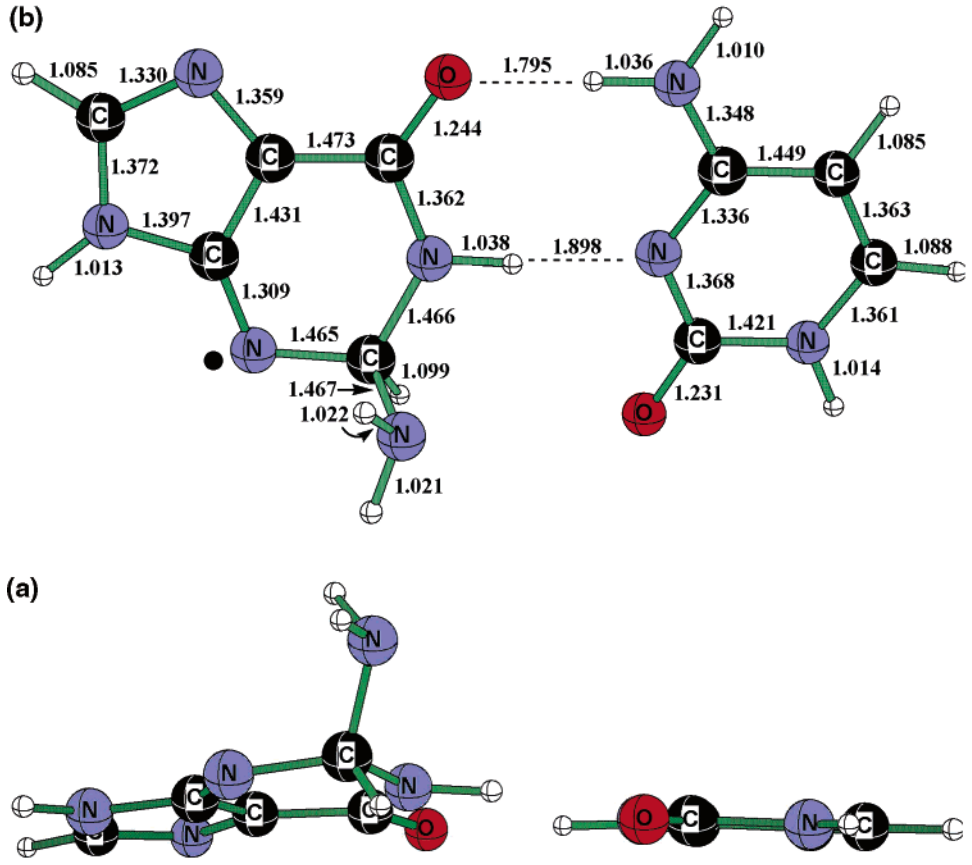

Figure 9. (a) Optimized geometry of the G(C2)−C base-pair radical at the B3LYP/DZP++ level of theory. There is a hydrogen atom attached to the guanine C2 position compared to G−C. (b) Side view of the G(C2)−C radical, showing that the amino group of guanine is significantly out of plane.

for each $(G + H)^{\bullet}-C$ and $G-(C + H)^{\bullet}$ hydrogenated base-pair radical.

The dissociation energies of the hydrogenated G-C radical base pairs were defined as

$$D_e = E[(G + H)^{\bullet}-C] - E[(G + H)^{\bullet}] - E(C) \qquad \text{(i)}$$

To analyze the unpaired electron distributions, Kohn−

$$D_e = E[G - (C + H)^{\bullet}] - E(G) - E[(C + H)^{\bullet}] \qquad \text{(ii)}$$

Sham molecular orbitals and spin-density plots were constructed from the appropriate B3LYP/DZP++ density. Natural population atomic charges were determined using the natural bond order analysis of Reed and co-workers.[60−63]

Results and Discussion

In this work, we use generic labels[33] to represent the 12 possible radical isomers resulting from hydrogen addition to the G−C base pair (Chart 1). The label G(N3)−C is meant to imply that a hydrogen atom has been added to the N3 site of the guanine moiety. The total and relative energies of the 12 radicals are reported in Table 1. The optimized geometries of the closed-shell G−C pair and the $(G + H)^{\bullet}-C$ and $G-(C + H)^{\bullet}$ radicals are schematically represented from Figures 1−13. Generally, hydrogen addition causes substantial distortions to the original C_s-symmetry G−C pair. Most H-atom addition products are of C_1 symmetry, except for G(C8)−C and G−C(C6), which retain planarity. For hydrogen-atom addition to the carbonyl group of each base, we discuss only the results associated with hydrogen-atom appendage to the oxygen atom. When a hydrogen atom adds to the carbon side of the carbonyl bond, the resulting oxygen radical will extract a hydrogen atom from its H-bond pair. The total energy of this radical is higher than that of most isomers we considered. Furthermore, as a complicated biochemical processes, the barrier-free hydrogen and proton transfers[42−46] are beyond the scope of this research.

1. Closed-Shell Guanine−Cytosine (G−C) Base Pair. The Watson−Crick model of the G−C base pair has been explored in this research using the B3LYP/DZP++ method. The optimized structure with C_s symmetry is shown in Figure 1. The three heavy-atom−heavy-atom distances associated with H bonds are predicted to be 2.767, 2.924, and 2.911 Å. It is well-known that these gas-phase H-bond distances do not match quantitatively with the crystallographic findings 2.91, 2.95, and 2.86 Å. The discrepancies between theoretical H-bond lengths and the crystal structure are due to the environmental effects, described in the important paper by Guerra et al.[47] The dissociation energies of G−C, $(G + H)^{\bullet}-C$, and $G-(C + H)^{\bullet}$ are reported in Table 2. The closed-shell G−C dissociation energy in this research is in good agreement with the definitive theoretical result of Sponer et al.[48]

2. Radicals. a. G(C8)−C. The lowest-energy radical in this series is the radical generated by the addition of hydrogen to atom C8 of guanine (Chart 1 and Figure 2). Qualitatively,

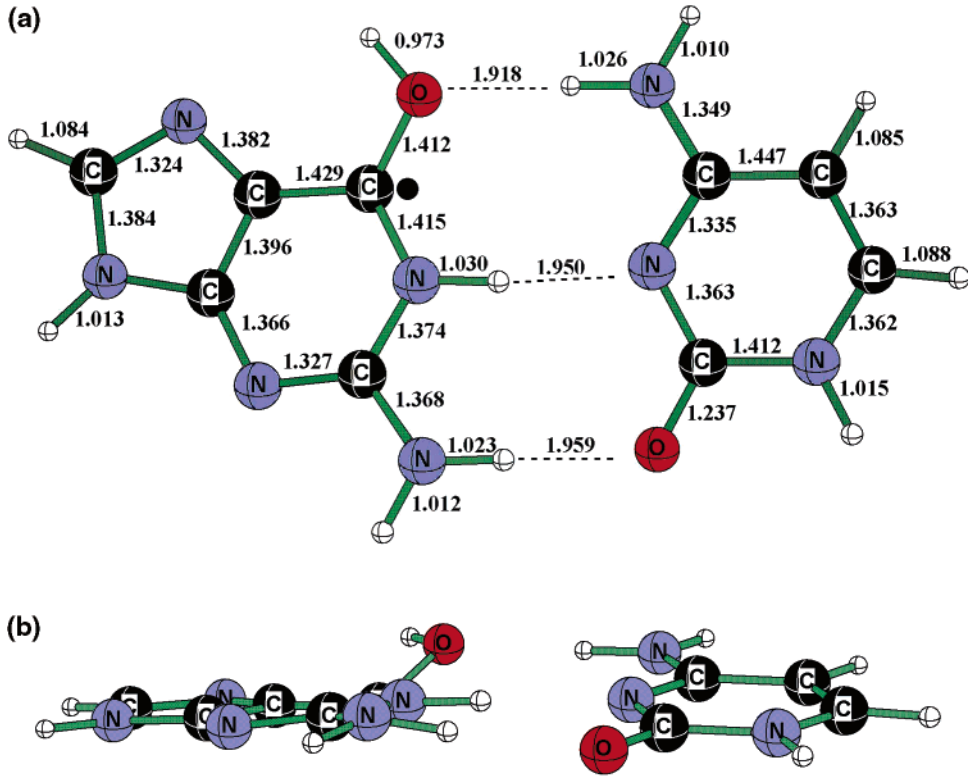

Figure 10. (a) Optimized geometry of the G(O6)−C radical at the B3LYP/DZP++ level of theory. A hydrogen atom is attached to guanine O6 position compared to G−C. (b) Side view of the G(O6)−C radical, showing the twisting of the guanine and cytosine rings.

atom C8 is changed to sp^3 hybridization from sp^2 in G−C after the C8═N7 double bond is broken. The resulting radical pair has relatively minor structural perturbations, keeping the C_s symmetry of closed-shell G−C. The (G + H)• radical is favorable to the formation of H bonds with cytosine, and the three H bonds are shortened by 0.009, 0.017, and 0.008 Å. Although the hydrogen-bonded distances are little changed from G−C to G(C8)−C, some heavy-atom−heavy-atom distances are substantially changed. Most notably, the C8═N7 distance (1.310 Å in G−C) is increased to 1.453 Å for G(C8)−C. This result is of course consistent with breaking the C8═N7 π bond of G−C. However, other structural changes are not so trivially explained. For example, the distance N9−C8 increases from 1.390 to 1.476 Å in going from G−C to the hydrogen-addition radical.

The G(C8)−C dissociation energy is 28.1 kcal/mol, slightly greater than the 27.2 kcal/mol predicted for closed-shell G−C.

b. G−C(N3). When a hydrogen atom adds to the N3 position of cytosine to break the N3═C4 double bond, the radical designated G−C(N3) is formed. Adding the hydrogen to this nitrogen atom necessarily results in the loss of the hydrogen bond N1H1···N3. The entire backbone shifts to form two new hydrogen bonds O6···H3N3 and N1H1···O2 (Figure 3). The new structure favors the formation of H bonds since the new N1H1···O2 bond length is significantly shorter (0.154 Å) than the old N2H2···O2 bond length of the original closed shell G−C pair. The pairing energy is 7.3 kcal/mol greater than that of radical G(C2)−C (Figure 9a), the other radical with two hydrogen bonds. As the second-lowest-energy radical, the total energy of the G−C(N3) radical is only 3.1 kcal/mol above that of the global minimum radical G(C8)−C (Figure 2), despite the reduction from three to two hydrogen bonds. The π bond N3═C4 increases in distance from 1.342 Å for G−C to 1.409 Å for the radical with the formal π bond broken.

c. G−C(C5). Structure G−C(C5) (Figure 4) is obtained when a hydrogen atom attaches to the cytosine C5 site. G−C(C5) is predicted to lie only 3.6 kcal/mol above the global minimum G(C8)−C (Figure 2). The G−C planarity disappears when the C5═C6 π bond is disrupted, increasing this distance from 1.362 to 1.494 Å. The adjacent C6−N1 bond length increases by 0.020 Å relative to G−C, possibly because the N1 lone-pair electrons interact with the unpaired electron formally located on cytosine C6 (with a spin density of 0.84). The overall geometrical structure of G−C(C5) preserves the original G−C pairing pattern. The two H-bond lengths O6···H4aN4 and N1H1···N3 are decreased by 0.023 and 0.009 Å, respectively, and the N2H2a···O2 H-bond distance is increased by 0.004 Å. The pairing energy is 27.9 kcal/mol, only 0.7 kcal/mol larger than that for closed-shell G−C at the same level of theory.

d. G−C(C6). This radical, shown in Figure 5, is achieved by adding a hydrogen atom to site C6 of cytosine. The total energy of G−C(C6) lies 5.8 kcal/mol above the lowest-energy radical isomer G(C8)−C (Figure 2). G−C(C6) maintains the original C_s symmetry of the closed-shell G−C pair. The spin density at atom C5 is predicted to be 0.87, indicating that most of the unpaired electron density resides in this region, consistent with our simple Lewis dot structure. As was the case for the previous radical G−C(C5) (Figure 4), the hydrogen-bond pattern is not greatly perturbed. The

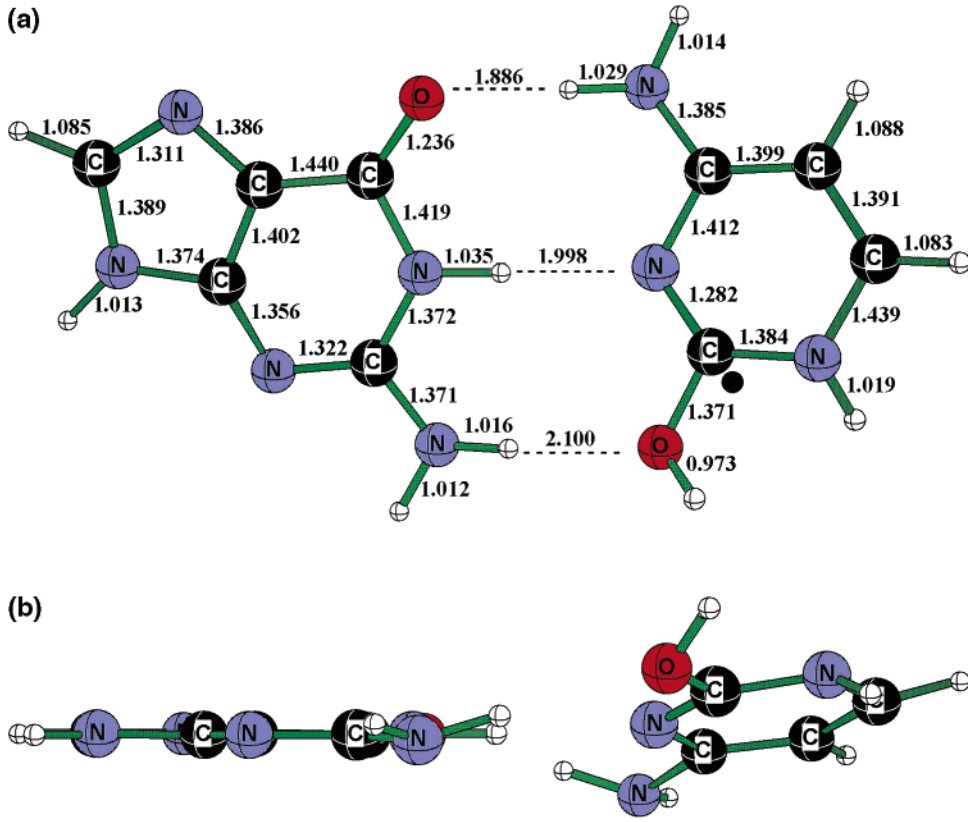

Figure 11. (a) Optimized geometry of the G−C(O2) base-pair radical at the B3LYP/DZP++ level of theory. There is a hydrogen atom attached to the cytosine O2 position compared to G−C neutral pair. (b) Side view of the G−C(O2) radical, showing the twisting of the guanine and cytosine rings.

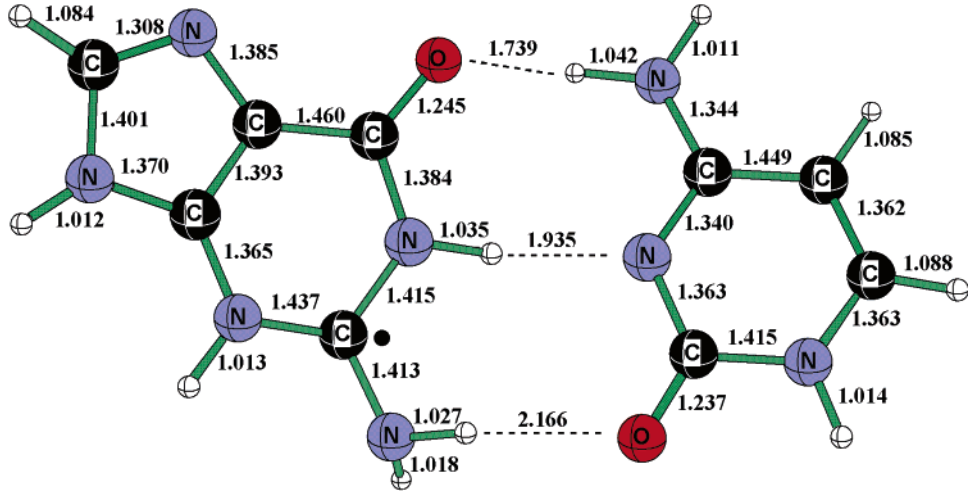

Figure 12. Optimized geometry of the G(N3)−C base-pair radical at the B3LYP/DZP++ level of theory. Relative to the neutral G−C, there is a hydrogen atom attached to guanine N3.

H-bond lengths decrease by only 0.011, 0.006, and 0.028 Å compared to G−C, while the dissociation energy is 27.7 kcal/mol, very close to that for the G−C pair (27.2 kcal/mol). The G−C double-bond distance C5═C6 (1.362 Å) increases to 1.486 Å upon qualitative breakage of the π bond.

e. G(N7)−C. This radical is formed by hydrogen-atom addition to the atom N7 of guanine (Figure 6). The π bond (C8═C7) that is broken lengthens from 1.310 Å (G−C) to 1.417 Å for the radical. Structure G(N7)−C is predicted to lie 12.1 kcal/mol higher than the global minimum G(C8)−C. The out-of-plane angle of the hydrogen attached to C8 is 43.5° (dihedral angle H8−C8−N7−C5 is 136.5°), because of the slight pyramidization of C8. The two hydrogen atoms H7 and H9 are both slightly out of plane. This radical is of biological relevance because it may be an intermediate in the formation of 8-oxo guanine, which has been found to resemble adenine and has been identified as a lesion generator.[63] As seen earlier in this research, the H-bond lengths shorten when they are away from the radical formation center. In this case, two of three H bonds are shorter by 0.025 and 0.021 Å, respectively, compared to the closed-shell G−C pair, and the third is longer by 0.021 Å.

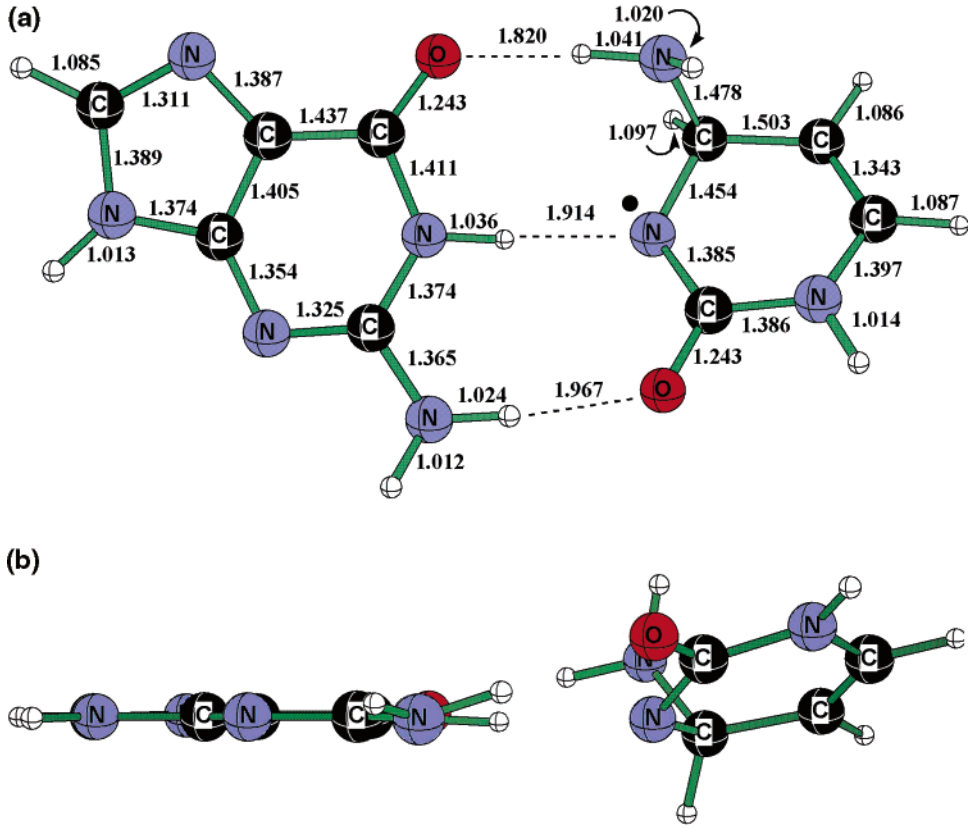

Figure 13. (a) Optimized geometry of the G−C(C4) base-pair radical at the B3LYP/DZP++ level of theory. Relative to neutral G−C, there is a hydrogen atom attached to cytosine C4. (b) Side view of the G−C(C4) radical, showing the twisting of the guanine and cytosine rings.

Table 2. Dissociation Energies of the GC Pair and the (G + H)•−C and G−(C + H)• Base-Pair Radicals (See Chart 1 for Atom Numbering)

		isolated species and energies (Eh)				
radicals	energies (Eh)	guanine/(+H) moiety		cytosine/(+H) moiety		dissociation energies (D_e) (kcal/mol)
G−C	−937.71869	G	−542.66217	C	−395.01323	27.2 27.5[a]
G(C8)−C	−938.28079	G(C8)	−543.22269	C	−395.01323	28.1
G−C(N3)	−938.27585	G	−542.66217	C(N3)	−395.57852	22.1
G−C(C5)	−938.27503	G	−542.66217	C(C5)	−395.56846	27.9
G−C(C6)	−938.27152	G	−542.66217	C(C6)	−395.56521	27.7
G(N7)−C	−938.26148	G(N7)	−543.20692	C	−395.01323	25.9
G(C4)−C	−938.25296	G(C4)	−543.20106	C	−395.01323	24.3
G(C5)−C	−938.24890	G(C5)	−543.19774	C	−395.01323	23.8
G(C2)−C	−938.24606	G(*C2*)	−543.20931	C	−395.01323	14.8
G(O6)−C	−938.24108	G(O6)	−543.19648	C	−395.01323	19.7
G−C(O2)	−938.23572	G	−542.66217	C(O2)	−395.56029	8.3
G(N3)−C	−938.23540	G(N3)	−543.19012	C	−395.01323	20.1
G−C(C4)	−938.22578	G	−542.66217	C(C4)	−395.53060	20.7

[a] This is the RI-MP2/(aDZ → aTZ) result selected by Sponer et al. in their latest ab initio research to compare with other theoretical results (see ref 48).

The pairing energy of 25.9 kcal/mol corresponds to the dissociation of the radical into the hydrogenated guanine radical plus neutral cytosine.

f. G(C4)−C. As a derivative of purine, guanine has both a six-membered ring and a five-membered ring. The two rings share the C4═C5 double bond. The radical isomer G(C4)−C (Figure 7) is produced when a hydrogen atom is added to position C4. The total energy of this isomer is 17.5 kcal/mol higher than the lowest-energy radical G(C8)−C (Figure 2). The geometrical perturbations with respect to closed-shell G−C are large because the planarity of both rings is destroyed when the shared double bond is diminished to a C−C single bond. In this process, the C4═C5 distance (1.405 Å) is increased to 1.513 Å for the radical. However, unlike the Lewis dot picture, the unpaired electron is partially distributed in the vicinity of C4 (spin density 0.42) and C2

Table 3. Relative Energies of Radicals Derived from Cytosine (C + H)• at the B3LYP/DZP++ Level of Theory

	non-ZPVE		ZPVE-corrected
radical	total energy (Eh)	relative energy (kcal/mol)	relative energy (kcal/mol)
C(N3)	−395.57852	0.0	0.0
C(C5)	−395.56846	6.3	6.2
C(C6)	−395.56521	8.4	8.0
C(O2)	−395.56029	11.4	11.0
C(C4)	−395.53060	30.1	29.8

Table 4. Relative Energies of Radicals Derived from Guanine (G + H)• at the B3LYP/DZP++ Level of Theory

	non-ZPVE		ZPVE-corrected
radical	total energy (Eh)	relative energy (kcal/mol)	relative energy (kcal/mol)
G(C8)	−543.22269	0.0	0.0
G(C2)	−543.20931	8.4	9.2
G(N7)	−543.20692	9.9	10.4
G(C4)	−543.20106	13.6	13.9
G(C5)	−543.19774	15.7	15.4
G(O6)	−543.19648	16.4	16.9
G(N3)	−543.19012	20.4	20.9

(spin density 0.26). All three hydrogen-bond distances increase, by 0.031, 0.036, and 0.051 Å compared to the closed-shell G−C, and the dissociation energy (Table 2) of G(C4)−C is 24.3 kcal/mol.

g. G(C5)−C. Structure G(C5)−C (Figure 8a) is the other radical isomer resulting when the shared double bond of the purine is broken. The resulting C4−C5 single bond distance (1.510 Å) is 0.003 Å less than that just discussed. The total energy of this isomer is 20.0 kcal/mol high than the lowest-energy radical G(C8)−C (Figure 2). As found for the previous radical isomer G(C4)−C, the inclined geometry of the two rings in guanine is not a simple open-book geometry but a twisted open-book structure (Figure 8b illustrates the nonplanarity). The three hydrogen-bond distances lengthen by 0.037, 0.008,and 0.058 Å compared to G−C. The predicted dissociation energy of 23.8 kcal/mol is also very comparable to that of G(C4)−C, namely, only 0.5 kcal/mol higher.

h. G(C2)−C. In the formation of this radical, the hydrogen atom breaks the guanine C2═N3 double bond by adding to the C2 site. The total energy of G(C2)−C is predicted to be 21.8 kcal/mol higher than the lowest-energy radical G(C8)−C. The resulting minimum undergoes a qualitative structural change (Figure 9a) compared to closed-shell G−C pair. In the new radical, the two hydrogen atoms of the guanine amino group are so far from the cytosine moiety that no N2H2a···O2 hydrogen bond remains. If this radical can be formed in the DNA damage process, the resulting adduct will cause significant strain (see Figure 9b) in the closely stacked base pairs (∼3.4 Å stacking distance[51]), which may result in an SSB of the DNA backbone. The remaining two H bonds (O6···H4aN4 and N1H1···N3) are somewhat elongated (by 0.072 and 0.012 Å) compared to those for the closed-shell G−C. The radical dissociation energy for G(C2)−C is low, 14.8 kcal/mol, since only the two hydrogen bonds remain (Table 2).

i. G(O6)−C. The radical resulting from hydrogen-atom addition to the carbonyl oxygen of guanine, structure G(O6)−C (Figure 10a), is predicted to lie 24.9 kcal/mol higher than the energy sink G(C8)−C on the potential surface for hydrogen addition to G−C. In structure G(O6)−C, atom O6 is transformed from the oxo to hydroxyl form. As a result, the C6−O6 bond distance is lengthened by 0.166 Å compared to closed-shell G−C. The atom C6 carries a spin density of 0.90, still lying in the purine plane, consistent with the simple Lewis dot structure. The three H-bond distances are 1.918, 1.950 and 1.959 Å. Nevertheless, the twisted pairing geometry (Figure 10b) is not favorable for hydrogen bonding. The pairing (dissociation) energy is only 19.7 kcal/mol, which is the second lowest among the radical isomers retaining three hydrogen bonds.

j. G−C(O2). Structure G−C(O2) (Figure 11a) is formed by hydrogen-atom addition to the oxygen atom of the cytosine carbonyl group. The G−C distance O2═C2 increases from 1.241 to 1.371 Å for the newly formed radical. The attached H2 atom on the O2 site is out of plane by 30.5° (dihedral angle H2−O2−C2−N1). Because of the steric interaction with atom H2, the cytosine H1 atom also shows an obvious distortion from the ring plane by 37.1° (dihedral angle H1−N1−C2−O2). The total energy of G−C(O2) is 28.3 kcal/mol above that of the global minimum G(C8)−C (Figure 2). The NAB pairing interaction also shows interesting features. The theoretical dissociation energy is only 8.3 kcal/mol, much lower than other radical isomers with three H bonds. Compared with the isolated hydrogen-added cytosine isomer C(O2 + H), the geometry of the cytosine moiety in G−C(O2) is different because of the steric interaction between the additional hydrogen atom H2 and the cytosine atom H1. The guanine and cytosine rings twist (Figure 11b), because of the pyramidization of the amino group in cytosine. As for G(O6)−C, the twisted pairing geometry is not favored for H-bond formation, with 0.163, 0.112, and 0.216 Å elongations in bond distances compared to the neutral G−C pair.

k. G(N3)−C. When the hydrogen atom adds to the guanine N3 atom, a radical formally centered at C2 is produced (Figure 12). Breaking the N3═C2 π bond increases this distance by 0.108 Å. This radical is predicted to lie 28.5 kcal/mol above the lowest-energy radical G(C8)−C. The guanine amino group changes from planar to nonplanar partly because of the new sp^3 hybridization at C2. The guanine amino group adjacent to the radical center moves slightly out of plane to diminish the nitrogen lone-pair electron interaction with the localized single electron of C2 (with a spin density of 0.88, following the simple valence picture). As a result, the C2−N2 bond rotation causes the N2H2a••·O2 distance to be lengthened by 0.282 Å. The other two H bonds are increased by 0.016 and 0.049 Å when compared with the closed-shell G−C pair (Figure 1). An energy of 20.1 kcal/mol (Table 2) is required to dissociate this radical base pair, compared to 27.2 kcal/mol for the neutral G−C base pair.

l. G-C(C4). Structure G−C(C4) (Figure 13a) is the isomer with the highest energy of our family of 12 radicals, lying 34.5 kcal/mol higher than the global minimum radical G(C8)−C structure. G−C(C4) is generated by attaching a hydrogen atom to the C4 position of cytosine. Like the previously discussed radical G(C2)−C (Figure 9a), the additional hydrogen causes the carbon atom which connects to the amino group to be transformed qualitatively from sp^2 to sp^3 hybridization. This lengthens the G−C bond N3═C4 from 1.342 to 1.454 Å for the new radical. The amino group thus moves out of the cytosine plane by 78.1° (dihedral angle N4−C4−C5−C6 is 101.9°). The H bond O6···H4aN4 still exists, unlike the vanishing N2H2a···O2 bond in G(C2)−C (Figure 9a). The dissociation energy is 20.7 kcal/mol, about 6.0 kcal/mol higher than that of the radical G(C2)−C with two H bonds. The planarity of the G−C pair has been lost in order to form three H bonds, since the cytosine amino group is significantly out of plane. The guanine and cytosine are twisted as seen in Figure 13b.

Conclusions

In general, the geometrical perturbations are significant in going from the neutral G−C pair to the (G + H)•−C and G−(C + H)• radicals. A total of 10 of the 12 isomers deviate from the original C_s symmetry of the G−C pair in the gas phase. The other two radical isomers, G(C8)−C and G−C(C6), maintain C_s symmetry. Classified by the hydrogen-atom addition site, the 12 radical isomers may be divided into three groups: (1) The hydrogen atom adds to an atom that is close to or directly related to the three interstrand H bonds of the base pair. (2) The hydrogen atom adds to the C4═C5 bridgehead double bond of guanine. (3) The hydrogen atom adds to one of the five atoms away from the G−C hydrogen bonds.

Among the three groups, radicals from the first group undergo the largest structural changes and are the least favored energetically [relative energies ranged 21.8∼34.5 kcal/mol, except for G-C(N3)]. The formation of radicals G(C2)−C (Figure 9a) and G−C(C4) (Figure 13a) may cause significant strain in $\pi-\pi$ stacked DNA bases. Another radical, G−C(N3) (Figure 3), which changes the base pairing sequence, may cause a major lesion of DNA, so as to shift the entire G−C backbone. Compared to the H bonds of neutral G−C (dissociation energy ∼27.0 kcal/mol), all [except G−C(N3)] H bonds in this group show longer bond distances and lower dissociation energies, which can be as small as 8.3 kcal/mol [G−C(O2)].

The two radicals, G(C4)−C (Figure 7) and G(C5)−C (Figure 8), classified in our second group show intermediate total energies (relative energies 17.5 and 20.0 kcal/mol) and pairing energies. The significant geometry disturbances are attributed to the "butterfly" structure of the two-ring guanine system. Since the planarity of guanine is gone, $\pi-\pi$ stacking conjugation may serve to create vertical base−base interactions.

The third group, composed of five radicals, has the lowest relative energies (below 12.1 kcal/mol) and highest dissociation energies (above 25.9 kcal/mol). The planar structure is maintained for radicals G(C8)−C and G−C(C6), since both are formed without qualitative perturbation of the three H bonds. Most H-bond lengths for these radical isomers are even shorter than those of G−C. As the most probable products of NAB lesion following hydrogen-atom attachment, these structures suggest that the formation of hydrogenated G−C pairs may not cause immediate G−C dissociation.

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Appendix

Tables 3 and 4 show the relative energies of radicals derived from cytosine (C + H)• and guanine (G + H)• at the B3LYP/DZP++ level of theory.

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