Type I Benzophenone-Mediated Nucleophilic Reaction of 5′-Amino-2′,5′-dideoxyguanosine. A Model System for the Investigation of Photosensitized Formation of DNA–Protein Cross-Links

Bénédicte Morin and Jean Cadet*

CEA/Département de Recherche Fondamentale sur la Matière Condensée, SESAM/LAN, F-38054 Grenoble Cedex 9, France

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5′-Amino-2′,5′-dideoxyguanosine has been synthesized in order to investigate the intramolecular reactivity of an amino group toward the guanine radical produced by type I photosensitization mechanism. Benzophenone-mediated photosensitization of 5′-amino-2′,5′-dideoxyguanosine in aerated aqueous solution results in the formation of a predominant cyclic nucleoside together with an unstable nucleoside precursor. The two modified nucleosides have been isolated by reverse phase high performance liquid chromatography and characterized by spectroscopic measurements including ^{13}C and ^{1}H NMR, fast atom bombardment mass spectroscopy, and UV absorption. The stable photoproduct has been identified as 9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-7-ol, 3-amino- (1*R-exo*), whereas its precursor has been assigned as acetic acid, [(7-hydroxy-9-oxa-2,4-diazabicyclo [4.2.1]non-2-en-3-yl)amino]oxo- (1*R-exo*). A reaction mechanism, involving nucleophilic addition of the sugar amino group to guanine radical intermediates, is proposed to explain the formation of the two photoproducts.

Introduction

Exposure of living organisms to solar radiation may induce mutagenic and lethal effects as the result of both direct pyrimidine dimerization and sensitized oxidative modifications to genomic DNA (*1–3*). At wavelengths within the UVA region (320–400 nm), the bulk of the biological effects are mediated by photodynamic processes involving endogenous and exogenous photosensitizers (*4–6*). Photoexcited sensitizers, mostly in their triplet excited states, are able to generate DNA oxidative modifications *via* two principal mechanisms (*7*). Type I reaction involves either proton abstraction or electron transfer by the excited photosensitizer to, or more generally from, the substrate. Type II mechanisms occur *via* the initial generation of $^{1}O_2$ by the excited photosensitizer and subsequent reaction of the latter reactive oxygen species with the substrate (2). Purine nucleic acid components, and particularly guanine, which presents the lowest ionization potential among DNA components, are more readily photooxidized than their pyrimidine analogs. It should be noted that DNA in eukaryotic cells is in close contact with proteins such as histones. So, it is conceivable that DNA–protein cross-links (*4*, *5*) induced by photodynamic processes are important contributors to the deleterious effects of solar radiation to cells. Several authors have reported that DNA–protein cross-links, in either human or Chinese hamster cells, are generated by various photosensitizers, including chloroaluminum phthalocyanine (*8*), hematoporphyrin derivatives (*9, 10*), and methylene blue (*10, 11*). The amino acids cysteine and tryptophan were found to photobind to DNA, most likely at guanine sites, as the result of photooxidation reactions mediated by hematoporphyrin derivatives (*12*). Van Vunakis *et al.* (*13*) have reported that Tris buffer (2-amino-2-methyl-1,3-propanediol), when present in an aerated aqueous solution of [^{14}C]-2′-deoxyguanosine 5′-monophosphate (5′-dGMP)[1] exposed to visible light in the presence of methylene blue, was able to covalently bind to oxidized purine intermediates.

However, there is still an almost complete lack of structural information on photosensitized DNA–protein cross-links as well as on the mechanism of their formation. Studies on closely related model systems would provide useful information that could be relevant for a better understanding of the chemical aspects of dye photosensitized formation of DNA–protein cross-links. We have recently showed that free hydroxyl group containing compounds may react with radical guanine intermediates (*14, 15*). The present work deals with the extension of the latter model systems on photosensitized DNA–protein cross-links by investigating the reactivity of an amino function, which is present, for example, in lysine residues within proteins. For this purpose, 5′-amino-2′,5′-dideoxyguanosine (5′-NH_2dGuo) has been synthesized in order to investigate the reactivity of an amino group toward photooxidized radical intermediates of the guanine moiety. The amino group, as a nucleophilic agent, is a model sytem for mimicking the amino group of the side chain of lysine and arginine in proteins. The regions of histones strongly involved in the binding of DNA to the nucleosome core in chromatin are believed to be those which are rich in lysine and arginine (*16–19*). We would like to report the isolation by high performance liquid chromatography of two cyclic nucleosides produced upon benzophenone-mediated photosensitization of 5′-NH_2dGuo in aerated aqueous solution. The

* To whom correspondence should be addressed. Phone: (33) 76-88-49-87; Fax: (33) 76-88-50-90.

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[1] Abbreviations: FAB/MS, fast atom bombardment mass spectrometry; dGuo, 2′-deoxyguanosine; 5′-dGMP, 2′-deoxyguanosine 5′-monophosphate; 5′-NH_2dGuo, 5′-amino-2′,5′-dideoxyguanosine; HMQC, heteronuclear multi-quantum coherence; HMBC, heteronuclear multibond correlation.

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characterization of the two photoproducts as 9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-7-ol, 3-amino- (1*R-exo*), and acetic acid, [(7-hydroxy-9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-3-yl)amino]oxo- (1*R-exo*), was achieved on the basis of extensive spectroscopic measurements including FAB/MS together with ^{1}H and ^{13}C NMR analyses.

Materials and Methods

Chemicals. 2′-Deoxyguanosine (dGuo) was purchased from Sigma Chemical Co. (St. Louis, MO). Pyridine, dimethylformamide, and dichloromethane were obtained from SDS (France); *p*-toluenesulfonyl chloride and sodium azide were from Aldrich (Aldrich Chemical Co. Ltd., U.K.). Palladium-charcoal activated (10% Pd), silica gel 60, and benzophenone were from Merck (Darmstadt, Germany). "Rectapur" ammonia solution (28%) was obtained from Prolabo (Paris, France). HPLC-grade methanol was purchased from Carlo Erba (Farmitalia Carlo Erba, Milan, Italy). Ammonium formate was from BDM Laboratory Supplies, Poole (U.K.).

5′-Amino-2′,5′-dideoxyguanosine Synthesis. 5′-NH₂dGuo was synthesized according to an adaptation of a procedure described by Prusoff *et al.* (*20, 21*). This involved the synthesis of the 5′-amino analog of 2′-deoxyguanosine, *via* the 5′-*O*-tosyl and 5′-azido derivatives.

N²-Isobutyryl-2′-deoxyguanosine. The protected nucleoside was prepared by using a previously described procedure (*22*). Yield 62%; 200.13 MHz ^{1}H NMR (DMSO-d_6) δ 1.23 (m, 6H, CH(CH_3)$_2$), 2.39 (m, 1H, H2″), 2.71 (m, 1H, CH(CH$_3$)$_2$), 3.85 (m, 1H, H2′), 3.65 (m, 2H, H5′,H5″), 3.95 (m, 1H, H4′), 4.52, (m, 1H, H3′), 5.15 (t, 1H, 5′OH), 5.45 (d, 1H, 3′OH), 6.32 (t, 1H, H1′), 8.32 (s, 1H, H8); (50.61 MHz) ^{13}C NMR (DMSO-d_6) δ 18.9, 19.2 (*C*H(*C*H$_3$)$_2$), 35.0 (C-2′), 61.5 (C-5′), 70.5 (C-3′), 82.9 (C-1′), 87.7 (C-4′), 120.1 (C-5), 137.2 (C-8), 148.6 (C-4), 149.6 (C-6), 155.2 (C-2), 180.4 (*C*O).

N²-Isobutyryl-5′-*O*-(*p*-tolylsulfonyl)-2′,5′-dideoxyguanosine. N²-Isobutyryl-2′-deoxyguanosine (1 g, 2.96 mmol) and *p*-toluenesulfonyl chloride (670 mg, 3.52 mmol) were dried under reduced pressure. The resulting residue was dissolved in 10 mL of anhydrous pyridine and stirred for 20 h at room temperature. After hydrolysis of the nonreacted *p*-toluenesulfonyl chloride, the resulting solution was evaporated to dryness under diminished pressure and the residue coevaporated several times with a mixture of toluene and ethanol (3:1). Then, the residue was purified by low pressure chromatography on a silica gel column. *p*-Toluenesulfonyl hydroxyl was first eluted with 300 mL of CH_2Cl_2. N²-Isobutyryl-3′,5′-di-*O*-(*p*-tolylsulfonyl)-2′,5′-dideoxyguanosine was removed from the column by 400 mL of CH_2Cl_2/CH_3OH (98:2), and finally N²-isobutyryl-5′-*O*-(*p*-tolylsulfonyl)-2′,5′-dideoxyguanosine was eluted by 400 mL of CH_2Cl_2/CH_3OH (95:5). After evaporation of the solvent, 1 g of N²-isobutyryl-5′-*O*-(*p*-tolylsulfonyl)-2′,5′-dideoxyguanosine (68%) was obtained as a white solid. 200.13 MHz ^{1}H NMR (DMSO-d_6) δ 1.25 (m, 6H, CH(CH_3)$_2$), 2.48 (m, 1H, H2″), 2.49 (s, 3H, CH_3), 2.84 (m, 1H, H2′), 2.87 (m, 1H, CH(CH$_3$)$_2$), 4.08 (m, 1H, H4′), 4.32 (m, 2H, H5′,H5″), 4.48 (m, 1H, H3′), 5.61 (d, 1H, 3′OH), 6.27 (t, 1H, H1′), 7.45 (d, 2H, aromatic), 7.79, (d, 2H, aromatic), 8.18 (s, 1H, H8), 9.17 (s, 1H, NH), 9.68 (s, 1H, NH); (50.61 MHz) ^{13}C NMR (DMSO-d_6) δ 18.5, 18.7, 18.8 (*C*H(*C*H$_3$)$_2$), 21.0 (*C*H$_3$), 34.7 (C-2′), 70.1, 70.3 (C-5′, C-3′), 83.1, 83.9 (C-1′, C-4′), 120.4 (C-5), 127.4, 129.8 (aromatic), 132.1 (C-1), 137.5 (C-8), 144.9 (C-4), 147.8 (C-6), 154.7 (C-2), 180.0 (*C*O); MS (FAB+) *m/z* 1005 (15, [2M + Na]), 983 (7, [2M + H]$^+$), 514 (85, [M + Na]), 492 (72, [M + H]$^+$), 222 (100, [B + 2H]$^+$).

N²-Isobutyryl-5′-azido-2′,5′-dideoxyguanosine. A solution of N²-isobutyryl-5′-*O*-(*p*-tolylsulfonyl)-2′,5′-dideoxyguanosine (0.6 g, 1.2 mmol) and sodium azide (0.7 g, 10 mmol, 9 equiv) in dry *N*,*N*-dimethylformamide (8 mL) was stirred magnetically for 3 h at 90 °C. Sodium azide was removed by filtration and washed with ethanol. The resulting limpid solution was evaporated under reduced pressure, providing 0.35 g of N²-isobutyryl-5′-azido-2′,5′-dideoxyguanosine (79%), which was used without further purification for the next step. 200.16 MHz ^{1}H NMR (DMSO-d_6) δ 1.20 (m, 6H, CH(CH_3)$_2$), 2.34 (m, 1H, H2″), 2.83 (m, 1H, CH(CH$_3$)$_2$), 2.97 (m, 1H, H2′), 3.61 (m, 2H, H5′,H5″), 3.78 (m, 1H, H4′), 4.07 (m, 1H, H3′), 4.48 (m, 1H, 3′OH), 6.40 (t, 1H, H1′), 8.23 (s, 1H, H8); (50.61 MHz)^{13}C NMR (DMSO-d_6) δ 19.1; 20.8 (*C*H(*C*H$_3$)$_2$), 35.0 (C-2′), 52.0 (C-5′), 71.2 (C-3′), 83.2, 85.4 (C-1′, C-4′), 120.4 (C-5), 137.2 (C-8), 145.5 (C-4), 149.1 (C-6), 150.0 (C-2), 179.6 (*C*O); MS (FAB+) *m/z* 385 (70, [M + Na]), 222 (30, [B + 2H]$^+$).

N²-Isobutyryl-5′-amino-2′,5′-dideoxyguanosine. A mixture of N²-isobutyryl-5′-azido-2′,5′-dideoxyguanosine and 10% palladium-on-charcoal (0.35 g) in 1:1 ethanol/water solution (60 mL) was hydrogenated for 6 h at room temperature at a pressure of 50 bars. Then, the catalyst was removed by filtration through a Celite pad, and the filtrate was evaporated to dryness under vacuum. The resulting residue was dissolved in 5 mL of water, prior to HPLC analysis. This was achieved on the ODS column (300 × 7.5 mm i.d., mean particle size 10 μm) from Macherey-Nagel (Düren, Germany) by using 25 mM NH_4HCO_2 buffer with a 8:2 (v/v) mixture of H_2O and CH_3OH as the isocratic eluent at a flow rate of 3 mL/min. The fraction, corresponding to the HPLC peak (k' = 4.3) provided 300 mg of N²-isobutyryl-5′-amino-2′,5′-dideoxyguanosine. 200.16 MHz ^{1}H NMR (DMSO-d_6) δ 1.24 (m, 6H, CH(CH_3)$_2$), 2.42 (m, 1H, H2″), 2.87 (m, 1H, H2′), 2.93 (m, 1H, CH(CH$_3$)$_2$), 3.17 (m, 2H, H5′, H5″), 4.05 (m, 1H, H4′), 4.67 (m, 1H, H3′), 6.36, (t, 1H, H1′), 8.31 (s, 1H, H8); 50.61 MHz ^{13}C NMR (DMSO-d_6) δ 18.9, 19.2 (*C*H(*C*H$_3$)$_2$), 34.8 (C-2′), 42.0 (C-5′), 70.8 (C-3′), 83.4, 85.4 (C-1′, C-4′), 120.7 (C-5), 138.2 (C-8), 148.1, 148.3 (C-4, C-6), 154.9 (C-2), 180.2 (*C*O); MS (FAB+) *m/z* 673 (15, [2M + H]$^+$), 359 (12, [M + Na]), 337 (32, [M + H]$^+$), 222 (100, [B + 2H]$^+$), 152 (37, [B − [COCH(CH$_3$)$_2$] + 2H]$^+$), 116 (23, S$^+$).

5′-Amino-2′,5′-dideoxyguanosine. The isobutyryl group of N²-isobutyryl-5′-amino-2′,5′-dideoxyguanosine (300 mg) was removed by treatment with 28% ammonia aqueous solution. The mixture was stirred magnetically for 10 days at room temperature. The resulting solution was evaporated to dryness and coevaporated several times with water to eliminate the ammonia. The residue was resuspended in 5 mL of water prior to HPLC analysis. The 5′-amino-2′,5′-dideoxyguanosine was purified by HPLC as described earlier. The fractions corresponding to the fastest eluting HPLC peak (k' = 1.3) were collected and lyophilized, giving 200 mg of 5′-amino-2′,5′-dideoxyguanosine as a white solid (60% with respect to N²-isobutyryl-5′-*O*-(*p*-tolylsulfonyl)-2′,5′-dideoxyguanosine). 400.13 MHz ^{1}H NMR (DMSO-d_6) δ 2.34 (m, 1H, H2″), 2.73 (m, 1H, H2′), 3.12 (s, 1H, H5″), 3.13 (s, 1H, H5′), 4.03 (m, 1H, H4′), 4.55 (m, 1H, H3′), 6.27 (dd, 1H, H1′), 6.97 (s, 2H, NH_2), 8.00 (s, 1H, H8); 100.61 MHz ^{13}C NMR (DMSO-d_6) δ 38.6 (C-2′), 41.7 (C-5′), 71.1 (C-3′), 83.2 (C-1′), 84.6 (C-4′), 117.3 (C-5), 135.9 (C-8), 147.5 (C-2), 150.6 (C-4), 154.1 (C-6); MS (FAB$^+$) *m/z* 289 (10, [M + Na]), 267 (90, [M + H]$^+$), 152 (100, [B + 2H]$^+$); HRMS: calcd. for $C_{10}H_{14}N_6O_3$, 267.1206; found 267.1200.

Acetylation of Photoproducts 7 and 8. The photoproducts **7** and **8** were dissolved in 0.2 mL of acetic anhydride and 1 mL of pyridine. The resulting solution was stirred magnetically for 6 h at 20 °C. Then, the pyridine was removed under vacuum and the mixture was lyophilized.

Photosensitization. A 50 mL of aqueous solution saturated with benzophenone (4×10^{-4} M) containing 50 mg of 5′-amino-2′,5′-dideoxyguanosine (**1**) in a 100 mL test tube was exposed for 2 h to 16 black lamps (λ_{max} = 350 nm) in a Rayonet photochemical reactor (Rayonet, Southern New England Ultraviolet Co., Hamden, CT). A continuous flow of air maintained the solution saturated with oxygen during the irradiation. Then, the solutions were evaporated to dryness under reduced pressure, and the resulting residue was resuspended in a minimun volume of water prior to HPLC analysis.

Chromatography of Photoproducts. Samples were injected onto a homemade semipreparative Nucleosil octadecylsilyl silica gel column (300 × 7.5 mm i.d., mean particle size 10 μm) from Macherey-Nagel (Düren, Germany). The separation of the photoproducts was achieved by using a 0.025 M ammonium formate aqueous solution as the isocratic eluent. The HPLC

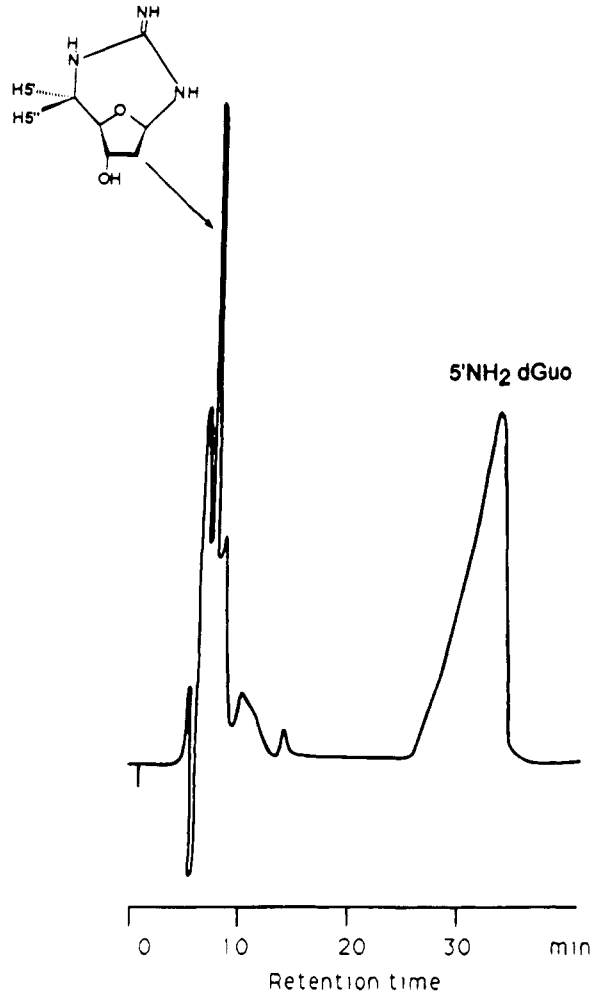

Figure 1. HPLC elution profile of the products of benzophenone-mediated photosensitization of 5′-amino-2′,5′-dideoxyguanosine (**1**) in aerated aqueous solution on an ODS column.

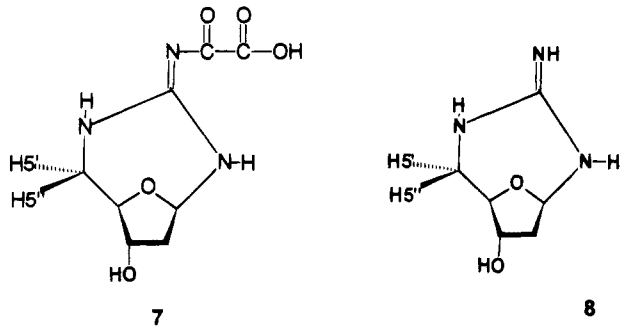

Figure 2. Structure of 5′-NH_2-dGuo photoproducts: acetic acid, [(7-hydroxy-9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-3-yl)amino]-oxo- (1*R-exo*) (**7**), and 9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-7-ol, 3-amino- (1*R-exo*) (**8**).

system consisted of a dual pump M 6000A (Waters Associates, Milford, MA) equipped with a Rheodyne Model 7125 (Berkeley, CA) injector loop and a differential refractometer Waters R401 (Millipore, Milford, MA). The major bands were collected, pooled,and concentrated by rotary evaporation. Then, the residue was lyophilized a minimun of three times in order to remove ammonium formate.

Spectroscopic Measurements. ^1H and ^{13}C NMR spectra were recorded on AM 400 and AM 200 Bruker spectrometers (Bruker, Wissembourg, France). The chemical shifts are expressed in ppm with respect to tetramethylsilane (TMS) used as internal reference in 99.8% DMSO-d_6. Two-dimensional $^1H-^{13}C$ heteronuclear correlated NMR experiments (HMQC) were performed on a Unity 400 Varian apparatus operating at 400.13 MHz for ^1H and 100.61 MHz for ^{13}C NMR analysis. Long-range $^1H-^{13}C$ coupling experiments (HMBC) were achieved on a Unity Varian 500 apparatus. The H5′ signal was assigned downfield to H5″ proton according to Remin and Shugar (*23*). The H2′/H2″ protons were further assigned on the basis of coupling constant arguments (*24, 25*). The spectra were computer simulated using the iterative LAOCOON III program in order to verify the assignments and to obtain accurate chemical shifts and coupling constants. FAB mass spectra were recorded in the positive mode by using a Model ZAB 2-SEQ spectrometer (Fisons-V.G, Manchester, United Kingdom) equipped with a LSIMS source. The molecules were dissolved in a thioglycerol matrix.

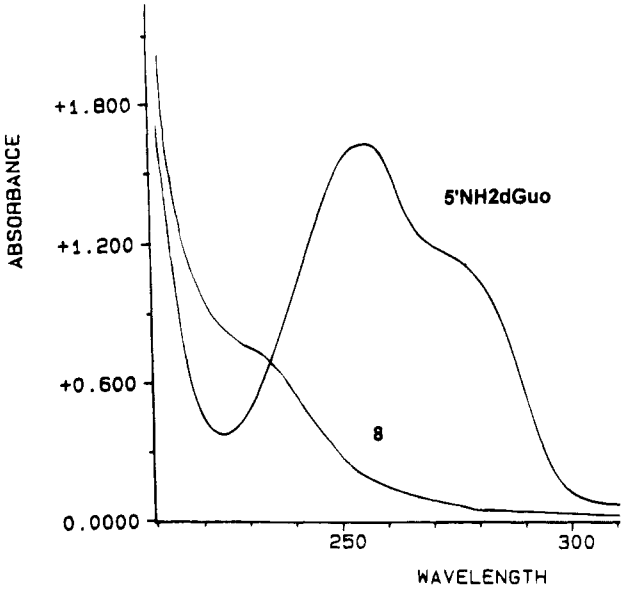

Figure 3. UV spectra of 5′-NH_2dGuo (**1**) and **8** in H_2O.

Results and Discussion

Attemps to induce type I photosensitized formation of adducts between 1 mM of dGuo and 1 mM of free amino acids such as lysine were unsuccessful. This may be explained by the predominance of nucleophilic reactions mediated by water molecules which lead to the formation of imidazolone and oxazolone compounds (*26*). Therefore, the choice of a proper model system appears critical in such studies. The model system utilized in the present study was 5′-amino-2′,5′-dideoxyguanosine (**1**). The purpose of substituting the 5′-hydroxymethyl of dGuo by an amino function was to mimic the close interaction between DNA and histones in cells.

Synthesis of 5′-Amino-2′,5′-dideoxyguanosine (1). 5′-Amino-2′,5′-dideoxyguanosine (**1**) which was synthesized *via* the 5′-*O*-tosyl and 5′-azido intermediates was isolated by reverse phase high performance liquid chromatography and characterized by ^1H and ^{13}C NMR and FAB mass spectroscopy. The FAB mass spectrum in the positive mode of **1** shows a pseudomolecular ion at *m/z* 267 $[M + H]^+$. The exact mass measurement of 5′-amino-2′,5′-dideoxyguanosine obtained from a high resolution FAB mass spectrum is 267.1200. This indicates an empirical formula of $C_{10}H_{14}O_3N_6$. It should be noted that only one signal for two NH_2 groups and one amide proton was obtained in the ^1H spectrum in DMSO-d_6 of 5′-NH_2-dGuo because these protons are in exchange with traces of water. The ^{13}C NMR spectrum exhibits 10 carbon resonance signals. Assignment of the ^{13}C signals was achieved by 2D $^1H-^{13}C$ correlated and DEPT NMR

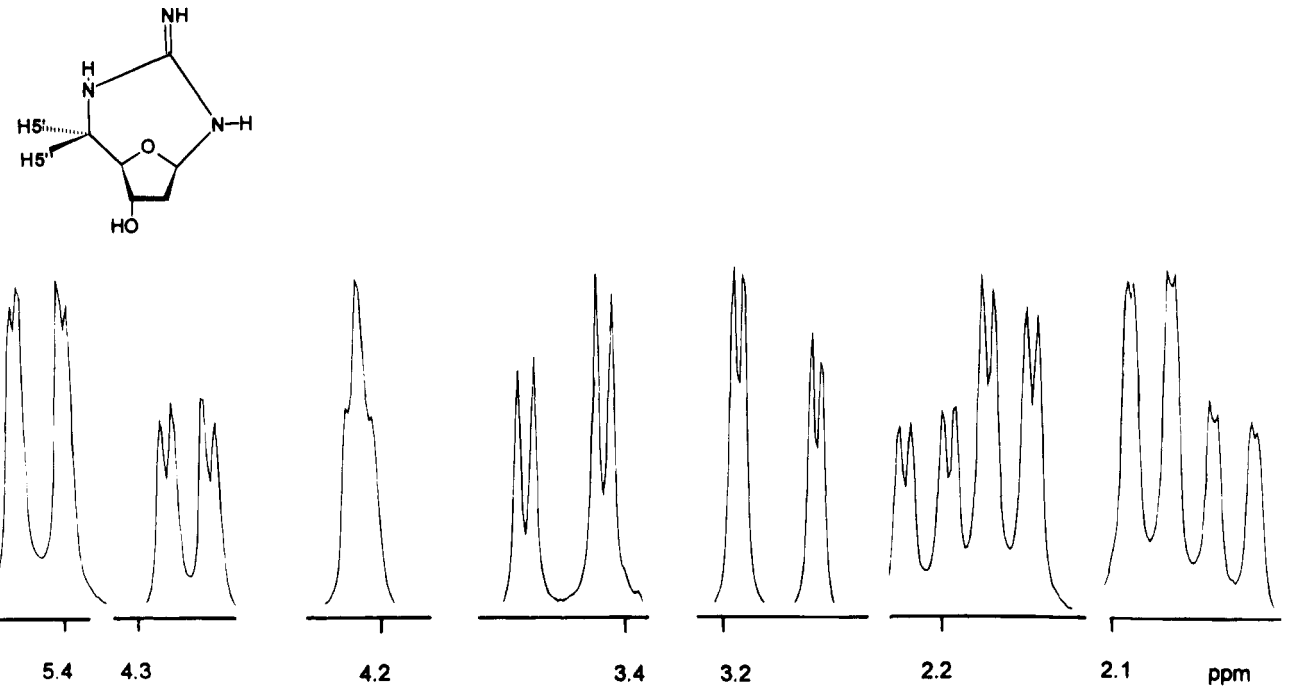

Figure 4. 400.13 MHz ^{1}H NMR spectrum of **8** in DMSO-d_6.

analysis (data not shown). Most noticeably, the C-5′ resonance (δ = 41.7 ppm) of 5′-amino-2′,5′-dideoxyguanosine (**1**) is shifted 20.4 ppm upfield relative to that of dGuo (δ = 62.1 ppm), as expected from the reduced electronegative effect of an amino group with respect to a hydroxyl function.

Isolation and Characterization of the Two Main Benzophenone-Photosensitized Decomposition Products. The HPLC elution profile of the benzophenone-mediated photosensitized oxidation products of 5′-NH_2dGuo (**1**) is illustrated in Figure 1. The fastest eluting HPLC fraction (retention time = 7 min) was found to contain the two main photoproducts **7** and **8**. The stable compound and its precursor (Figure 2) are identified as 9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-7-ol, 3-amino- (1*R-exo*) (**8**), and acetic acid, [(7-hydroxy-9-oxa-2,4-diazabicyclo[4.2.1]non-2-en-3-yl)amino]oxo- (1*R-exo*) (**7**), respectively, on the basis of extensive spectroscopic measurements (*vide infra*). The relative rate of **7** and **8** was found to depend on the length of the irradiation. Photoproduct **7** is not very stable in aqueous solution since it was totally converted into **8** within 5 h when left in aqueous solution at 20 °C. It should be noted that the two photoproducts **7** and **8** are more rapidly eluted on the ODS column than 5′-NH_2dG (**1**) since they had lost their aromatic character (*vide infra*). In addition, there is no detectable amount of other photooxidized products of **1** in the HPLC elution profile.

FAB Mass Spectrometry. The molecular weight of **8** was determined to be 157 as inferred from mass spectrometry measurements: (1) the FAB mass spectrum in the positive mode of **8** exhibits a pseudomolecular ion at *m/z* 158 $[M + H]^+$ and an ion at *m/z* 250 corresponding to a glycerol adduct; (2) the exact mass measurement of the molecular ion of **8** as inferred from high resolution FAB mass analysis provided a molecular weight of 158.0942. This suggests an empirical formula of $C_6H_{11}N_3O_2$; (3) when **8** was acetylated, the positive mode FAB mass spectrum revealed the presence of two peaks at *m/z* 200 $[M + OAc + H]^+$ and *m/z* 242 $[M + 2OAc + H]^+$. The latter observations may be rationalized in terms of two structures corresponding to a mono- and the di-O-acetylated derivatives of **8**, respectively.

Table 1. Proton Chemical Shifts δ (ppm) and Coupling Constants *J* (Hz) for 5′-NH_2dGuo (1) and Photoproducts 7 and 8 Obtained at 400.13 MHz in DMSO-d_6 (δ, ppm from TMS) as Inferred from Computer Iterative Analysis (LAOCOON III Program)

	Chemical Shifts δ (ppm)								
	H1′	H2′	H2″	H3′	H4′	H5′	H5″	H8	NH_2/NH
5′-NH_2dGuo	6.27	2.73	2.34	4.55	4.03	3.13	3.12	8.00	6.97
7	5.65	2.16	2.12	4.28	4.27	3.70	3.29		
8	5.41	2.06	2.19	4.28	4.21	3.42	3.18		7.70
	Coupling Constants *J* (Hz)								
	$J_{1'2'}$	$J_{1'2''}$	$J_{2'2''}$	$J_{2'3'}$	$J_{2''3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$
5′-NH_2dGuo	7.5	6.5	−13.4	6.0	3.5	3.1	3.2	8.5	−13.5
7	2.3	6.5	−14.4	6.4	2.2	0.4	2.7	2.3	−14.2
8	0.9	7.7	−14.4	7.2	1.9	0.3	2.9	1.8	−14.2

A pseudomolecular ion was observed at *m/z* = 230 in the FAB mass spectrum of **7**. This is indicative of a molecular mass of 229. The highest mass fragment (*m/z* = 252) which differs by 22 mass units from the molecular peak is characteristic of a quasimolecular ion [M + Na]. FAB/MS of the monoacetylated product exhibited a pseudomolecular ion at *m/z* = 272 which differs by 42 mass units from that of **7**. It should be noted that a pseudomolecular ion at *m/z* = 294 $[M + OAc + Na]^+$ was also observed. This suggests $C_8H_{11}O_5N_3$ as the empirical formula for **7**. However, no exact mass measurement was obtained because of the weak ionization of photoproduct **7** from the thioglycerol matrix.

UV Absorption. The UV absorption spectra of 5′-NH_2dGuo (**1**) and **8** in H_2O are shown in Figure 3. It should be noted that, in contrast to 5′-NH_2dGuo (**1**), photoproduct **8** did not exibit any absorption band around 280 and 250 nm. However, we may observe a gentle increase in absorption toward shorter wavenlengths. This strongly suggests that the base residue in **8** is modified and has lost considerable aromaticity.

Proton NMR. The 400.13 MHz ^{1}H NMR spectrum of **8** is illustrated in Figure 4. The proton chemical shifts

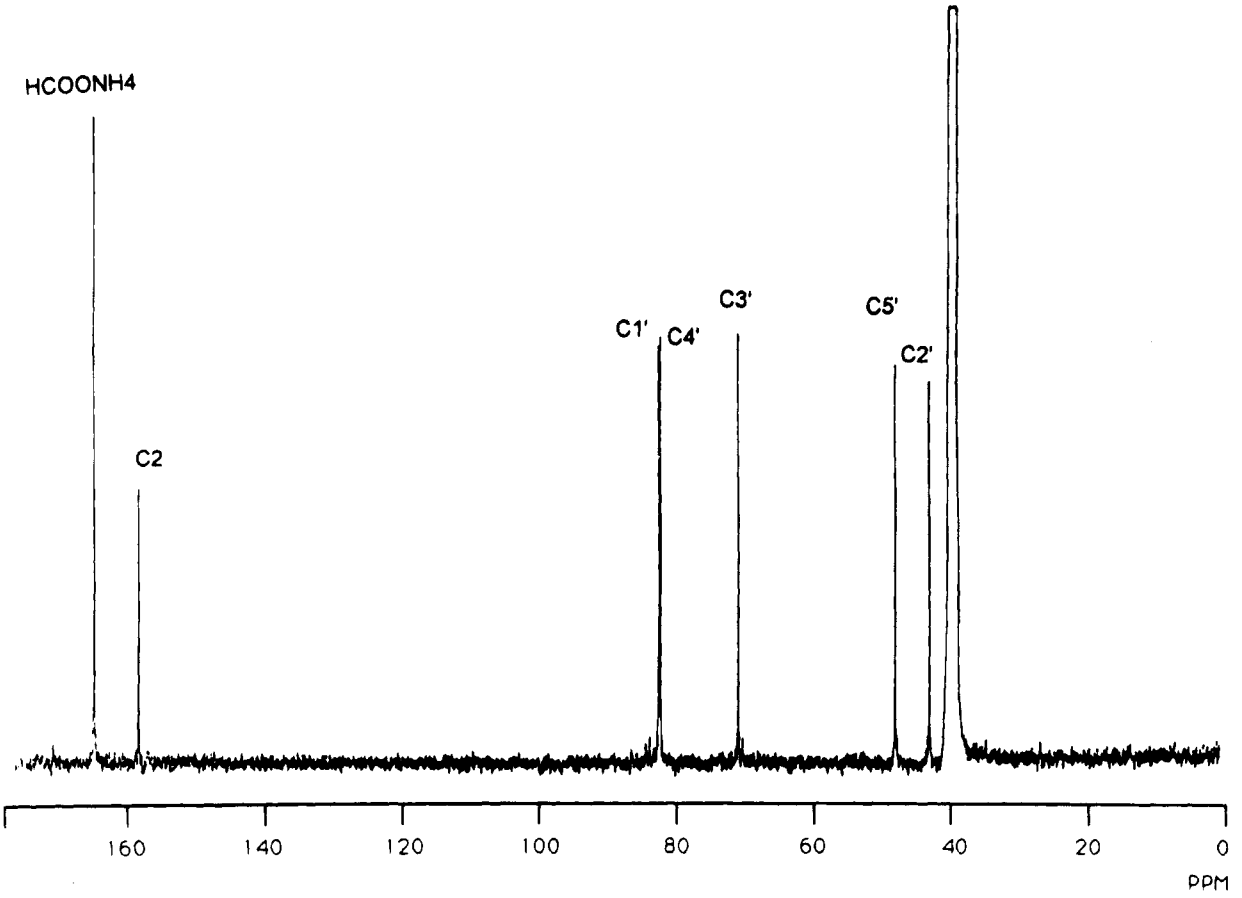

Figure 5. 100.13 MHz proton-decoupled ^{13}C NMR spectrum of **8** in DMSO-d_6.

and coupling constants of 5′-NH_2dGuo (**1**) and photoproducts **7** and **8** are listed in Table 1. First, it should be noted that the ^1H NMR spectrum of **8** shows the loss of the H8 proton. Evidence that **8** has a rigid constrained structure is given by the low magnitude of the *trans* $J_{1'2'}$ and $J_{3'4'}$ coupling constants as already observed for other cyclic nucleosides involving the 5′-hydroxymethyl groups (*27–32*). The low magnitude of the $J_{1'2'}$ and $J_{3'4'}$ coupling constants is due to a severe distortion within the sugar moiety induced by the additional sugar–base covalent bond which forces the sugar ring to adopt a coplanar conformation. The consequence of such a conformation is that all vicinal *trans* protons have dihedral angles close to 90°, as inferred from the low magnitude of $J_{1'2'}$ and $J_{3'4'}$ coupling constants. Further confirmation of the assignment of **8** is provided by the unusual low values of $J_{4'5'}$ and $J_{4'5''}$ constants that were determined to be 2.9 and 1.8 Hz, respectively. This suggests a staggered *gauche-gauche* (gg) orientation about the C4′–C5′ bond. Examination of molecular models shows that the presence of a C2–O5′ covalent bond limits the orientation about the C4′–C5′ bond to gg rotamers. Such a structure is confirmed by the determination of the *gauche-gauche* (gg), *trans-gauche* (tg), and *gauche-trans* (gt) conformer populations using the Karplus equation (*33*). Photoproduct **7** exhibits similar ^1H NMR features to **8** with only slight differences, suggesting a closely related structure. In particular, the ^1H NMR spectrum of **7** shows also the lack of the H8 proton. The ^1H NMR chemical shifts of the sugar protons of **7** are similar to those of **8** with, however, a slight downfield shift for the H1′, H4′, H5′, and H5″ signals. The magnitude of *trans* $J_{1'2'}$, $J_{2''3'}$, and $J_{3'4'}$ coupling constants is also low. This is indicative of a rigid structure for the sugar moiety, part of which is also involved in a seven-membered ring. It may be concluded that **7** is a cyclic nucleoside as confirmed by

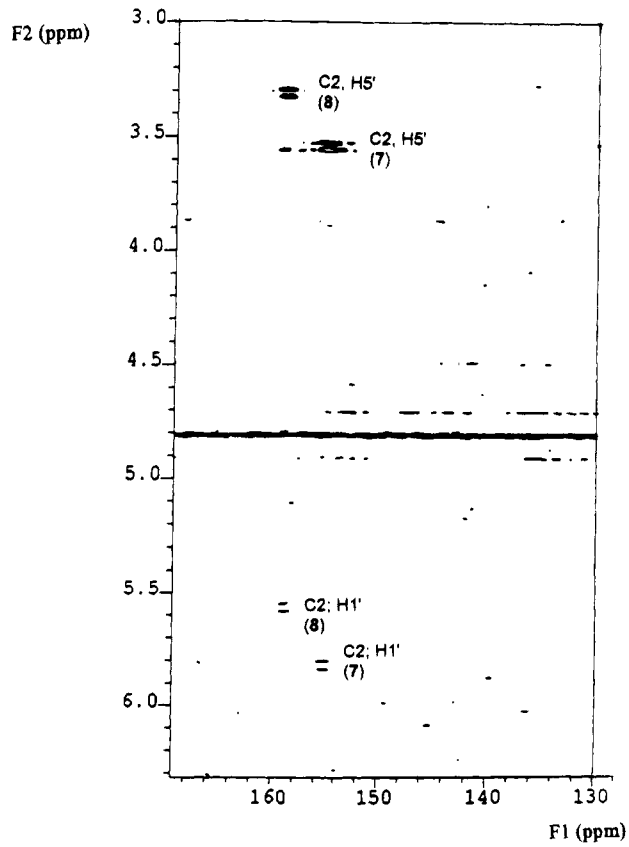

Figure 6. Long-range coupling experiment (HMBC) of a mixture of photoproducts **7** and **8** in D_2O .

Table 2. Carbon Chemical Shifts δ (ppm) for 5′-NH_2dGuo (1) and 7 and 8 Obtained at 100.61 MHz in DMSO-d_6 (δ, ppm from TMS)[a]

δ[a] (ppm)	C-1′	C-2′	C-3′	C-4′	C-5′	C-2	C-4	C-5	C-6	C-8 (C-2)
5′-NH_2dGuo	83.2	38.6	71.1	84.6	41.7	147.5	150.6	117.3	154.1	135.9
7	82.8	42.9	71.2	84.3	48.4					154.0
8	83.0	43.1	71.5	83.3	48.2					159.8

[a] The C-8 carbon is now the C-2 carbon of **7** and **8**.

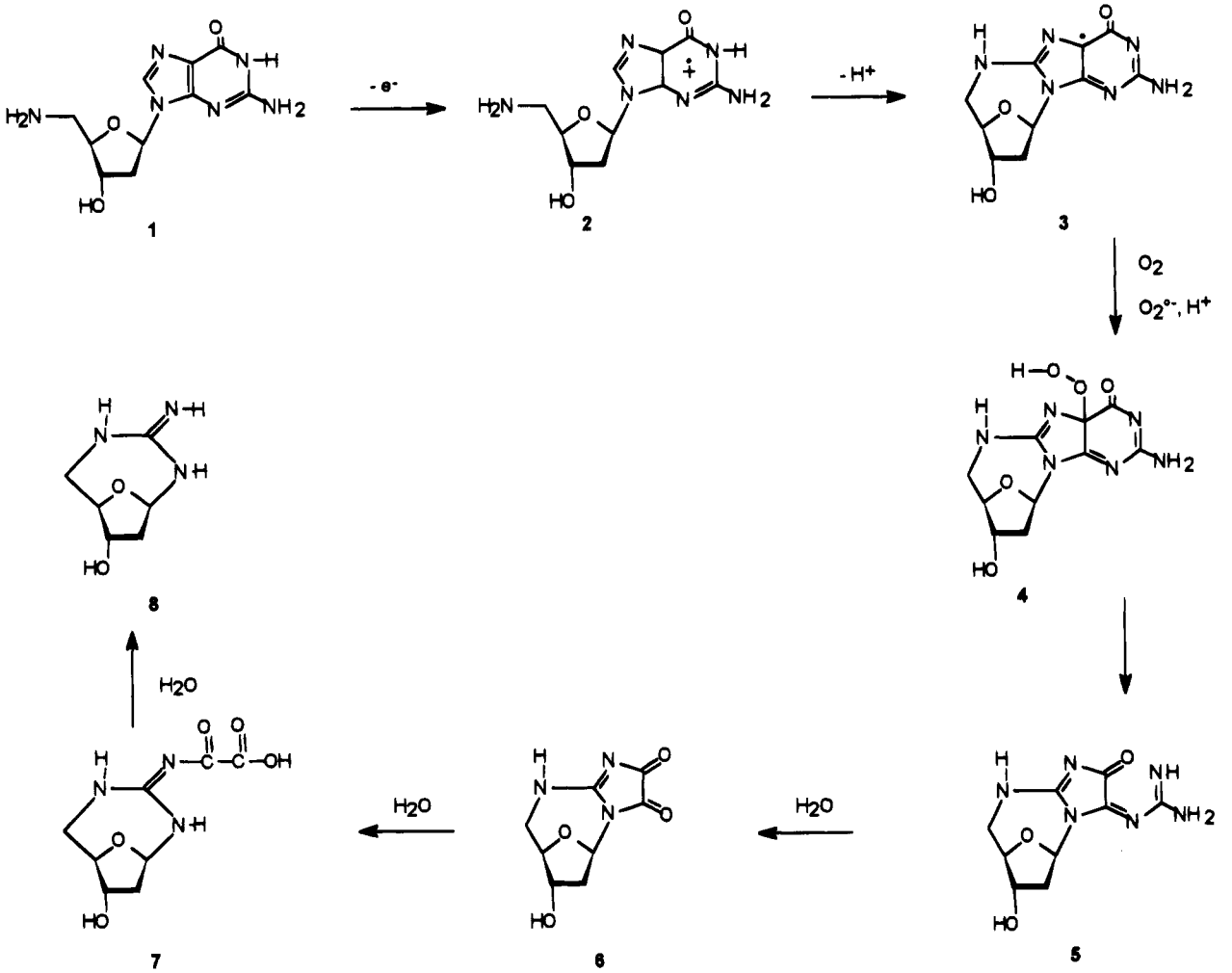

Figure 7. Proposed reaction mechanism for the generation of **7** and **8**.

the consideration of the results of a long-range $^1H-^{13}C$ scalar coupling experiment (*vide infra*).

Carbon NMR. There are six resonance signals in the ^{13}C NMR spectrum of the modified nucleoside **8**, as illustrated in Figure 5. Five of them were assigned as the sugar carbons from a 2D $^1H-^{13}C$ correlated NMR analysis. As a result, there is only one signal left for the base moiety, which appears in the downfield region of the spectrum (δ = 159.8 ppm). The missing resonance signals of **8** with respect to that of 5′-NH_2dGuo (**1**) can be attributed to base carbons as inferred from a 2D $^1H-^{13}C$ correlated NMR analysis (data not shown). The long range ^1H-^{13}C scalar coupling experiment (Figure 6) has been carried out in order to confirm the suggested cyclic structure of **8**. Interestingly, a cross peak is observed between H1′ (5.5 ppm) and the low-field carbon (δ = 159.8 ppm) assigned as C-2. The main cross peak corresponds to a correlation between the H5′,H5″ protons (3.3 ppm) and the carbon C-2. Therefore, H1′ and H5′,H5″ are correlated with the same carbon C-2 which is located three bonds away. This is in agreement with a cyclic structure for **8**. Futhermore, a comparison of the carbon chemical shifts of 5′-NH_2dGuo (**1**) with those of **8** (Table 2) provides additional structural information for the latter nucleoside. The C-5′ resonance of **8** is 6.5 ppm downfield shifted with respect to that of 5′-NH_2dGuo (**1**), suggesting that the amino group is involved in a covalent bond with an electronegative function. Interestingly, the chemical shift of the only base carbon left (159.8 ppm) of **8** is similar to the signal observed for the guanidinium carbon of arginine (157.8 ppm) (*34*) and (2*S*)-2′,5′-anhydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-guanidinylidene-2-hydroxy-4-oxoimidazolidine (*14*).

In the case of **7**, only five resonance signals have been observed in the upfield region of the ^{13}C NMR spectrum. They have been assigned as the sugar resonances. However, further information concerning the base moiety can be obtained from the long-range $^1H-^{13}C$ scalar coupling experiment. The H1′ proton (5.65 ppm) and the H5′,H5″ protons (3.70, 3.29 ppm) exhibit a long-range heteronuclear scalar correlation with a carbon that was assigned as those of the base moiety (δ(C-2) = 154 ppm). This provides further confirmation for a cyclic stucture for **7**. The C-2 resonance of **7** is 5 ppm upfield relative

to that of **8**. This suggested that an electronegative group is attached to the imino function of the base.

Mechanism of Formation of Photocycloadducts 7 and 8. A possible mechanism for the benzophenone-photosensitized formation of 9-oxa-2,4-diazabicyclo[4.2.1]-non-2-en-7-ol, 3-amino- (1*R-exo*) (**8**), is depicted in Figure 7. Benzophenone has been shown to be an efficient photosensitizer of guanine nucleic acid components, acting through a Type I mechanism (*2*). Electron transfer from the guanine moiety to triplet excited benzophenone rather than hydrogen abstraction is likely to be the predominant reaction leading to the formation of the purine radical cation **2**. As was shown on the basis of pulse radiolysis experiments (*35, 36*) and indirectly confirmed by final product analysis (*14, 15, 26*), the radical cation **2** derived from dGuo predominantly undergoes a deprotonation reaction. Nucleophilic addition of either a water molecule or a hydroxyl group to the latter intermediate was found to lead to 7,8-dihydroimidazole adducts. The situation appears quite different for 5′-amino-2′,5′-dideoxyguanosine (**1**). The main difference observed was the loss of the H8 proton of the base moiety. A likely mechanism would be that deprotonation of the guanine radical cation **2** is prevented by a nucleophilic substitution of the purine C-8 by the amino group. This would lead to the transient formation of the cyclic nucleoside radical **3**.

Kasai et *al.* (*37*) had recently elucidated the mechanism of formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine within native DNA upon riboflavin photosensitization. Interestingly, the authors proposed that the transient guanine radical undergoes a competitive hydratation reaction with respect to deprotonation. This may be explained by stacking interactions which prevent, at least partly, the latter reaction. It is interesting to note that the formation of imidazolone and oxazolone compounds (*26*) which arises from the deprotonation of the radical cation and subsequent addition of water was not observed in the benzophenone-mediated photooxidation of 5′-NH_2-dGuo. This is likely to be explained by the overwhelming intramolecular nucleophilic substitution of the C-8 of the guanine radical cation by the 5′-amino group. The intramolecular reaction is facilitated by the high nucleophilicity of the amino group. On the other hand, the 5′-(hydroxymethyl) group of dGuo has been shown to undergo intramolecular nucleophilic addition to the neutral radical subsequent to deprotonation of the guanine radical cation **2**. It should be noted that a similar mechanism has been proposed to explain the nucleophilic substitution of aromatic olefin by an amino group consecutive to photoinduced electron transfer (*38, 39*).

Then, oxygen addition to **3** at C-5 is likely to generate the hydroperoxide **4**. It should be noted that molecular oxygen is required to obtain the cyclic nucleoside **8**. This is indirectly supported by the absence of formation of **7** and **8**, or any cyclic nucleoside, when 5′-amino-2′,5′-dideoxyguanosine (**1**) was photosensitized in oxygen-free aqueous solution. The transient keto hydroperoxide **4** is likely to undergo a β scission within the pyrimidine ring at the C5−C6 bond with subsequent elimination of CO_2. In a subsequent step, the resulting nucleoside **5** loses its guanidine group through hydrolysis. The next step would involve the opening of the imidazolidinedione ring, giving rise to compound **7** with an oxalic acid residue attached to the imino function N=C2. Hydrolysis of **7** was found to quantitatively generate the final product **8**.

Conclusion

In conclusion, we reported that a free amino group attached to the sugar moiety of guanine nucleoside is able to intramolecularly react with the base moiety upon one-electron oxidation. This photoreaction constitutes an interesting model to be used for further investigating the photosensitized formation of DNA−protein cross-links. In this respect, work is in progress using a lysine residue tethered to the 5′-hydroxyl group of 2′-deoxyguanosine through the carboxyl group of the amino acid.

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