# Chemical Aspects of the Benzophenone-Photosensitized Formation of Two Lysine−2′-Deoxyguanosine Cross-Links†

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**Abstract:** L-Lysine, 5′-ester with 2′-deoxyguanosine (5′-Lys-dGuo), has been synthesized in order to investigate the photosensitized formation of lysine−2′-deoxyguanosine (lysine−dGuo) adducts. The purpose of tethering a lysine residue to the 5′-hydroxyl group of dGuo was to mimic the close interaction between DNA and amino acids of histones within cells. Benzophenone-mediated photosensitization of 5′-Lys-dGuo in aerated aqueous solution was found to give rise to two main intramolecular adducts involving the α-amino function of the lysine residue and the C8 position of the guanine moiety. The two modified nucleosides were isolated by reversed-phase high-performance liquid chromatography and characterized by extensive spectroscopic measurements including $^{13}C$ and $^{1}H$ NMR analyses together with fast atom bombardment mass spectroscopy (FAB-MS). They were identified as L-lysine, $N^2$-[5-[(aminoiminomethyl)imino]-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-oxoimidazolidinyl]-, intramolecular 1,5′-ester (**6**) and L-lysine, $N^2$-[1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, intramolecular 1,5′-ester (**10**). The structure assignment of the two photoadducts is indicative of the occurrence of two mechanisms. One is likely to involve a nucleophilic substitution of the guanine radical cation by the α-amino group of the lysine residue. On the other hand, the formation of **6** may be explained in terms of an intramolecular addition of the α-amino function to the 7,8-double bond of a neutral guanine radical.

## Introduction

It is now generally accepted that oxidative DNA lesions are implicated in various deleterious biological effects including lethality, mutagenesis, and carcinogenesis.[1] Oxidative damage to DNA includes base modifications, abasic sites, DNA strand breaks, and DNA−protein cross-links.[2,3] At wavelengths within the UVA region (320−400 nm), the bulk of the biological effects is mediated by photodynamic processes involving endogenous and exogenous photosensitizers.[4−7] Such photosensitization processes occur through two competitive mechanisms.[8] The type I reaction involves either proton abstraction or electron transfer by the excited photosensitizer to, or more generally from, the substrate. The type II mechanism occurs *via* the initial generation of $^{1}O_2$ by the excited photosensitizer and subsequent reaction of the latter reactive oxygen species with the substrate.[3] Purine nucleic acid components, and particularly guanine, which presents the lowest ionization potential among DNA components, are usually more readily photooxidized than their pyrimidine analogues.[3,7,9] Ionizing radiation,[10−15] chemicals,[16−19] and visible light in the presence of photosensitizers can produce nucleobase−amino acid cross-links in the DNA−protein complexes.[20−28]

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† Abbreviations: dGuo, 2′-deoxyguanosine; 5′-Lys-dGuo, L-lysine, 5′-ester with 2′-deoxyguanosine; **6**, L-lysine, $N^2$-[5-[(aminoiminomethyl)imino]-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-oxoimidazolidin-2-yl]-, intramolecular 1,5′-ester; **10**, L-lysine, $N^2$-[1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, intramolecular 1,5′-ester; 5′-Cbz-Lys-dGuo, 5′-[*N,N*′-bis(dibenzyloxycarbonyl)-L-lysine]-2′-deoxyguanosine; Cbz, benzyloxycarbonyl; HMQC, heteronuclear multiquantum coherence; HMBC, heteronuclear multibond correlation; DQF-COSY, double quantum filtered correlated spectroscopy; NOE, nuclear overhauser effect; FAB-MS, fast atom bombardment mass spectrometry.

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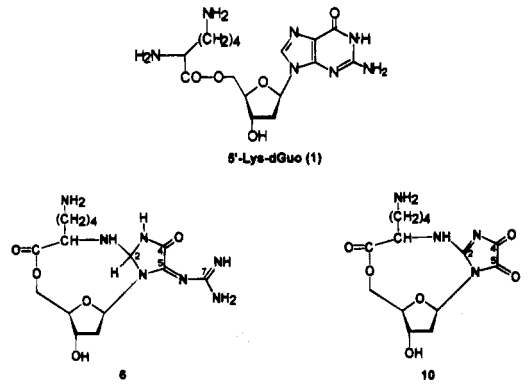


**Figure 1.** Structure of 5′-Lys-dGuo (**1**) and photoproducts **6** and **10**.

However, knowledge of the chemical nature of the adducts formed in dye-photosensitized DNA−protein systems is scarce. In previous investigations, it has been established that hydroxyl groups, which can be regarded as models for threonine and serine side chains, may react with radical guanine intermediates.[29,30] More recently, we have found that the sugar amino group of 5′-amino-2′,5′-dideoxyguanosine, which was used to mimick the side chain of lysine within proteins, may react intramolecularly with the C8 position of the guanine moiety upon one-electron oxidation.[31]

Lysine is of particular interest as a potential participant in protein−nucleic acid cross-linking because of its occurrence in relatively large amounts in the histones, one of the main protein components of eukaryotic chromatin. The regions of the histones H2A, H2B, H3, and H4 that are most strongly involved in the binding of DNA to the core nucleosome in chromatin are generally believed to be those that are rich in lysine and arginine. The potential proximity of these residues to the guanine bases in DNA makes these amino acids of interest as possible participants in the photosensitized formation of DNA−histone cross-links. One major difficulty in the actual identification of nucleic acid−amino acid adducts is associated with the relatively low reactivity of guanine bases toward lysine residues when they are irradiated as free compounds in aqueous solutions. Therefore, the choice of a suitable model system appears critical in such studies.

Therefore, we have synthesized L-lysine, 5′-ester with 2′-deoxyguanosine (5′-Lys-dGuo) (Figure 1), as a model system in order to investigate the dye-photosensitized formation of lysine−guanine cross-links. The purpose of substituting the 5′-hydroxymethyl of dGuo by a lysine residue through its carboxyl group was to mimic the intimate association between DNA and histones in nucleosomes. This would allow the reactivity of the lysine amino group toward the guanine moiety upon one-electron oxidation to be studied. We would like to report the isolation by high-performance liquid chromatography of two main photoproducts arising from the addition of the α-amino group of lysine to the C8 of the guanine base upon benzophenone-mediated photosensitization of 5′-Lys-dGuo in aerated aqueous solution. The characterization of the two photoproducts as L-lysine, $N^2$-[5-[(aminoiminomethyl)imino]-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-oxoimidazolidinyl]-, intramolmolecular 1,5′-ester (**6**), and L-lysine, $N^2$-[1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, intramolecular 1,5′-ester (**10**) (Figure 1), was achieved on the basis of extensive spectroscopic measurements including fast atom bombardment mass spectrometry (FAB-MS) together with $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) analyses.

## Experimental Section

**Chemicals.** 2′-Deoxyguanosine (dGuo) and *N,N′*-di-Cbz-L-lysine were purchased from Sigma Chemical Co. (St. Louis, MO). *N,N*-Dimethylformamide (DMF), dichloromethane ($CH_2Cl_2$), and chloroform ($CHCl_3$) were obtained from SDS (France). Tributylamine, *N,N′*-dicyclohexylcarbodiimide, palladium-charcoal activated (10% Pd), benzophenone, and silica gel PF 254 were from Merck (Darmstadt, Germany). 1-Hydroxybenzotriazole was purchased from Janssen (Belgium). HPLC grade methanol ($CH_3OH$), ethanol, and anhydrous sodium sulfate ($Na_2SO_4$) were obtained from Carlo Erba (Farmitalia Carlo Erba, Milan, Italy). Ammonium formate was from BDM Laboratory Supplies Poole (U.K.).

**High-Performance Liquid Chromatography.** The HPLC 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 semipreparative (300 × 7.5 mm i.d.) reversed-phase column was home-made with 10 μm Nucleosil octadecylsilyl silica gel (Nucleosil 100-10 $C_{18}$, Macherey-Nagel, Düren, Germany). The separation of 5′-Lys-dGuo (**1**) was achieved by using a 0.025 M ammonium formate $H_2O-CH_3OH$ (90:10) solution as the eluent. The separation of the photoproducts was achieved by using a 0.025 M ammonium formate $H_2O-CH_3OH$ (95:5) solution as the isocratic eluent. The major bands were collected, pooled, and concentrated by rotary evaporation. Then, the resulting residues were lyophilized a minimum of three times in order to remove ammonium formate.

**Spectroscopic Measurements.** Ultraviolet absorption spectra were obtained in water with a Hewlett-Packard 8542A diode array spectrophotometer (Amsterdam, The Netherlands). FAB mass spectra were recorded in a positive mode by using a Model ZAB 2-SEQ spectrometer (Fisons-V.G., Manchester, U.K.) equipped with an LSIMS source. The molecules were dissolved in a thioglycerol matrix containing 0.1 M NaI. The $^1H$ and $^{13}C$ NMR spectra were recorded in $D_2O$ in the Fourier transform mode by using AM 400 and AC 200 Brüker apparatus. The $D_2O$ samples were first lyophilized in 99.8% $D_2O$ and then redissolved in 99.99% $D_2O$. The chemical shifts were expressed in parts per million with respect to the 3-(trimethylsilyl)propionic acid (TPS) used as an internal reference in 99.99% deuterium oxide, $D_2O$. Heteronuclear multiquantum coherence (HMQC), heteronuclear multibond correlation (HMBC), and $^1H-^1H$ homonuclear (DQF-COSY) correlated NMR experiments were performed on a U 400 Varian apparatus. Theoretically, the optimum delay required to observe long-range coupling correlations in the HMBC experiments is $(1/2)^nJ(C,H)$, where $^nJ(C,H)$ are the long-range coupling constants. The $^2J(C,H)$ coupling in an aromatic system is usually small (2−3 Hz) whereas $^3J(C,H)$ coupling is within the range 7−15 Hz. Consequently, we used 100, 80, and 60 ms as the delays for the HMBC analyses. The assignment of the nonexchangeable proton signals was inferred from the DQF-COSY spectra. The H5′ signal was assigned downfield to the H5″ proton according to Remin and Shugar.[32] The H2′/H2″ protons were further assigned on the basis of coupling constants arguments.[33,34]

**Synthesis of L-Lysine, 5′-Ester with 2′-Deoxyguanosine (1). (a) *N,N′*-Di-Cbz-L-lysine, 5′-Ester with 2′-Deoxyguanosine.** dGuo (0.4 g, 1.5 mmol), tributylamine (360 μL, 1.5 mmol), and 1-hydroxybenzotriazole (0.2 g, 1.5 mmol) were dissolved in 15 mL of anhydrous DMF. DMF was used instead of dichloromethane used previously in order to solubilize a sufficient amount of dGuo. To this solution were added *N,N′*-di-Cbz-L-lysine (0.62 g, 1.5 mmol) and *N,N′*-dicyclohexyl-

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carbodiimide (0.36 g, 1.7 mmol). Then, the resulting solution was kept at 23 °C overnight. Subsequently the precipited 1,3-dicyclohexylurea was removed by filtration and washed with $CH_2Cl_2$. Then, the filtrate was evaporated to dryness under diminished pressure. The resulting residue was resuspended in 30 mL of $CH_2Cl_2$. The unreacted dGuo was extracted with $H_2O$ (3 × 20 mL). The organic phase was dried with $Na_2SO_4$ and then evaporated to dryness. The resulting viscous residue was subjected to flash column chromatography (silica gel, 30 g) with a step solvent gradient starting from $CHCl_3$ and finishing with $CH_3OH$. *N,N'*-di-Cbz-L-lysine, 3',5'-diester with 2'-deoxyguanosine, was first eluted with 600 mL of $CHCl_3-CH_3OH$ (96:4). *N,N'*-Di-Cbz-L-lysine, 3'-ester with 2'-deoxyguanosine, was removed from the column by 600 mL of $CHCl_3-CH_3OH$ (92:8), and finally *N,N'*-di-Cbz-L-lysine, 5'-ester with 2'-deoxyguanosine, was eluted with 600 mL of $CHCl_3-CH_3OH$ (88:12). After evaporation of the solvent, 0.26 g (26%) of *N,N'*-di-Cbz-L-lysine, 5'-ester with 2'-deoxyguanosine, and 0.11 g (11%) of *N,N'*-di-Cbz-L-lysine, 3'-ester with 2'-deoxyguanosine, were obtained as white solids. Data for *N,N'*-di-Cbz-L-lysine, 5'-ester with 2'-deoxyguanosine: MS (FAB+) *m/z* 686 (20, [M + Na]), 664 (20, [M + H]$^+$), 152 (15, [B + 2H]$^+$), 90 (10, [Bn]$^+$). Data for *N,N'*-di-Cbz-L-lysine, 3'-ester with 2'-deoxyguanosine: MS (FAB$^+$) *m/z* 686 (10, [M + Na]), 664 (10, [M + H]$^+$), 152 (70, [B + 2H]$^+$), 90 (55, [Bn]$^+$).

**(b) L-Lysine, 5'-Ester with 2'-Deoxyguanosine (1).** A mixture of *N,N'*-di-Cbz-L-lysine, 5'-ester with 2'-deoxyguanosine (0.26 g), and 10% palladium-on-charcoal (0.1 g) in 1:1 ethanol−water solution (30 mL) was hydrogenated for 2 h at room temperature at a pressure of 20 bar. 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 by using 25 mM ammonium formate buffer with a 9:1 (v/v) mixture of $H_2O$ and $CH_3OH$ as the isocratic eluent at a flow rate of 3 mL/min. The fractions corresponding to the fastest eluting HPLC peak ($k' = 0.7$) were collected and lyophilized, giving 60 mg of 5'-L-lysine, 5'-ester with 2'-deoxyguanosine (38%): $^1$H NMR (400.13 MHz, $D_2O$) $\delta$ 1.42 (m, 2H, $CH_2$), 1.64 (m, 2H, $CH_2$), 1.91 (m, 2H, $CH_2$), 2.67 (m, 1H, H2''), 2.94 (t, 2H, $CH_2$), 3.11 (m, 1H, H2'), 4.12 (t, 1H, CH), 4.42 (m, 1H, H4'), 4.54 (dd, 1H, H5''), 4.67 (dd, 1H, H5'), 4.75 (m, 1H, H3'), 6.43 (t, 1H, H1'), 8.10 (s, 1H, H8); $^{13}$C NMR (50.61 MHz, $D_2O$) $\delta$ 21.3 ($CH_2$), 26.2 ($CH_2$), 29.3 ($CH_2$), 37.4 (C2'), 38.8 ($CH_2$), 52.4 (CH), 65.6 (C5'), 70.9 (C3'), 83.6 (C4'), 84.3 (C1'), 116.6 (C5), 138.0 (C8), 151.5 (C4), 153.7 (C2), 159.0 (C6), 169.9 (*C*O); MS (FAB$^+$) *m/z* 418 (5, [M + Na]), 396 (100, [M + H]$^+$), 245 (5, [5'-Lys-sugar]), 152 (65, [B + 2H]$^+$); HRMS for $C_{16}H_{26}N_7O_5$, calcd 396.1995, found 396.1952.

**Acetylation of Photoproduct 6.** The photoproduct (1 mg) was dissolved in 0.2 mL of acetic anhydride and 1 mL of pyridine. The resulting solution was stirred magnetically for 2 h at 20 °C. Then, the pyridine was removed under diminished pressure, and the mixture was lyophilized prior to FAB-MS analysis.

**Photosensitization.** An aqueous solution (30 mL) saturated with 0.4 mM benzophenone containing 30 mg of 5'-Lys-dGuo (**1**) in a 50 mL test tube was exposed for 2 h to 16 black lamps ($\lambda_{max}$ = 350 nm; 24 W each) in a Rayonet photochemical reactor (Rayonet, Southern New England Ultraviolet Co., Hamden, CT). The irradiations were carried out at neutral pH. 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 minimum volume of water prior to HPLC analysis.

**L-Lysine, $N^2$-[5-[(Aminoiminomethyl)imino]-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-oxoimidazolidinyl]-, Intramolecular 1,5'-Ester (6).** The HPLC fractions containing the fastest eluting product (retention time = 5.6 min) were combined and evaporated to dryness, giving a homogeneous compound (2% yield) which was characterized as L-lysine, $N^2$-[5-[(aminoiminomethyl)imino]-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-oxoimidazolidinyl]-, intramolecular 1,5'-ester (**6**): $^1$H NMR (400.13 MHz, $D_2O$) $\delta$ 1.51 (m, 2H, $CH_2$), 1.75 (m, 2H, $CH_2$), 1.87 (m, 2H, $CH_2$), 2.31 (m, 1H, H2''), 3.06 (t, 2H, $CH_2$), 3.67 (m, 1H, H2'), 3.77 (q, 1H, CH), 4.04 (dd, 1H, H5''), 4.17 (m, 1H, H4'), 4.80 (m, 1H, H3'), 5.19 (dd, 1H, H5'), 5.87 (q, 1H, H1'), 5.49 (s, 1H, H2); $^{13}$C NMR (100.61 MHz, $D_2O$) $\delta$ 24.1 ($CH_2$), 29.0 ($CH_2$), 34.0 ($CH_2$), 38.2 (C2'), 42.0 ($CH_2$), 55.9 (*C*H), 64.4 (C5'), 70.5 (C3'), 85.0 (C4'), 87.4 (C1'), 66.6 (C2), 158.5 (C5), 166.2 (C4), nd (C7), 179.3 (*C*O); MS (FAB$^+$) *m/z* 428 (15, [M + 2Na]); 406 (10, [M + Na]); 384 (82, [M + H]$^+$); HRMS for $C_{15}H_{25}N_7O_5$, calcd 384.1995, found 384.1965.

**L-Lysine, $N^2$-[1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, Intramolecular 1,5'-Ester (10).** The HPLC fractions (retention time 7.8 min) were combined and evaporated to dryness, giving a homogeneous compound (2% yield) which was characterized as L-lysine, $N^2$-[1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, intramolecular 1,5'-ester (**10**): $^1$H NMR (400.13 MHz, $D_2O$) $\delta$ 1.58 (m, 2H, $CH_2$), 1.79 (m, 2H, $CH_2$), 2.06 (m, 2H, $CH_2$), 2.39 (m, 1H, H2''), 2.72 (m, 1H, H2'), 3.09 (m, 2H, $CH_2$), 4.00 (m, 1H, H4'), 4.26 (dd, 1H, H5''), 4.60 (t, 1H, CH), 4.79 (m, 1H, H3'), 5.00 (dd, 1H, H5'), 5.58 (t, 1H, H1'); $^{13}$C NMR (100.61 MHz, $D_2O$) $\delta$ 25.2 ($CH_2$), 29.1 ($CH_2$), 32.0 ($CH_2$), 40.6 (C2'), 42.9 ($CH_2$), 56.3 (*C*H), 63.1 (C5'), 70.7 (C3'), 84.2 (C1'), 85.8 (C4'), nd (C2, C5, C4), 176.8 (*C*O); MS (FAB$^+$) (acetylated sample) *m/z* 425 (10, [M + 2OAc + H]$^+$); 383 (25, [M + OAc + H]$^+$); HRMS for $C_{16}H_{23}N_4O_7$, calcd 383.1566, found 383.1643.

## Results and Discussion

We have recently shown that the 5'-amino group of 5'-amino-2',5'-dideoxyguanosine may react with the purine radical cation produced by type I photosensitization. As a more relevant model system for investigating the photosensitized formation of DNA−protein cross-links, we have synthesized 5'-Lys-dGuo (**1**).

**Synthesis of 5'-Lys-dGuo (1).** The purpose of the synthesis was to tether a lysine residue through its carboxylic group to the sugar moiety of dGuo. The exocyclic amine of dGuo has to be protected as well as the α- and ε-amino functions of L-lysine. It is important to recall that the protecting groups of the latter product have to be removed under conditions in which the N-glycosidic bond of dGuo and the ester function between the sugar moiety and the lysine residue are preserved. Therefore, we explored the possibility of utilizing coupling reagents involved in peptide synthesis.

The *tert*-butyl chloroformate (Boc) group was used for the protection of the exocyclic amino group of the guanine base. Unfortunately, the N-glycosidic bond of dGuo was cleaved during the Boc deprotection that involves the use of trifluoroacetic acid−water solution. Attempts to protect the 2'-amino group of dGuo with either benzyl chloroformate or benzyl chloride failed, and no detectable protected product was observed. Therefore, we decided to leave the 2'-amino group free.

The two amino groups of L-lysine were blocked by benzyloxycarbonyl protecting groups. We chose the Cbz protection since it could be easily removed under neutral hydrogenolysis conditions.[35] The yield of the condensation reaction of the lysine residue to the 5' position of the sugar moiety is 10% with respect to the starting dGuo.

5'-Lys-dGuo (**1**) was isolated by reversed-phase high-performance liquid chromatography and characterized by $^1$H and $^{13}$C NMR and fast atom bombardment mass spectrometry. The FAB mass spectrum in the positive mode of 5'-Lys-dGuo (**1**) shows a pseudomolecular ion at *m/z* 396 [M + H]$^+$. The exact mass measurement of 5'-Lys-dGuo (**1**) obtained from a high-resolution FAB mass spectrum is 396.1952. This is indicative of an empirical formula of $C_{16}H_{26}N_7O_5$. Most noticeably, the $^1$H NMR spectrum of 5'-Lys-dGuo (**1**) shows downfield shifts of 0.85 and 0.78 ppm for the H5' ($\delta$ = 4.67 ppm) and the H5'' resonances ($\delta$ = 4.55 ppm) relative to that of dGuo (H5', $\delta$ = 3.82 ppm; H5'', $\delta$ = 3.77 ppm), respectively. The $^{13}$C NMR spectrum exhibits 16 carbon resonance signals.

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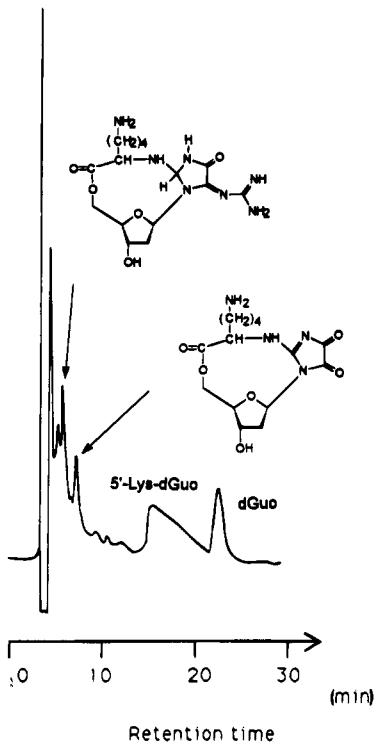


**Figure 2.** HPLC elution profile on an ODS column of the products of benzophenone-mediated photosensitization of 5'-Lys-dGuo (**1**) in aerated aqueous solution.

Assignment of the $^{13}C$ signals was achieved by a 2D $^{1}H-^{13}C$ correlated experiment (data not shown). Six carbon resonance signals were assigned as those of the lysine moiety. The C5' resonance ($\delta$ = 65.6 ppm) signal of 5'-Lys-dGuo is shifted 3.4 ppm downfield relative to that of dGuo ($\delta$ = 62.1 ppm) as expected from the presence of an ester function at the C5' position of the sugar moiety.

**Isolation of the Photoproducts 6 and 10.** Benzophenone-photosensitized oxidation of 5'-Lys-dGuo (**1**) in aerated aqueous solution led to the formation of two main photoproducts, which were efficiently separated by reversed-phase HPLC (Figure 2). The fastest eluting HPLC fraction (retention time 5.6 min) was found to contain an intramolecular photoadduct which was identified as L-lysine, $N^2$-[5-[(aminoiminomethyl)imino]-1-(2-deoxy-$\beta$-D-*erythro*-pentofuranosyl)-4-oxoimidazolidinyl]-, intramolecular 1,5'-ester (**6**). A second photoadduct (retention times 7.8 min) which is more retained on the ODS column was identified as L-lysine, $N^2$-[1-(2-deoxy-$\beta$-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, intramolecular 1,5'-ester (**10**). The two photoadducts are formed in a similar yield, which is about 2% with respect to the starting 5'-Lys-dGuo (**1**). We observe also the presence of dGuo (30% degradation with respect to the starting 5'-Lys-dGuo) in the HPLC elution profile. This may be explained by the hydrolysis of the ester function linking the lysine residue to the sugar moiety. The two photoadducts **6** and **10** are the main photoproducts formed during the irradiation with respect to the degradation yield (40%) of the 5'-Lys-dGuo. The two photoproducts are more rapidly eluted on the ODS column than 5'-Lys-dGuo (**1**) as expected from the fact that they have lost, at least partly, their aromatic character (*vide infra*).

**Structural Assignment of L-Lysine, $N^2$-[5-[(Aminoiminomethyl)imino]-1-(2-deoxy-$\beta$-D-*erythro*-pentofuranosyl)-4-oxoimidazolidinyl]-, Intramolecular 1,5'-Ester (6). (a) Spectroscopic Measurements.** The molecular weight of **6** was determined to be 383 as inferred from mass spectrometry measurements. The FAB mass spectrum in the positive mode exhibits a pseudomolecular ion at m/z 384 $[M + H]^+$ and high quasimolecular ions at *m/z* 406 [M + Na] and *m/z* 428 [M + 2Na]. High-resolution mass spectrometry measurement provided a molecular weight of 384.1965 for the modified nucleoside. This is indicative of an elemental composition of $C_{15}H_{25}N_7O_5$ for **6**. Further support for the structure of **6** was provided by detailed $^{1}H$ NMR spectroscopic analysis (Table 1). Firstly, we observe the presence of a lysine residue in the photoproduct as inferred from the characteristic signals of the methinic proton ($\delta$ = 3.77 ppm) and the four methylene protons of the side chain of lysine ($\delta$ = 3.06, 1.87, 1.75, and 1.51 ppm). Interestingly, we may note in the downfield region of the $^{1}H$ NMR spectrum (Figure 3) of **6** the presence of a singlet (H2) at 5.49 ppm which is shifted upfield by 2.61 ppm with respect to the related H8 proton resonance of 5'-Lys-dGuo (**1**). Confirmation of the $^{1}H$ assignments was provided by two-dimensional $^{1}H-^{1}H$ COSY analysis (data not shown). A pronounced downfield shift effect ($\delta$ = 0.52 ppm) is observed for the H5' proton of **6** with respect to the corresponding signal of 5'-Lys-dGuo (**1**). In contrast, we note an upfield shift ($\delta$ = 0.5 ppm) for the H5'' of **6**. This may be explained by the magnetic anisotropy of the carbonyl group that causes a downfield shift of the H5' proton lying in a deshielding cone whose axis is along the CO bond. This also explains the shielding of H5'' which is outside the cone.[36] Another interesting feature deals with the downfield shift of the resonance signal of the H2' proton (0.54 ppm) with respect to the corresponding signal of 5'-Lys-dGuo (**1**). To our knowledge, such a drastic deshielding effect has not been previously observed for guanine nucleosides. Furthermore, this could not be attributed to an aromatic ring current of the base moiety because of the loss of the aromatic character of the photoproduct **6** (*vide infra*). This is most probably due to the presence of the guanidine residue of the modified base in the close proximity of the H2'' proton.

Inspection of the $^{13}C$ NMR data provides complementary relevant structural information (Table 2). The 2D $^{13}C-^{1}H$ NMR spectrum of **6** allowed the assignment of all the protonated carbons (data not shown). Interestingly, the C2 resonance signal of **6** ($\delta$ = 66.0 ppm) is in fact 72.0 ppm upfield shifted relative to the C8 resonance of 5'-Lys-dGuo (**1**). Such a large upfield shift indicates a change in the carbon hybridization from $sp^2$ to $sp^3$. The sugar carbon signals appear within the range 38–87 ppm, which, typically, correspond to the expected signals of a 2-deoxy-*erythro*-pentofuranose residue of 2'-deoxyribonucleosides.

At this stage, it was important to determine which of the free amino groups, i.e., either the $\alpha$-amino function that is usually implicated in the peptide bond of proteins or the free $\epsilon$-amino group of the side chain of lysine in proteins, was involved in the covalent bond with the guanine moiety. Support for the $\alpha$-amino function is provided by the consideration of the results of long-range $^{1}H-^{13}C$ scalar coupling experiments (Figure 5). Covalent binding through the $\epsilon$-amino group would have necessitated the implication of four additional bonds. The proton-coupled $^{13}C$ NMR spectrum shows $^{3}J$(C,H) coupling constants between H2 and the methinic carbon of the lysine residue. This provides further confirmation of a cyclic photoadduct as the result of a covalent cross-link formation between the $\alpha$-amino group of the lysine and the C2 of the former guanine moiety. It should be noted that no photoadduct involving the free $\epsilon$-amino group in a covalent bond with the base moiety was detected. The C5 carbon atom ($\delta$ = 158.5

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**Table 1.** $^1$H NMR (400.13 MHz) Chemical Shifts and Coupling Constants of 5′-Lys-dGuo (**1**) and Photoproducts **6** and **10** in $D_2O$

Chemical Shifts

| | H1′ | H2′ | H2″ | H3′ | H4′ | H5′ | H5″ | H8 or H2 | CH | CH2 | CH2 | CH2 | CH2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5′-Lys-dGuo | 6.43 | 3.13 | 2.67 | 4.75 | 4.44 | 4.67 | 4.54 | 8.10 | 4.12 | 2.94 | 1.91 | 1.64 | 1.42 |
| **6** | 5.87 | 3.67 | 2.31 | 4.80 | 4.17 | 5.19 | 4.04 | 5.49 | 3.77 | 3.06 | 1.87 | 1.75 | 1.51 |
| **10** | 5.58 | 2.72 | 2.39 | 4.79 | 4.00 | 5.00 | 4.26 | – | 4.60 | 3.09 | 2.06 | 1.79 | 1.58 |

Coupling Constants

| | 1′,2′ | 1′,2″ | 2′,2″ | 2′,3′ | 2″,3′ | 3′,4′ | 4′,5′ | 4′,5″ | 5″,5″ |
|---|---|---|---|---|---|---|---|---|---|
| 5′Lys-dGuo | 6.7 | 6.6 | −14.3 | 6.1 | 4.1 | 3.4 | 7.9 | 3.3 | -11.9 |
| **6** | <0.5 | 6.6 | -12.9 | 6.5 | 10.7 | 6.9 | 2.8 | <0.5 | -12.4 |
| **10** | 3.7 | 9.2 | -13.8 | 9.0 | 7.9 | 8.0 | 1.9 | 0.6 | -13.4 |

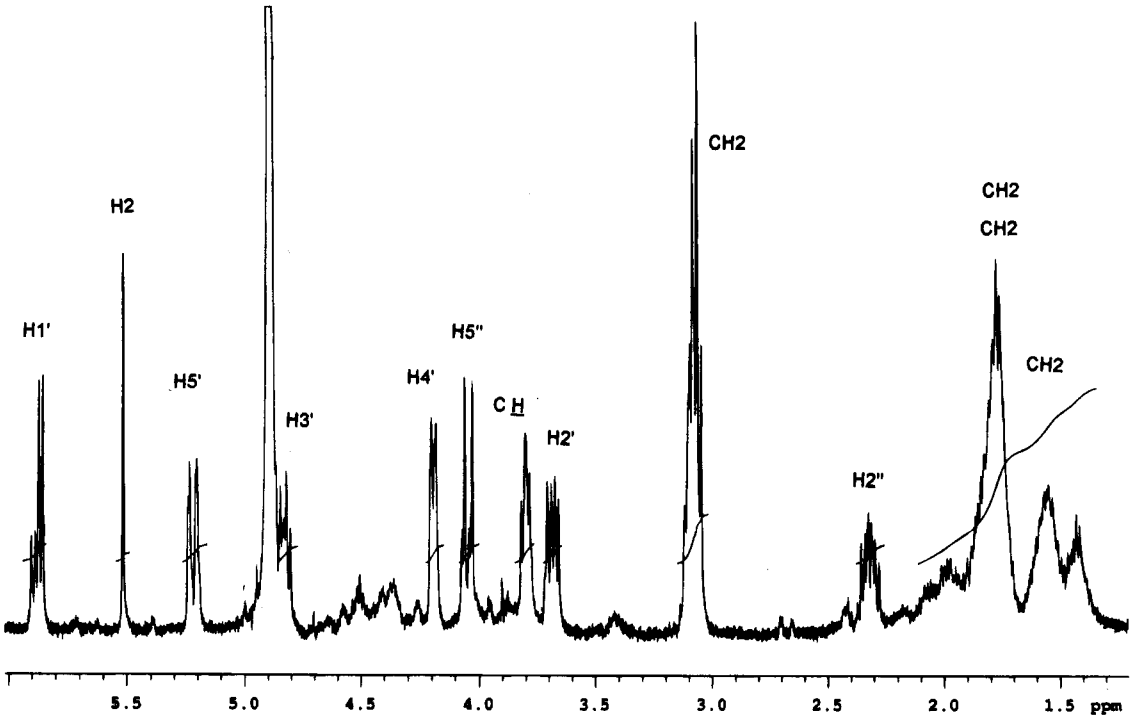


**Figure 3.** $^1$H NMR (400.13 MHz) spectrum of **6** in $D_2O$.

**Table 2.** $^{13}$C NMR (100.61 MHz) Chemical Shifts for 5′-Lys dGuo (**1**) and Photoproducts **6** and **10** Obtained in $D_2O$

| | 5′-Lys-dGuo | **6** | **10** |
|---|---|---|---|
| C1′ | 84.3 | 87.4 | 84.2 |
| C2′ | 37.4 | 38.2 | 40.6 |
| C3′ | 70.9 | 70.5 | 70.7 |
| C4′ | 83.6 | 85.0 | 85.8 |
| C5′ | 65.6 | 64.4 | 63.1 |
| CH | 52.4 | 55.9 | 56.3 |
| $CH_2$ | 21.3 | 24.1 | 25.2 |
| $CH_2$ | 26.2 | 29.0 | 29.1 |
| $CH_2$ | 29.3 | 34.0 | 32.0 |
| $CH_2$ | 38.8 | 42.0 | 42.9 |
| CO | 169.9 | 179.3 | 176.8 |
| C8 (C2) | 138.0 | 66.6 | nd |
| C6 | 159.0 | – | – |
| C5 (C5) | 116.6 | 158.5 | nd |
| C4 (C4) | 151.5 | 166.2 | nd |
| C2 (C7) | 153.7 | nd | – |

ppm) of the modified base was assigned, taking into consideration the three-bond correlations of the latter carbon with the H1′ and the H2 protons. The H2 proton is involved in another three-bond $^1$H−$^{13}$C correlation with a carbon which was assigned as C4 ($\delta$ = 166.2 ppm). We may also note a $^3J$(C,H) correlation between the H5″ proton and the CO of the carboxylic function. The UV absorption spectrum of 5′-Lys-C2H-dGuo (**6**) indicates that the guanine base has lost considerable aromaticity with the absence of strong absorption bands around 254 nm.

**(b) Conformational Analysis of the Adduct.** Consideration of the $^3J$(H,H) coupling constants involving the sugar ring provides information on the dihedral angles between the related protons and, therefore, on the conformational properties of the molecule. The dihedral angles are calculated according to the modified Karplus equation:[38] $^3J$(H,H) = 10.5 $\cos^2 \phi$(H,H) − 1.2 $\cos \phi$(H,H) where $\phi$(H,H) is the dihedral angle. The coupling constant between the H2″ proton and the H3′ proton exhibits an unusually high value of 10.7 Hz. This may be rationalized in terms of *trans* diaxial orientation. The H2′ proton exhibits a coupling value of 6.5 Hz with the vicinal H3′ proton. This is indicative of a dihedral angle of about 30° with the H3′ proton. The coupling constants involving the H1′ on one hand and the H2′ and H2″ protons on the other hand are 0.5 and 6.6 Hz, respectively. This may be accounted for by dihedral angles close to 90° and 30°, respectively. It should be added that the coupling constant between the H3′ and H4′ protons is 6.9 Hz. This may be explained by a dihedral angle of 150°, showing an equatorial orientation for the H4′ proton with respect to the mean plane of the sugar ring. Altogether, the overall informa-

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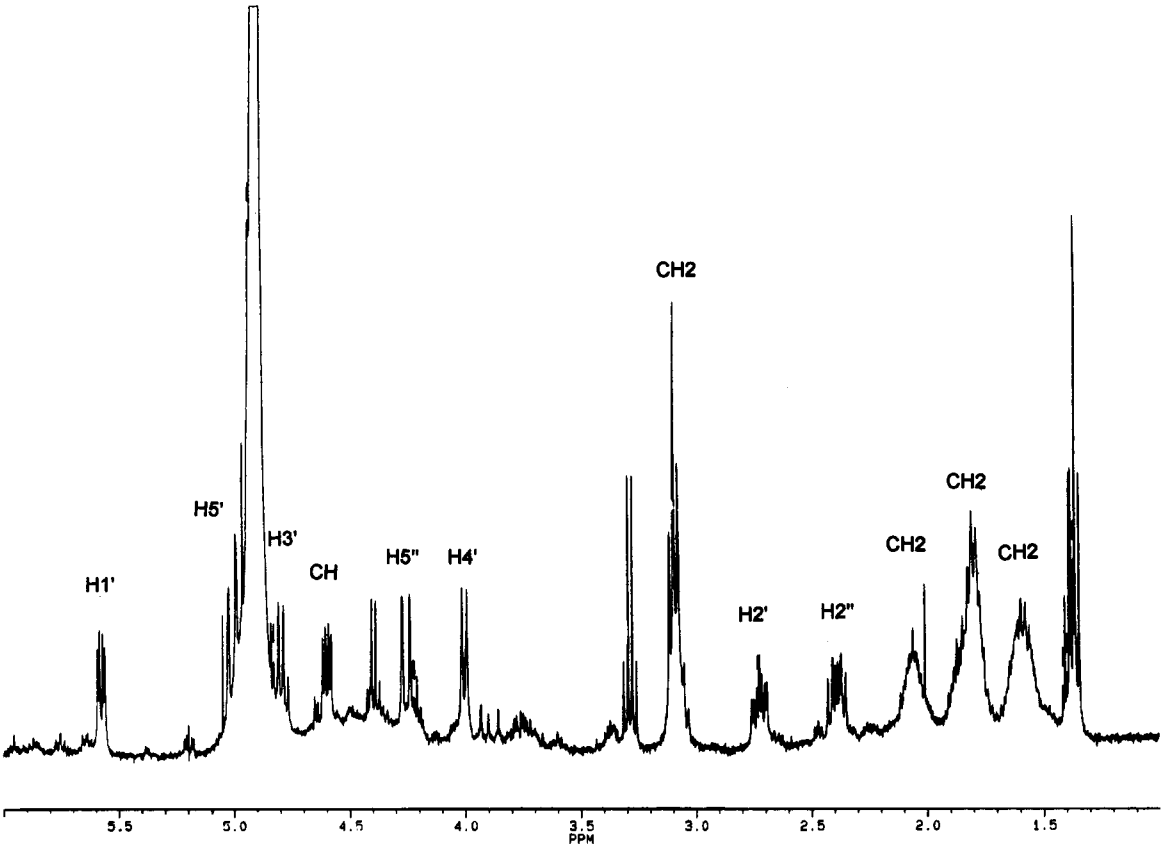


**Figure 4.** $^1$H NMR (400.13 MHz) spectrum of **10** in $D_2O$.

tion shows that the sugar ring of **6** adopts a C3′-*endo*−C2′-*exo* puckered form.

Further support for a cyclic structure of **6** is provided by the observed magnitudes of 2.8 and 0.5 Hz, respectively, for $J$(4′,5′) and $J$(4′,5″) coupling constants. Similar values are expected for an almost *gg* staggered rotamer of the exocyclic hydroxymethyl group about the C4′−C5′ bond.[39] Such an orientation is confirmed by the calculation of the *trans*−*gauche*, *gauche*−*trans*, and *gauche*−*gauche* conformer populations using the Karplus equations.[40]

The pronounced upfield shift observed for the H2′ proton (*vide supra*) strongly suggests that the guanidine group of the base moiety is probably directed toward the H2′ proton. This is indicative of a preferential *anti* conformation of the base with respect to the sugar moiety.

**Structural Determination of L-Lysine, $N^2$-[1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4,5-dihydro-4,5-dioxo-1*H*-imidazol-2-yl]-, Intramolecular 1,5′-Ester (10). (a) Spectroscopic Features.** Photoproduct **10** has to be acetylated in order to enhance its ionization for FAB-MS analysis in a thioglycerol matrix. The acetylated sample comprised a mixture of mono- and diacetylated derivatives of **10** which gave rise to molecular ions at *m/z* 383 and 425, respectively. In addition, the exact mass measurement of the monoacetylated molecule as inferred from high-resolution FAB mass spectral analysis provided a molecular weight of 383.1643. This suggests an empirical formula of $C_{16}H_{23}N_4O_7$. Confirmation of the unusual structure of this photoproduct was provided by detailed $^1$H (Table 1) and $^{13}$ NMR (Table 2) analyses. First, it should be noted that the spectrum of **10** shows the loss of the H8 proton (Figure 4). A similar photoproduct was isolated when a dGuo structural analog, the 5′-amino-2′,5′-dideoxyguanosine, was photooxidized in aerated aqueous solution.[31] 2D scalar correlated $^1$H NMR analysis was used to assign $^1$H resonance signals (data not shown). A pronounced downfield shift effect ($\delta$ = 0.33 ppm) was observed for the H5′ proton with respect to that of the starting product **1**. In contrast, a significant upfield shift effect was noted for the H5″ proton ($\delta$ = 0.28 ppm). This is indicative of a shielding effect exerted by the carbonyl group of the ester function. Another interesting feature deals with the upfield shift of the H2′ and H2″ resonance protons with respect to that of 5′-Lys-dGuo (**1**). This may be explained by a decrease in the aromatic ring current of the base moiety with respect to that of 5′-Lys-dGuo (**1**). However, it is likely that the base orientation does not allow an interaction of the H2′ proton with a deshielding group of the modified guanine. Interestingly, the methinic proton signal of the lysine residue resonating at $\delta$ = 4.60 ppm is 0.48 and 0.83 ppm shifted downfield relative to that of the related proton of 5′-Lys-dGuo (**1**) and **6**, respectively. Two possibilities, including either the α- or ϵ-amino function, have to be considered for the binding site of the lysine to the guanine moiety. The significant downfield shift effect observed on the methinic proton strongly supports the occurrence of a covalent bond between the α-amino group to the C2 position of the base. Indeed, the proximity of the unsaturated bond between the C2 and the N3 of the modified guanine is likely to explain the deshielding of the CH proton. However, it should be noted that further support for this hypothesis cannot be obtained from $^1$H−$^{13}$C NMR long-range coupling experiments (HMBC) since no relevant correlations were observed. On the other hand, the 2D $^{13}$C−$^1$H NMR spectrum (HMQC analysis) of **10** allowed the assignment of all the protonated carbons (Figure 6). Photoproduct **10** exhibits $^{13}$C NMR features similar to those of **6**, suggesting a closely related structure. The

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(40) Haasnot, C. A. G.; De Leeuw, F. A. A. M.; De Leeuw, H. P. M.; Altona, C., *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 576−577.

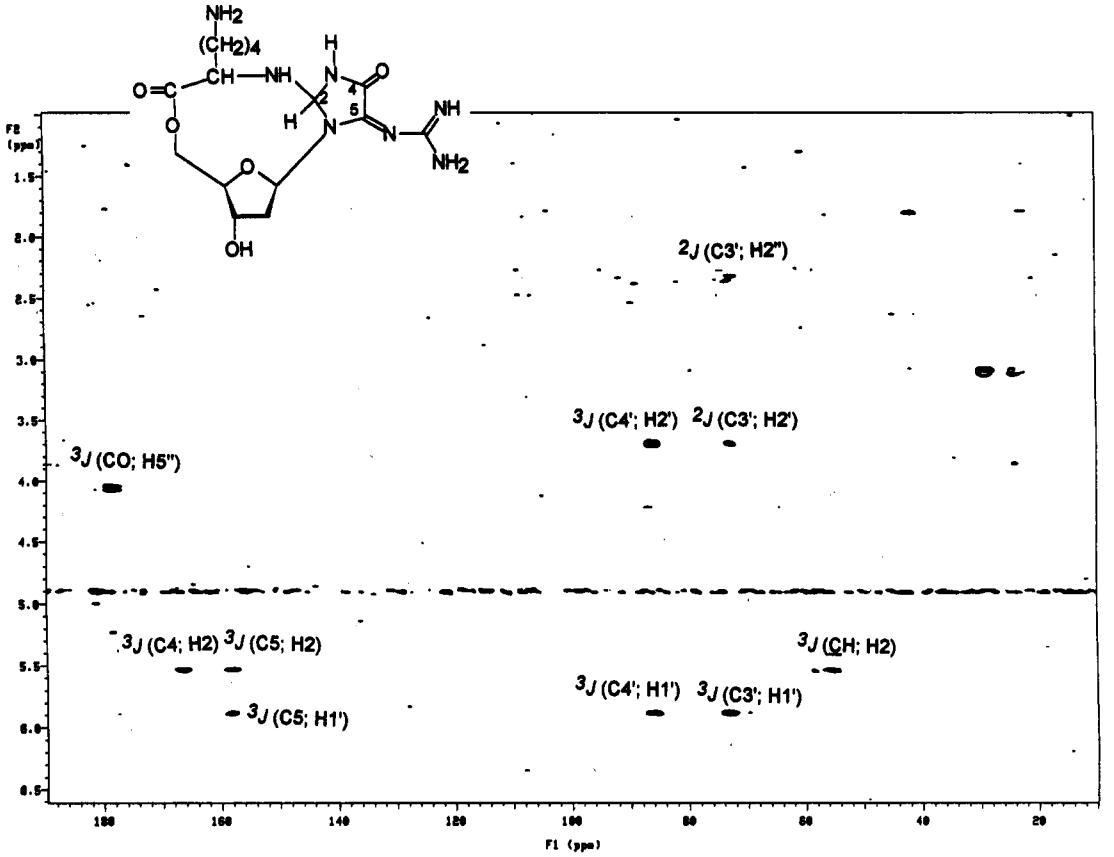


**Figure 5.** Long-range coupling experiment (HMBC) of **6** in $D_2O$.

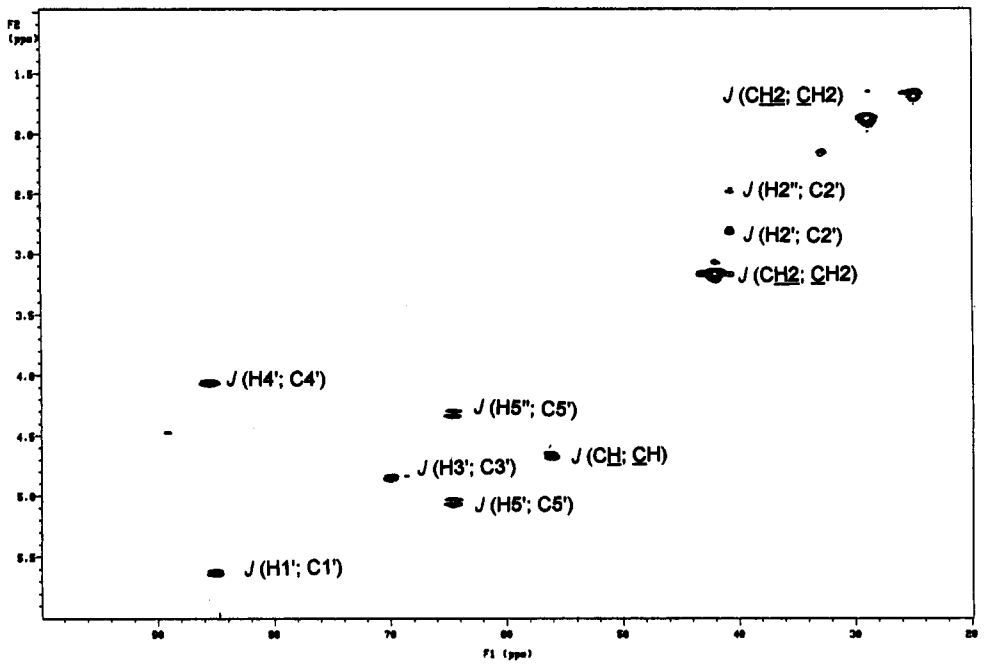


**Figure 6.** $^1H-^{13}C$ heteronuclear coupling experiment (HMQC) of **10** in $D_2O$.

absorption peak centered at 252 nm in the UV spectrum of **10** indicates that the modified guanine moiety has kept a partial aromaticity.

**(b) Conformational Properties of the Adduct.** Evidence that **10** has a cyclic structure is given by the unusually low values of $J(4',5')$ and $J(4',5'')$ coupling constants that were determined to be 1.9 and 0.6 Hz, respectively. This suggests a staggered *gauche–gauche* orientation about the C4′–C5′ bond.

The sugar coupling constants of **10** are partly different from those of **6**. The coupling constant between the H3′ proton on one hand and either the H4′ or H2″ proton on the other hand exhibits a value of ~8.0 Hz. This may be rationalized in terms of a dihedral angle of about 150°. It may be concluded that the sugar moiety exhibits a preferential C3′-*endo*–C2′-*exo* conformation as is the case for photoadduct **6**.

**Mechanisms of Formation of Photoadducts 6 and 10.** The structure assignment of the two photoadducts **6** and **10** which was achieved on the basis of extensive mass and NMR spectroscopic measurements (*vide supra*) is indicative of the occurrence of two different mechanisms (Figure 7). Benzophe-

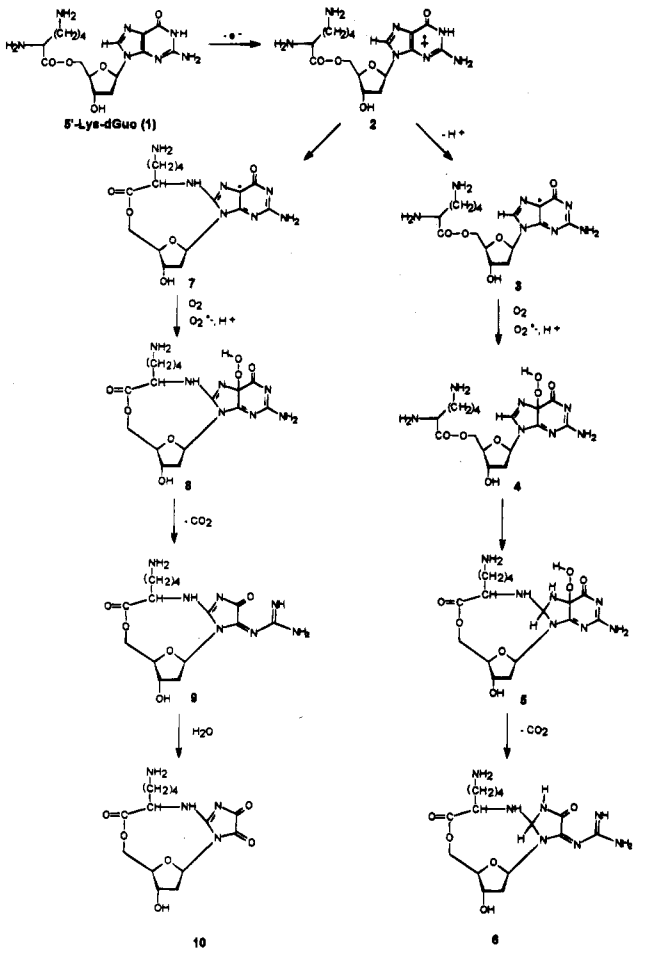


**Figure 7.** Proposed reaction mechanisms for the photosensitized generation of photoproducts **6** and **10**.

none has been shown to be an efficient photosensitizer of guanine nucleic acid components, acting through a type I mechanism.[3] The formation of **6** and **10** upon exposure of 5'-Lys-dGuo (**1**) to photoexcited type I photososensitizer is likely to involve a common intermediate. Such an intermediate is a guanine radical cation, **2**, which is generated though electron abstraction from 5'-Lys-dGuo (**1**) by triplet photoexcited benzophenone. Indeed 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[41,42] and indirectly supported by final product analysis,[29,30,43] 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-dihydroguanine adducts.[29,30,43] The photoproduct **6** may be formed by the same mechanism. It is the first characterized guanine photoadduct which involves a nucleophilic addition of an amino function to the radical **2**. It is believed to be formed when a molecule of oxygen reacts with the guanine radical **3**. Then, the resulting peroxyl radical or related hydroperoxide **4** may be subjected to a nucleophilic attack at the C2 position by the α-amino group of lysine. Molecular oxygen is required to allow the nucleophilic addition by the amino function. This is indirectly supported by the absence of formation of photoproducts **6** and **10**, when 5'-Lys-dGuo was photosensitized under oxygen-free conditions. The transient keto hydroperoxide **5** is likely to undergo an α-keto cleavage which leads to the opening of the pyrimidine ring at the C5−C6 bond with subsequent elimination of $CO_2$, giving rise to **6**.

The situation appears quite different for the formation of **10**. An interesting structural feature of **10** deals with the absence of the H8 proton within the base moiety. A similar observation was recently made for the main photoproduct which arises from intramolecular reaction of the free amino group of 5'-amino-2',5'-dideoxyguanosine to the C8 position of the guanine moiety upon one-electron oxidation.[31] A possible mechanism for the formation of **10** would be that deprotonation of the guanine radical cation **2** is prevented by the efficient nucleophilic substitution of the purine C8 by the α-amino function of the lysine moiety. This would lead to the transient formation of a cyclic nucleoside radical cation. It should be noted that a similar mechanism has been proposed to explain the nucleophilic photosubstitution of aromatic olefins by an amino function consecutive to photoinduced electron transfer.[44−47] In this step the hydrogen H8 is substituted by the nucleophilic amino group. However, at the present time, the mechanism of the reaction still remains tentative. Then, the cyclic nucleoside radical cation may undergo a deprotonation reaction. Oxygen addition to **7** at C5 is expected to generate the hydroperoxide **8** through a transient peroxyl radical. The latter keto hydroperoxide **8** is likely to undergo a β-scission within the pyrimidine ring at the C5−C6 bond with subsequent elimination of $CO_2$. Then the resulting nucleoside **9** loses its guanidine group through hydrolysis to give the final photoproduct **10**. It is interesting to note that the formation of imidazolidone and oxazolone compounds[43] which involves initial deprotonation of the radical cation **2** and subsequent addition of water was not observed in the benzophenone-mediated photooxidation of 5'-Lys-dGuo (**1**). This could be explained by both the high nucleophilicity of the amino group and the close proximity between the α-amino group of the lysine and the C8 position of the guanine moiety.

## Conclusion

The presently reported photoreactions between guanine and lysine residues constitute model systems which should be useful to further investigate the photosensitized formation of DNA−protein cross-links.

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