J|A|C|S
ARTICLES
Published on Web 04/05/2002

# Formation of Transient Intermediates in Low-Temperature Photosensitized Oxidation of an 8-$^{13}$C-Guanosine Derivative

Ping Kang and Christopher S. Foote*

*Contribution from the Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90095-1569*

Received August 23, 2001. Revised Manuscript Received January 18, 2002

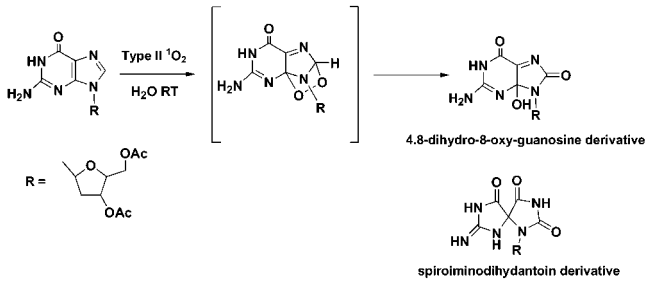
***Abstract:*** An 8-$^{13}$C-labeled guanosine derivative, 2′,3′,5′-*O*-*tert*-butyldimethylsilyl-*N*-*tert*-butyldimethylsilyl-8-$^{13}$C-guanosine, was synthesized and its photosensitized oxidation with singlet oxygen carried out below −100 °C. Two transient intermediates that decompose directly to the final major product **5** and $CO_2$ were detected by $^{13}$C NMR between −100 and −43 °C. The two intermediates are carbamic acids based on $^{13}$C NMR and 2D NMR (HMQC, HMBC) spectra and the formation of final product **5** and of 8-$CO_2$. No endoperoxide intermediate could be detected by low-temperature NMR spectroscopy even at −100 °C. A reaction mechanism is proposed involving initial [4 + 2] cycloaddition of singlet oxygen to the imidazole ring to form an unstable endoperoxide, subsequent rearrangement of the endoperoxide to a dioxirane, and decomposition of the dioxirane to the two observed intermediates. Both oxygen atoms of $CO_2$ are derived from a single oxygen molecule, which strongly supports a dioxirane structure for the precursor of the two observed intermediates. The distribution of products estimated by $^{13}$C NMR accounts for all the $^{13}$C-containing products in the reaction mixture.

## Introduction

Photodynamic action involves the oxidation of biologically important molecules upon exposure to visible light with air in the presence of photosensitizer.[1−11] Photodynamic therapy (PDT) has been used to treat certain skin diseases and several types of malignant tumors.[12−16] Carcinogenic or mutagenic effects result from photooxidative modifications of DNA.[4,17,18]

Guanine, as the free base or in nucleosides and nucleotides, has been shown to be virtually the only base that is reactive with singlet oxygen.[19−22] Many attempts have been made to isolate and characterize intermediates and products of photosensitized oxidation of guanine derivatives. However, because of the low solubility of guanine derivatives in most solvents, the instability of the intermediates, and analytical difficulties in the separation and characterization of the polar and unstable photoproducts, the mechanism of photosensitized oxidation of guanine and its nucleosides is still not clear.

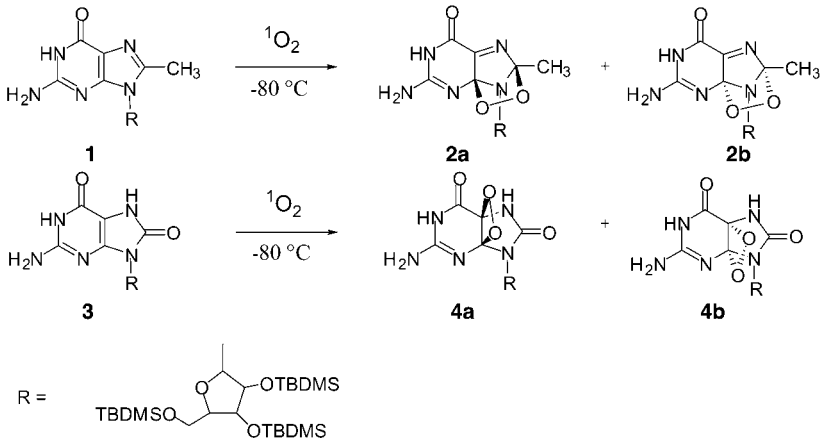
Diastereomers of 9-(3′,5′-di-)acetyl-2′-deoxy-β-D-*erythro*-pentofuranosyl-4,8-dihydro-4-hydroxy-8-oxoguanine (4-hydroxy-8-oxo-dG) were reported by Cadet and co-workers as the main products from photosensitized oxidation of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine.[1−3,8,23,24] It was suggested that the two diastereomers are formed from unstable diastereomeric intermediate endoperoxides from [4 + 2] cycloaddition with $^1O_2$ to the imidazole ring. Recently these compounds have been shown to be diastereomeric spiroiminodihydantoins.[25−27]



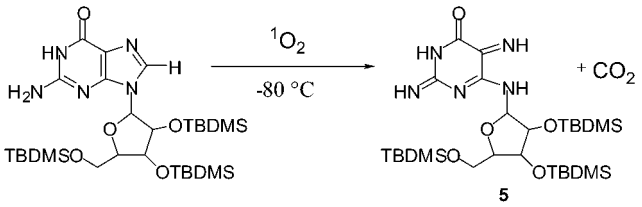

Photosensitized oxidation of guanosine in aqueous medium above 0 °C cannot provide direct information on reaction

(1) Cadet, J.; Vigny, P. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley & Sons: New York, 1990; Vol. 1, pp 1−272.
(2) Cadet, J.; Decarroz, C.; Wang, S. Y.; Midden, W. R. *Isr. J. Chem.* **1983**, *23*, 420−429.
(3) Cadet, J.; Berger, M.; Decarroz, C.; Wagner, J. R.; van Lier, J. E.; Ginot, Y. M.; Vigny, P. *Biochimie* **1986**, *68*, 813−834.
(4) Piette, J. *J. Photochem. Photobiol., B: Biol.* **1990**, *4*, 335−342.
(5) Piette, J. *J. Photochem. Photobiol.* **1991**, *11*, 241−260.
(6) Sies, H. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1058−1071.
(7) Marx, J. L. *Science* **1987**, *235*, 529−531.
(8) Cadet, J.; Teoule, R. *Photochem. Photobiol.* **1978**, *28*, 661−667.
(9) He, Y. Y.; An, J. Y.; Jiang, L. J. *J. Photochem. Photobiol., A: Chem.* **1999**, *120*, 191−199.
(10) Salet, C.; Moreno, G.; Ricchelli, F.; Bernardi, P. *J. Biol. Chem.* **1997**, *272*, 21938−21943.
(11) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 474−477.
(12) Dougherty, T. J.; Potter, W. R.; Weishaupt, K. R. In *Porphyrin Localization and Treatment of Tumors*; Doiron, D. R., Gomer, C. J., Eds.; Alan R. Liss: New York, 1984; Progress in Clinical and Biological Research Vol. 170, pp 301−314.
(13) Dougherty, T. J. *Photochem. Photobiol.* **1993**, *58*, 895−900.
(14) Shevchuk, I.; Chekulayev, V.; Chekulayeva, L. *J. Photochem. Photobiol. B: Biol.* **1998**, *45*, 136−143.
(15) Phillip, M. J.; McMahon, J. D.; O'Hara, M. D.; Hetzel, F. W.; Amsterdamsky, C.; Schaap, A. P. In *Porphyrin Localization and Treatment of Tumors*; Doiron, D. R., Gomer, C. J., Eds.; Alan R. Liss: New York, 1984; Progress in Clinical and Biological Research Vol. 170, pp 563−570.
(16) Fischer, F.; MaierBorst, W.; Lorenz, W. J. *J. Photochem. Photobiol., B: Biol.* **1998**, *43*, 27−33.
(17) Ribeiro, D. T.; Madzak, C.; Sarasin, A.; Dimascio, P.; Sies, H.; Menck, C. F. M. *Photochem. Photobiol.* **1992**, *55*, 39−45.
(18) Spikes, J. D. *J. Photochem. Photobiol. B: Biol.* **1998**, *42*, 1−11.
(19) Clagett, D. C.; Galen, T. J. *Arch. Biochem. Biophys.* **1971**, *146*, 196−201.
(20) Rosenthal, I.; Pitts, J. N., Jr. *Biophys. J.* **1971**, *11*, 963.
(21) Sastry, K. S.; Gordon, M. P. *Biochim. Biophys. Acta* **1966**, *129*, 42.
(22) Prat, F.; Hou, C. C.; Foote, C. S. *J. Am. Chem. Soc.* **1997**, *119*, 5051−5052.

***Scheme 1***




***Scheme 2***




intermediates because of their instability at this temperature and their reactivity with water. In a previous study, we obtained organic-soluble guanosine derivatives by functionalizing the hydroxyl groups on the ribose, which allowed us to carry out low-temperature photosensitized oxidation and detect transient intermediates.[11,28] Using low-temperature NMR, Sheu and Foote reported the identification of diastereomeric endoperoxides (**2a, 2b**) in the low-temperature photosensitized oxidation (−80 °C) of 2′,3′,5′-*O*-(*tert*-butyldimethylsilyl)-8-methylguanosine (**1**).[28] Additionally, diastereomeric dioxetanes **4a** and **4b** were identified in the photosensitized oxidation (−80 °C) of 2′,3′,5′-*O*-(*tert*-butyldimethylsilyl)-7,8-dihydro-8-oxoguanosine (**3**) (Scheme 1).[11] A major final product with an oxidized imidazole ring was characterized from the photosensitized oxidation of 2′,3′,5′-*O*-(*tert*-butyldimethylsilyl)guanosine.[29] However, no transient intermediates were detected (Scheme 2).

In the present paper, we report the results of the photosensitized oxidation (<−100 °C) of an 8-$^{13}$C-labeled derivative, 2′,3′,5′-*O*-*tert*-butyldimethylsilyl-*N*-*tert*-butyldimethylsilyl-8-$^{13}$C-guanosine (8-$^{13}$C-guanosine; Scheme 3). Two transient intermediates were detected by $^{13}$C NMR and assigned carbamic acid structures. Both intermediates decompose to form 8-$CO_2$ and the imidazole ring-opened product **5** characterized previously.[29] A mechanism that involves endoperoxide and dioxirane intermediates is proposed based on our experimental results (Schemes 5−7). In previous studies, HPLC and silica gel column chromatography were used to isolate the products of photosensitized oxidation before characterization.[23,29] The yields of the products thus isolated are usually low (less than 60%), which suggests there are always products that elude observation by these techniques. With 8-$^{13}$C-guanosine, by quantitative analysis of $^{13}$C NMR, we are able to account for all 8-carbon-containing products in the reaction mixture.

Previous study in our laboratory of the low-temperature (−80 °C) photosensitized oxidation of 2′,3′,5′-*O*-*tert*-butyldimethylsilylguanosine showed that **5** was the major product.[29] We carried out the same reaction at lower temperature (<−100 °C) in $CBr_2F_2$ and found that $CO_2$ is the other major product (by NMR) and is most likely derived from the 8-C (Scheme 2). However, due to the line broadening and the low sensitivity of NMR signals of the reaction mixture at −100 °C, we could not obtain clear NMR spectra in reasonably short times. Prolonged NMR acquisition yielded the spectrum of the final product **5** along with a complex mixture of other products.

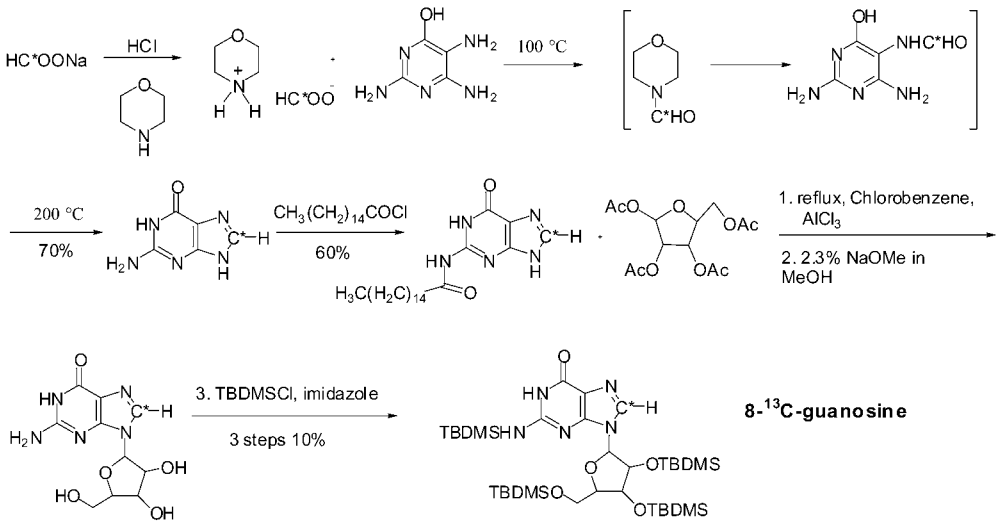
There are several advantages of using 8-$^{13}$C-labeled guanosine. (1) The sensitivity of the $^{13}$C NMR signal is greatly increased, making it suitable for detection of unstable intermediates in a short time. (2) Because of the large differences in intensity between the 8-$^{13}$C and other unlabeled carbons in the reaction mixture, we could greatly simplify the spectrum of the reaction mixture by choosing conditions under which only the signals of the $^{13}$C-labeled carbons are visible. (3) The one-bond and long-range C−H scalar couplings between the 8-$^{13}$C and various H atoms (which cannot be obtained in unlabeled guanosine at low temperatures within a short time) should provide valuable information about the structures of the transient intermediates. (4) Detection of $^{13}CO_2$ would unambiguously confirm that it derives from the 8-C.

## Results

**Synthesis of 8-$^{13}$C-Guanosine.** Taking into consideration the fact that that 8-C of guanosine is the carbon where many photooxidations take place,[11,23,28,30−33] we synthesized ‴8-$^{13}$C-guanosine using a modification of literature procedures (Scheme 3).[34,35] Treatment of sodium $^{13}$C-formate with morpholine and HCl gave morpholinium $^{13}$C-formate, which, when heated with

(23) Ravanat, J. L.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 379−388.
(24) Ravanat, J.-L.; Berger, M.; Benard, F.; Langlois, R.; Ouellet, R.; van Lier, J. E.; Cadet, J. *Photochem. Photobiol.* **1992**, *55*, 809−814.
(25) Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. *Org. Lett.* **2001**, *3*, 963−966.
(26) Luo, W. C.; Muller, J. G.; Rachlin, E. M.; Burrows, C. J. *Chem. Res. Toxicol.* **2001**, *14*, 927−938.
(27) Adam, W. personal communication, 2001.
(28) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 10446−10447.
(29) Sheu, C. M.; Kang, P.; Saeed, K.; Foote, C. S. *J. Am. Chem. Soc.*, in press.
(30) Adam, W.; Treiber, A. *J. Am. Chem. Soc.* **1995**, *117*, 2686−2693.
(31) Adam, W.; Saha-Möller, C. R.; Schönberger, A.; Berger, M.; Cadet, J. *Photochem. Photobiol.* **1995**, *62*, 231−238.
(32) Adam, W.; Andler, S.; Nau, W. M.; Saha-Möller, C. R. *J. Am. Chem. Soc.* **1998**, *120*, 3549−3559.
(33) Morin, B.; Cadet, J. *J. Am. Chem. Soc.* **1995**, *117*, 12408−12415.
(34) Sharma, M.; Alderfer, J. L.; Box, H. C. *J. Labelled Compd. Radiopharm.* **1983**, *20*, 1219.
(35) Furukawa, Y.; Honjo, M. *Chem. Pharm. Bull.* **1968**, *16*, 1076.

***Scheme 3***[a]




[a] C* is $^{13}C$.

2,4,5-triamino-6-hydroxypyrimidine sulfate at 100 °C, gave formyl 2,4,5-triamino-6-hydroxypyrimidine, presumably via *N*-formylmorpholine. The reaction mixture was further heated to 200 °C, resulting in 8-$^{13}$C-guanine. Protecting the amino group of 8-$^{13}$C-guanine with a palmitoyl group and then coupling with tetraacetylribofuranose followed by deprotection of the sugar gave 8-$^{13}$C-guanosine. Without separation, the 8-$^{13}$C-guanosine was treated with a large excess of TBDMS chloride, which allows easier separation and purification of the final 8-$^{13}$C-guanosine.

The 8-$^{13}$C-labeled guanosine derivative was fully characterized by $^1$H NMR, $^{13}$C NMR, and MS (see Experimental Section and Supporting Information for details). The C8−H is split into a doublet by the adjacent 8-$^{13}$C ($J_{C-H}$ = 212.3 Hz). The corresponding 8-$^{13}$C is also split by the C8−H ($J_{C-H}$ = 212.0 Hz). The $^1$H−$^{13}$C HMQC spectrum shows coupling between C8−H and 8-$^{13}$C. The C1′−H becomes a doublet of doublets ($J_1$ = 5.2 Hz, $J_2$ = 7.8 Hz), indicating a three-bond coupling between the 8-$^{13}$C and the C1′−H, which is confirmed by the $^1$H−$^{13}$C HMBC spectrum showing the cross-peak between 8-$^{13}$C and C1′−H. The $^{13}$C abundance estimated from MS is almost 96%.

**Low-Temperature Photooxidation.** The photosensitized oxidation of 8-$^{13}$C-guanosine was carried out in $CBr_2F_2$ at <−100 °C with 2,9,16,23-tetra-*tert*-butyl-19*H*,31*H*-phthalocyanine as photosensitizer, using a chromium glass filter to cut off light with wavelength below 540 nm, with a Cermax 300-W xenon lamp as the light source. After several hours of photolysis, the reaction mixture was kept in liquid $N_2$ and then placed in the precooled NMR probe for analysis. The $^{13}$C NMR spectrum shows clear peaks at low temperature and was used in this study. $^1$H NMR is not informative because of the severe line broadening at low temperature.

The $^{13}$C NMR spectra were taken at temperatures from −100 °C (173 K) to room temperature. Five NMR peaks were detected at −100 °C, 124.6, 134.6, 145.5, 154.3, and 164.0 ppm. Under the NMR acquisition conditions (~0.5 h), only the labeled $^{13}$C peaks appear, each peak representing one compound. The 134.6 ppm peak corresponds to the unreacted guanosine derivative. The 124.6 ppm peak is characteristic of $CO_2$. The other three peaks belong to new products. As the temperature rises from

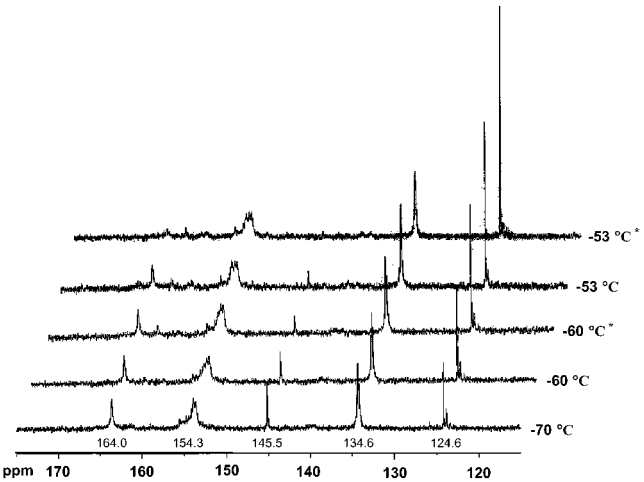



***Figure 1.*** Disappearance of two transient intermediates at 145.5 and 164.0 ppm and increase of $CO_2$ at 124.6 ppm with temperature and time. Asterisk indicates spectrum retaken 1 h after the first spectrum at the same temperature.

−100 °C, these peaks remain unchanged until −70 °C (203 K) where the peaks at 145.5 and 164.0 ppm begin to decrease and the $CO_2$ peak begins to increase. The two transient peaks completely disappear at −43 °C (230 K). The 154.3 ppm peak remains unchanged, and no other peaks are formed during this process. Figure 1 is the stacked spectra of the reaction mixture from −70 (203 K) to −53 °C (223 K) and shows the disappearance of the two transient peaks and the increase of $CO_2$ (124.6 ppm) with temperature and time. On the basis of the fact that no new $^{13}$C peaks appear during the decomposition of the two transient compounds and only $CO_2$ increases, we conclude that both transient compounds decompose to form $CO_2$. Product **5** was also formed in the reaction and was spectroscopically identical to our previous report.[29] Taking into account the formation of product **5**, we propose that the decompositions of the two transient compounds lead to both **5** and $CO_2$ directly (Scheme 4).

To further explore this suggestion, we quantitatively followed the NMR peak changes of the reaction mixture with temperature and time by integrating the $^{13}$C NMR peaks. Figure 2 shows more clearly that, as the temperature changes, the intensities of

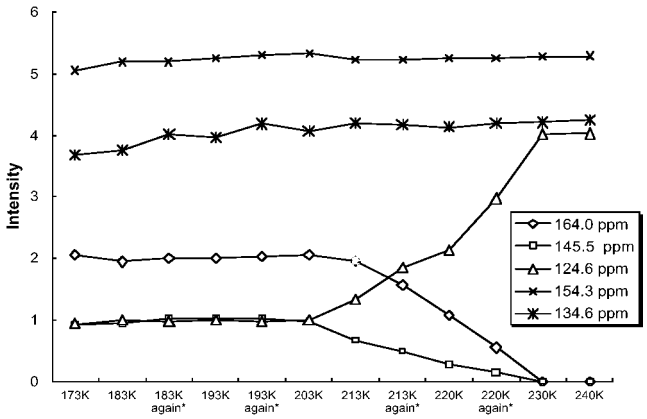
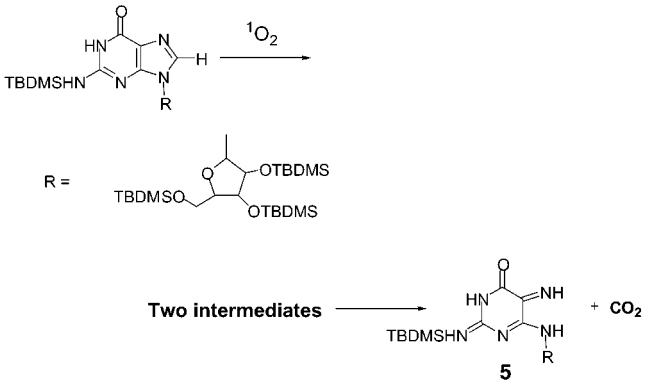



***Figure 2.*** Change of NMR peaks with temperature and time. Intensity relative to internal acetone-$d_6$ (207.1 ppm, intensity set to 10). Asterisks indicate spectra retaken 1 h later.

***Scheme 4***




the two transient peaks at 164.0 and 145.5 ppm remain constant until −70 °C (203 K), where both peaks begin to decrease and completely disappear at −43 °C (230 K). The total area of the transient peaks at 145.5 and 164.0 ppm decrease at the same rate as the increase of the $CO_2$ peak at 124.6 ppm. The $CO_2$ peak does not change from −43 to −33 °C (240 K), which indicates that no more $CO_2$ is formed after the complete decomposition of the two transient compounds. Figure 2 also suggests that the two intermediates have similar lifetimes. The peaks of the unreacted guanosine at 134.6 ppm and the other product at 154.3 ppm do not change during the whole process.

Our suggestion that $CO_2$ comes solely from the decompositions of the two transient intermediates is further confirmed by Figure 3, in which we compare the total intensity decrease of the two transient peaks at 164.0 and 145.5 ppm with the intensity increase of $CO_2$ at 124.6 ppm as a function of temperature and time. It is apparent that the intensity gain of $CO_2$ at 124.6 ppm is almost the same as the total intensity loss of peaks at 164.0 and 145.5 ppm as temperature or time changes. After the complete decomposition of the two transient intermediates, the intensity of the $CO_2$ peak does not change.

If $CO_2$ comes solely from the decomposition of the two transient intermediates, the total intensity of $CO_2$ and two transient peaks should not change during the process. Figure 4 shows that total intensity of the two transient peaks at 164.0 and 145.5 ppm and $CO_2$ at 124.6 ppm does not change with temperature and time, nor does that of product at 154.3 ppm. We estimate from Figure 4 that ∼45% of $^{13}C$ atoms are incorporated into $CO_2$ and ∼55% into the product at 154.3 ppm.

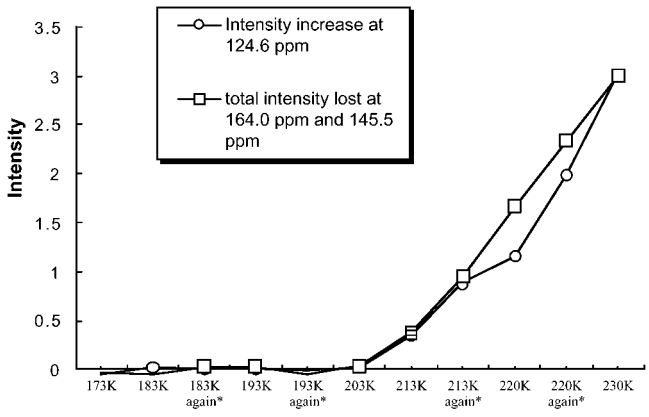



***Figure 3.*** Comparison of intensity increase of $CO_2$ at 124.6 ppm with the total intensity loss at 164.0 and 145.5 ppm as the temperature and time change. Intensity relative to internal acetone-$d_6$ (207.1 ppm, intensity set to 10). Asterisks indicate spectra retaken 1 h later.

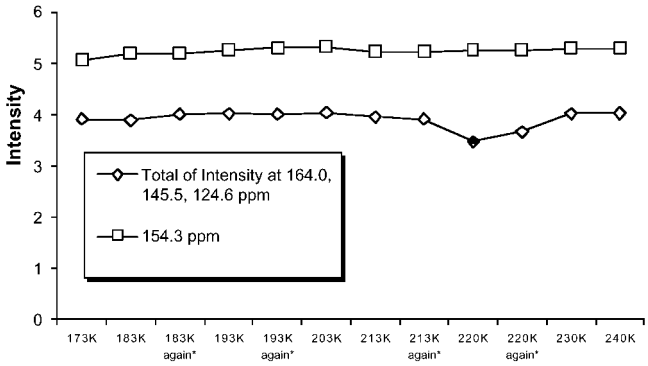



***Figure 4.*** Comparison of total intensity of two transient intermediate peaks at 164.0 ppm 145.5 ppm and $CO_2$ at 124.6 ppm with the intensity of peak at 154.3 ppm as the temperature and time change. Intensity relative to internal acetone-$d_6$ (207.1 ppm, intensity set to 10). * Spectra retaken 1 h later.

From the $^1H$ NMR spectrum, we also obtained 40−60% yield of the final product **5**. The two matched yields and the observation that the decomposition of two transient intermediates at 145.5 and 164.0 ppm and production of $CO_2$ occur simultaneously lead us to the conclusion that **5** and $CO_2$ are directly derived from the two transient intermediates (Scheme 4).

2D NMR spectra ($^1H-^{13}C$ HMQC and $^1H-^{13}C$ HMBC) of 8-$^{13}C$-guanosine show one-bond C−H coupling between 8-$^{13}C$ and 8-H and three-bond coupling between 8-$^{13}C$ and 1′-H of the sugar. After photolysis, HMQC at −100 °C shows that the one-bond couplings are lost for all the resulting peaks in the reaction mixture except that of the starting guanosine, which indicates that 8-H is lost in all the products even at −100 °C. The $^1H-^{13}C$ HMBC of the reaction mixture at −70 °C (203 K) displays a long-range coupling between the 1′-H and the $^{13}C$ in only one of the two transient intermediates, the one with the resonance at 145.5 ppm. No long-range coupling is observed for the product with the chemical shift at 164.0 ppm. Since the NMR spectra of the final product **5** shows no loss of the sugar moiety, and there is no loss of sugar peaks in the FAB-MS spectrum of the reaction mixture, the 8-C−9-N bond must have broken for the product with chemical shift at 164.0 ppm.

**$CO_2$ Formation.** The formation of $CO_2$ was confirmed by GC/MS. $CO_2$ (44 *m*/*z*) was detected by warming the reaction mixture after the photosensitized oxidation of the unlabeled guanosine derivative at below −100 or −80 °C. $^{13}CO_2$ (45 *m*/*z*)

***Table 1.*** Distribution of Isotopic $CO_2$ Resulting from Photosensitized Oxidation of Guanosine **14**

| | $^{44}CO_2$ | $:^{45}CO_2$ | $:^{46}CO_2$ | $:^{47}CO_2$ | $:^{48}CO_2$ |
|---|---|---|---|---|---|
| experiment[b] | 100 | : 1.22 | : 0.90 | : 0 | : 2.24 |
| 2-O[c,d] | 100 | : 1.15 | : 0.64 | : 0 | : 2.28 |
| 1-O[d,e] | 100 | : 2.09 | : 4.80 | : 0.09 | : 0.12 |

[a] See Experimental Section for the details of the calculations. [b] Reaction mixture at room temperature for 2 h after photolysis at $<-100$ °C, after correction for $CO_2$ in air. [c] 2-O was calculated based on the assumption that both O atoms come from one $O_2$ molecule. [d] $O_2$ experimental abundance $^{32}O_2:^{33}O_2:^{34}O_2:^{36}O_2 = 100:0:0.98:5.40$, natural $^{12}C:^{13}C = 100:1.10$, based on the assumption that $^{48}CO_2$ comes solely from the 8-C, $^{45}CO_2:^{44}CO_2 = 1:1.37$ at room temperature, natural $CO_2$ distribution $^{44}CO_2:^{45}CO_2:^{46}CO_2:^{48}CO_2 = 100:1.18:0.4:0$. [e] 1-O was calculated on the basis of the assumption that the two O atoms come from two different $O_2$ molecules.

was observed from the reaction mixture of 8-$^{13}$C-guanosine, which provides additional support for our suggestion that the 8-C is converted to $^{13}CO_2$.

To examine how $O_2$ is incorporated into the resulting $CO_2$, we carried out photosensitized oxidation of the guanosine derivative with $^{18}O_2$-enriched $O_2$ ($^{32}O_2:^{34}O_2:^{36}O = 100:0.98:5.40$). The resulting isotopic $CO_2$ was analyzed by GC/MS, and the results are summarized in Table 1. The experimental distribution of $CO_2$ isotopomers ("experiment") is almost the same as that calculated by assuming that the two oxygen atoms of $CO_2$ come solely from the same oxygen molecule (2-O). On the other hand, the $CO_2$ distribution calculated on the assumption that the two oxygen atoms come from two different oxygen molecules (1-O) is very different from our experimental data, especially the fraction of $^{46}CO_2$, which is 5 times larger, and that of $^{48}CO_2$, which is 18 times smaller than experiment. It is apparent from this result that the two oxygen atoms of $CO_2$ are derived solely from one oxygen molecule.

**Trapping Study.** We carried out several trapping studies in order to trap any possible transient oxidizing intermediates. $Me_2S$ was introduced to the reaction mixture immediately after photolysis and no $Me_2SO$ was detected. When we added $PPh_3$ to the reaction mixture at $-80$ °C, all the $^{13}$C peaks remained intact. We also carried out co-photooxidation of guanosine with $Ph_2S$, and no $Ph_2SO$ was observed. The trapping studies suggest that the two transient intermediates with chemical shifts at 145.5 and 164.0 ppm are not oxidizing since none of the trapping agents were oxidized and neither peak changed in the presence of $PPh_3$. The fact that $Ph_2S$ was not oxidized in the co-photooxidation of guanosine indicates that the species preceding the two intermediates is either not oxidizing or decomposes so rapidly that oxidative pathways cannot compete.

**Structure of the Stable Major Product.** The other major product (chemical shift 154.3 ppm) was generated at $-100$ °C. The 8-$^{13}$C peak was broad and probably consisted of multiple peaks with similar chemical shifts. Upon warming to $-43$ °C, this peak split into a sharper peak at 155.5 ppm and a broad peak at 155.0 ppm. The sharper peak persisted as we raised the temperature to room temperature. At the same time, the broad peak became broader and several smaller peaks appeared between 153.0 and 164.0 ppm. The sharper peak (155.5 ppm) is very similar to that of the compound originally assigned the structure 4-hydroxy-8-oxo-dG (155.6 ppm for C8) by Ravanat and Cadet[23] and more recently reassigned as diastereomeric spiroiminodihydantoins.[26,27] The peaks at 154.3 ppm could also be identical to the 4,8-dihydro-4-hydroxy-8-oxoguanosine diastereomers formed in the photooxidation of 7,8-dihydro-8-oxoguanosine, which has resonances at 155.0 and 155.4 ppm but has significant differences from the spirodiiminohydantoin in other parts of the spectrum.[11] The two diastereomers of this compound could account for some of the complexity of the peak. We were not able to resolve the other peaks, but note that many of the products of further degradation that have been observed (guanidinohydantoin (Gh) and iminoallantoin (Ia) (156.3, 157.2 ppm)[26]) have peaks in this region.

## Discussion

Singlet oxygen reacts with nucleic acids almost exclusively at the guanine residue, and the five-membered imidazole ring is the reactive site. Both [4 + 2] and [2 + 2] cycloaddition to form endoperoxide and dioxetane intermediates have been suggested for photosensitized oxidation of guanosine.[2,3,23] We did not succeed in our attempt to detect directly either intermediate in the photooxidation of the guanosine at $-100$ °C, suggesting that any such intermediate must rearrange very rapidly even at this temperature, if it is formed at all. However, we observed two transient intermediates which are most probably derived from the endoperoxide.

According to the experimental observations, the two transient intermediates have chemical shifts at 145.5 and 164.0 ppm, have similar lifetimes, and are not oxidative. Both decompose directly to **5** and $CO_2$, and neither has H attached to the 8-C. The intermediate with a chemical shift of 145.5 ppm has a three-bond C−H coupling between 8-C and 1′-H of the sugar, but the intermediate at 164.0 ppm has no such coupling. On the basis of these results, we propose the carbamic acid structures **6** and **7** for the two transient intermediates (Chart 1). It has been reported that the chemical shifts of carbonyls in compounds with similar C═N−COO structures are above 161 ppm [36−38] and the chemical shifts of carbonyls in compounds with NH−COO structures are ∼150 ppm [39−42] (Chart 2), which correlate very well with our structure assignments. Compounds **6** and **7** readily decompose to form **5** and $CO_2$. They would not be expected to react with either dimethyl sulfide or triphenylphosphine. Neither has H directly connected to 8-C. The proposed structures are further substantiated by the observation in the HMBC that coupling between the 8-$^{13}$C and 1′-H exists in **6** (145.5 ppm) but not in **7** (164.0 ppm), which shows the 8-C−9-N bond exists in **6** but is broken in **7**. Two other possible structures for the two transient intermediates are **8** and **9**, which can decompose to **5** and $CO_2$. We rule out these structures because the chemical shifts of **8** and **9** are not expected to differ by 20 ppm. Furthermore, **8** and **9** would not be expected to give $CO_2$ at these low temperatures, and there is no obvious route for their formation. In the photosensitized oxidation of a $^{13}$C-labeled imidazole, we identified the ring-closed form (Scheme 9, **B**) of the carbamic acid as the precursor to $CO_2$ formation. [29] It is possible that **6** and **7** are also in the closed

(36) Swenton, J. S.; Biggs, T. N.; Clark, W. M. *J. Org. Chem.* **1993**, *58*, 5607−5614.
(37) Vidal, J.; Guy, L.; Sterin, S.; Collet, A. *J. Org. Chem.* **1993**, *58*, 4791−4793.
(38) Kanazawa, A. M.; Denis, J. N.; Greene, A. E. *J. Org. Chem.* **1994**, *59*, 1238−1240.
(39) Davidsen, S. K.; May, P. D.; Summers, J. B. *J. Org. Chem.* **1991**, *56*, 5482−5485.
(40) Lützen, A.; Köll, P. *Tetrahedron: Asymmetry* **1997**, *8*, 1193−1206.
(41) Morales-Ríos, M. S.; Martínez-Galero, N. X.; Loeza-Coria, M.; Joseph-Nathan, P. *J. Org. Chem.* **1995**, *60*, 6194−6197.
(42) Suárez-Castillo, O. R.; GarcíaVelgara, M.; Morales-Ríos, M. S.; Joseph-Nathan, P. *Can. J. Chem.* **1997**, *75*, 959−964.

*Chart 1*

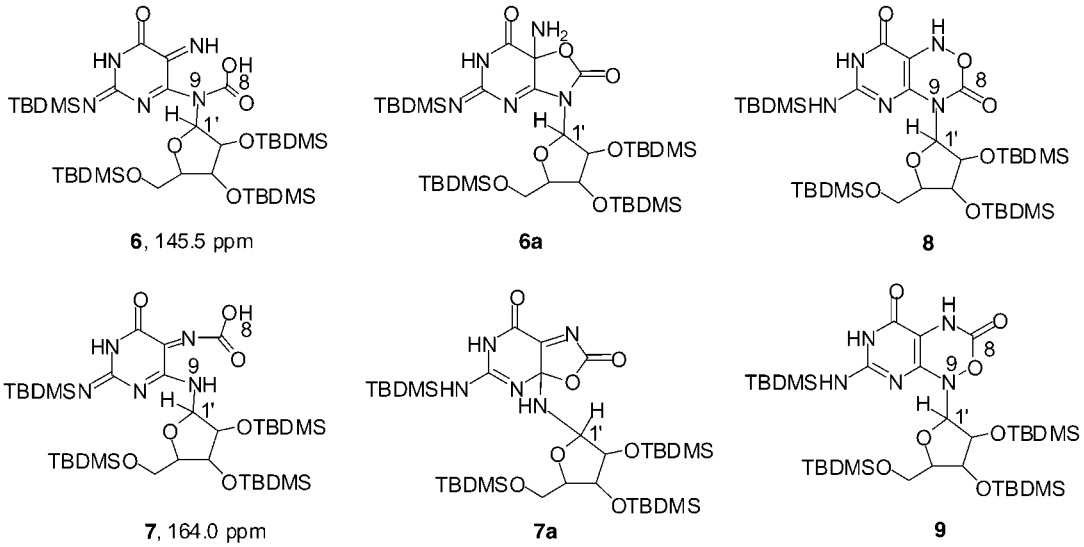
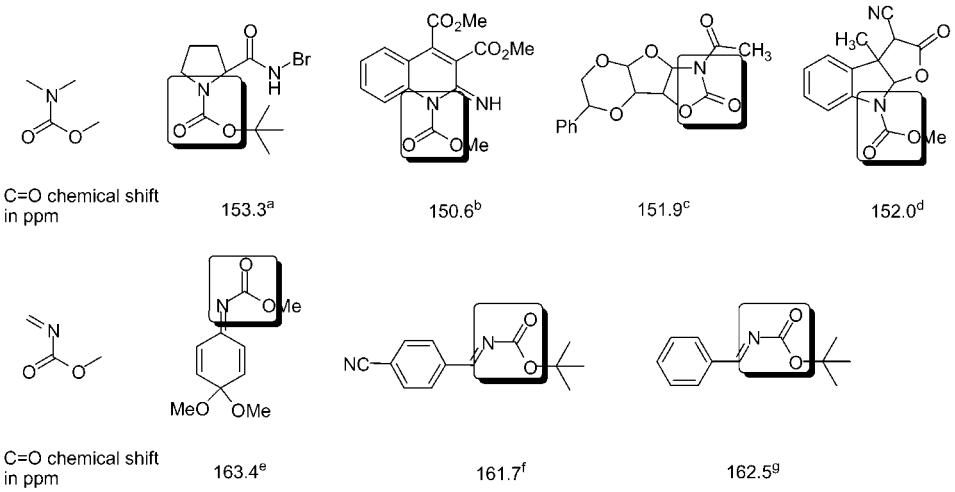



*Chart 2*




[a] Reference 39. [b] Reference 41. [c] Reference 40. [d] Reference 42. [e] Reference 36. [f] Reference 38. [g] Reference 37.

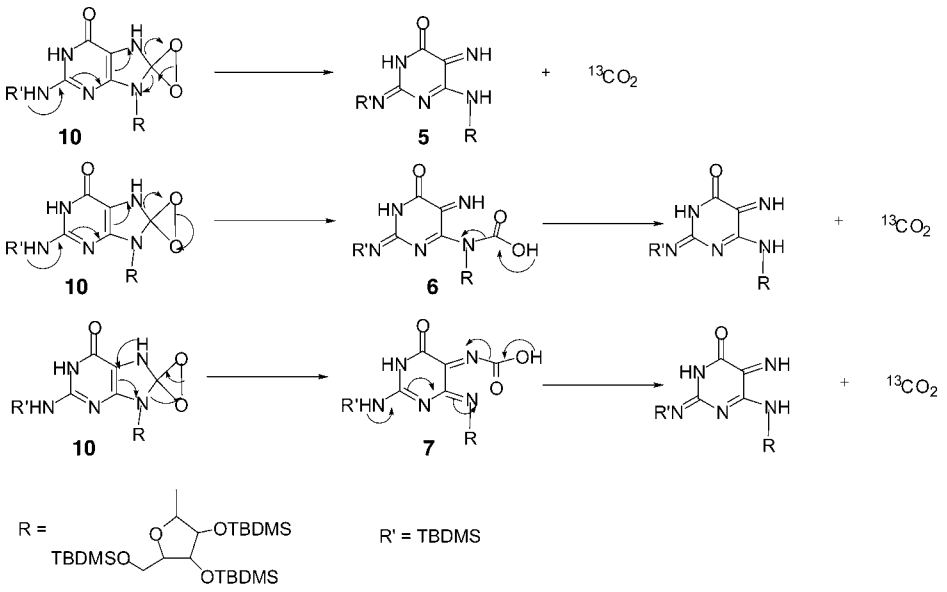
form **6a** and **7a**. However, we cannot differentiate between the open and the closed forms based solely on 8-$^{13}$C NMR data.

Taking into account the results from the $^{18}$O experiment that the two oxygen atoms of 8-$CO_2$ come solely from one $O_2$ molecule, the most likely precursor to the two observed intermediates (**6** and **7)** could have a dioxirane structure **10**. Initially one O atom of $O_2$ attaches to the 8-C, and then the second O atom must attach to the same carbon without breaking the O−O bond. If the O−O bond breaks before the second O attacks the 8-C, we would expect $^{18}$O scrambling in the $CO_2$. Under the experimental conditions, **10** does not build up in the reaction mixture but rapidly rearranges to **6** and **7**. Some probably also decomposes directly to 8-$CO_2$ and **5**, since we observed that, even at −100 °C, there is already a certain amount of $CO_2$ and **5** present in the reaction mixture (Scheme 5). An alternative explanation (as pointed out by a reviewer) for the incorporation of two O atoms from a single $O_2$ into the $CO_2$ involves the formation of a caged pair (radical or ionic) from O−O cleavage of the hydroperoxide **11**. This could in principle produce **6** and **7**. The absence of scrambling of the O-atom label in the $CO_2$ formed requires that there be almost no diffusion out of the cage, which could be possible at low temperature.

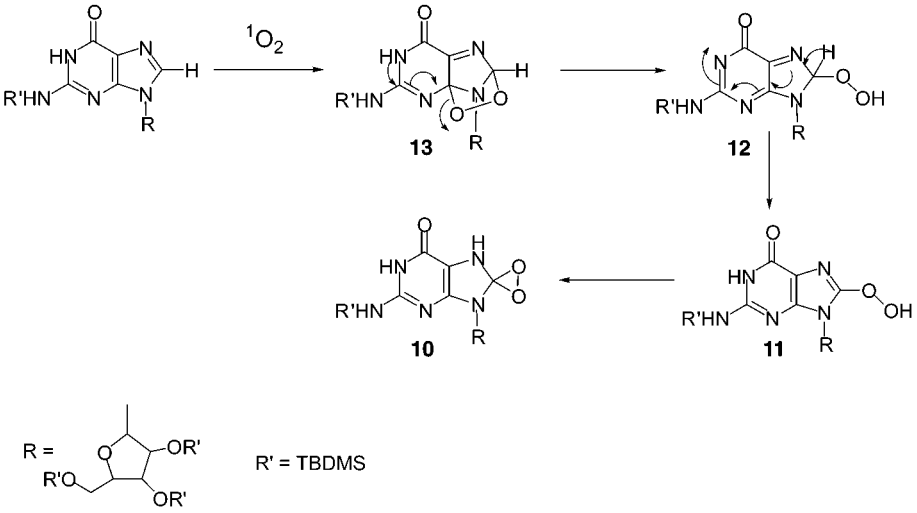
Calculations by Hoffmann et al.[43] show that the dioxirane $CO_2(NH_2)_2$ is a high-energy metastable molecule. Adiabatic decomposition of this compound to $CO_2$, $N_2$, and $H_2$ is accompanied by the release of ∼180 kcal/mol energy. The decomposition pathway is so efficient that the dioxirane cannot accumulate in the reaction mixture. The absence of oxidized trapping agents may be due to the low concentration of the dioxirane during the photooxidation as a result of its instability. Compound **11** could be formed by rearrangement of endoperoxide **13** via intermediate **12** (a similar intermediate was shown to be the rearranged product of the 2,5-endoperoxide in the low-temperature photosensitized oxidation of 4,5-diphenylimidazole[44]) or directly from **13** (Scheme 6). Support for the rearrangement from **13** to **11** comes from the fact that this guanosine derivative reacts with the singlet oxygen mimics, *N*-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and *N*-methyl-1,2,4-triazoline-3,5-dione (MTAD) to form hydroperoxide analogues whose structure was established by X-ray crystallography

(43) Song, J.; Khait, Y. G.; Hoffmann, M. R. *J. Phys. Chem. A* **1999**, *103*, 521−526.
(44) Kang, P.; Foote, C. S. Submitted to *J. Am. Chem. Soc.*

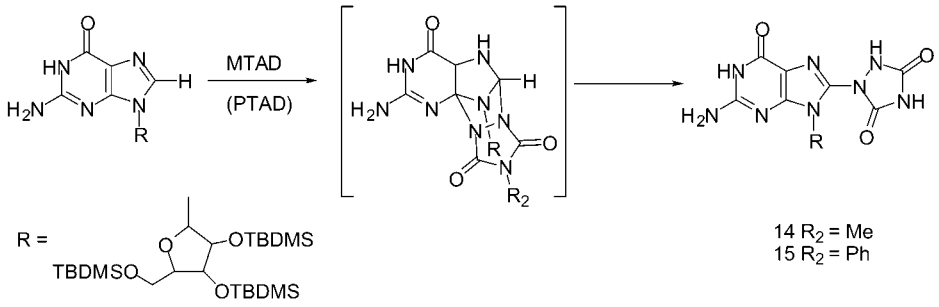
***Scheme 5***




***Scheme 6***




***Scheme 7***

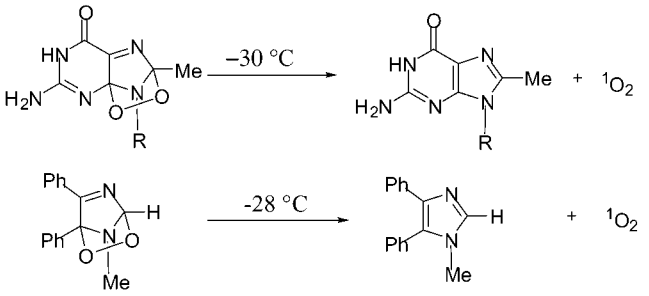



(Scheme 7).[29] Hydroperoxide **11** undergoes ring closure to give dioxirane **10**.

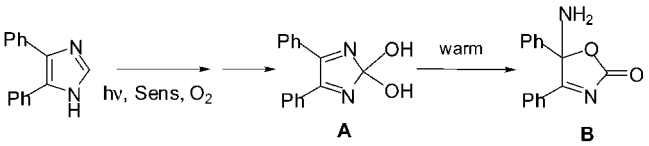
The presence of a labile 8-H or NH is important for the rearrangement of the endoperoxide to form the decomposed products. Substitution of the 8-H with methyl in the photosensitized oxidation of 8-methylguanosine TBDMS derivative causes the endoperoxide formed at low temperature to be relatively stable and undergo a retro-Diels−Alder reaction to regenerate the starting 8-methylguanosine and singlet oxygen upon warming.[28] The 2,5-endoperoxide of *N*-methyl-4,5-diphenylimidazole decomposes to the starting material while the 2,5-endoperoxide of 4,5-diphenylimidazole decomposes to a complex mixture of products (Scheme 8).[45] When the labile hydrogens are not present, the endoperoxides are more stable. The 2,5-endoperoxide of 4,5-diphenylimidazole begins to decompose at −88 °C whereas that from *N*-methyl-4,5-diphenylimidazole is stable until −28 °C. The endoperoxide of the 8-methylguanosine was observed at −80 °C and did not

(45) Kang, P.; Foote, C. S. *Tetrahedron Lett.* **2000**, *41*, 9623−9626.

**Scheme 8**




**Scheme 9**




decompose until −30 °C. In the guanosine derivative, where both a labile 8-H and a labile NH on the six-membered ring are present, we could not observe the endoperoxide even at −100 °C.

Recently Tannenbaum et al. found the major product of photosensitized oxidation of guanosine in aqueous media to be spiroiminodihydantoin instead of 4,8-dihydro-4-hydroxy-8-oxoguanosine previously assigned by Cadet.[23] The structure of spiroiminodihydantoin was unequivocally confirmed by a *Selec*tive Inade*quate* experiment carried out by Adam et al.[27] The spiroiminodihydantoin is the ubiquitous oxidation product of dG by metal-based one-electron oxidants, triplet ketones, singlet oxygen, and oxyl radicals. However, the mechanism of the formation of spiroiminodihydantoin from a singlet oxygen reaction is not certain. A major product from photosensitized oxidation of guanosine in nonpolar organic solvent under very mild conditions is **5**, whereas the main products from photosensitized oxidation of 2′-deoxyguanosine in aqueous medium are the diastereomers of spiroiminodihydantoin. The reason for this difference is not clear. However, in both cases, the yields are less than 50%.

In a detailed mechanistic study of low-temperature photosensitized oxidation of an isotope-labeled imidazole,[44] we found many similarities with the oxidation of guanosine. In that reaction, we characterized a transient intermediate as a diol (**A**) that decomposes to $CO_2$ via a carbamate derivative (**B**) (Scheme 9). It is possible that a diol is the intermediate in the guanosine reaction instead of the dioxirane.

The peaks at 155.5 ppm may well be that of the spiroiminodihydantoin[23] or of the 4,8-dihydro-4-hydroxy-8-oxoguanosine (155.0 ppm) observed by Sheu et al. in the low-temperature photooxidation of 7,8-dihydro-8-oxoguanosine derivative. The FAB-MS spectrum of the reaction mixture did not show this peak, and the only observed peak was that of **5**. However, our conditions could have caused loss of this compound. Other possible products in the complex peak could be Gh and Ia (156.3, 157.2 ppm) reported by Burrows et al. from the one-electron oxidation of 7,8-dihydro-8-oxoguanosine.[26] Further experiments are needed to identify these products.

## Conclusion

In the reaction of singlet oxygen with an 8-$^{13}$C-labeled guanosine derivative at low temperature (<−100 °C), we observed two transient intermediates that decompose directly to the final major product **5** and $CO_2$. Carbamic acid structures are proposed for the two intermediates based on the $^{13}$C NMR and 2D NMR ($^1$H−$^{13}$C HMQC, HMBC) spectra and the formation of final product **5** and 8-$CO_2$. Although neither endoperoxide nor dioxetane intermediates were directly detected by low-temperature NMR spectroscopy even at −100 °C, we propose a reaction mechanism that involves initial [4 + 2] cycloaddition of singlet oxygen with the imidazole ring to form an unstable endoperoxide, subsequent rearrangement of the endoperoxide to a dioxirane via a ring-opened peroxide, and decomposition of the dioxirane to the two observed intermediates. The $^{18}$O experiment strongly suggests that a dioxirane is a likely structure for the precursor of the two observed intermediates. This novel dioxirane distinguishes itself from other dioxiranes by decomposition without appreciable oxidative ability at low temperature. The distribution of products estimated by $^{13}$C NMR accounts for all the $^{13}$C-containing products. The elucidation of the structure of the compound (probably a diastereomeric mixture) with the $^{13}$C chemical shift of 155.5 ppm requires further investigation, but seems consistent with the spiroiminodihydantoin.

## Experimental Section

$^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker ARX-400, ARX-500, and Avance 500 spectrometers. Low-temperature NMR spectra were taken in a precooled probe maintained at the desired temperature. The solvent for low-temperature NMR was $CBr_2F_2$, with several drops of acetone-$d_6$ added as reference (the chemical shift of the carbonyl was set to 207.1 ppm) and locking solvent. GC/MS spectra were recorded on a HP G1800 A GCD with HP-5 GC column (cross-linked 5% phenyl methyl siloxane, 30 m × 0.25 mm × 0.25 $\mu$m). Positive ion FAB mass spectra were collected on a VG ZAB-SE reverse geometry, extended mass range, magnetic sector mass spectrometer. Thin-layer chromatography (TLC) was done using either DC-Fertigplatten Kieselgel 60 F254 or DC-Plastikfolien Kieselgel 60 F254 from E. Merck. Column chromatography was performed on silica gel 60, 70−230 mesh or 230−400 mesh from E. Merck.

**Materials.** $CaH_2$, $AlCl_3$, palmitoyl chloride, *N*,*N*-dimethylformamide (DMF), chlorobenzene, and dibromodifluoromethane were from Aldrich. 2,4,5-Triamino-6-hydroxypyridine sulfate, 1,2,3,5-tetra-*O*-acetyl-$\beta$-D-ribofuranose, morpholine, and *tert*-butyldimethylsilyl chloride (TBDMSCl) were from Sigma. $^{13}$C-Sodium formate ($^{13}$C >99%) was from Isotec Inc. $Na_2SO_4$, KOH, MeOH, EtOAc, $CH_2Cl_2$, and $CH_3CN$ were from Fisher. Deuterated solvents were from Cambridge Isotope Laboratory. Anhydrous DMF was prepared by stirring DMF with $CaH_2$ overnight and distilled under reduced pressure. Chlorobenzene was dried with Linde 4A molecular sieves. Dry pyridine was prepared by standing with solid KOH (20 g/kg) followed by distillation. $CBr_2F_2$ was purified by distillation.

**Preparation of Morpholinium $^{13}$C-Formate.** To sodium $^{13}$C-formate (0.500 g, 7.3 mmol) in 5 mL of distilled water was added 0.75 mL of 12 M HCl. Freshly distilled morpholine (0.696 g, 8.0 mmol) was added dropwise to the acid solution at 0 °C. The solution was stirred for 1.5 h and evaporated to dryness under reduced pressure. Absolute ethanol was added to the solid residue and filtered to separate NaCl. The filtrate was washed with ethanol. The combined solution was evaporated and used directly for the next step: $^1$H NMR ($CDCl_3$ $\delta$ 7.26 ppm) $\delta$ 8.41 (1H, $J_{C-H}$ = 200.6 Hz, H-$^{13}$C═O), 8.06 (2H, s, $NH_2$), 3.97 (4H, t, $J$ = 5.0 Hz, $CH_2O$), 3.21 (4H, t, $J$ = 5.0 Hz, $CH_2N$); $^{13}$C NMR ($CDCl_3$, $\delta$ 77.0 ppm) $\delta$ 168.40 (1C, $^{13}$C═O, $J_{C-H}$ = 200.7 Hz), 63.74 (2C, $CH_2O$), 43.32 (2C, $CH_2N$).

**Preparation of 8-$^{13}$C-Guanine.** 2,4,5-Triamino-6-hydroxypyrimidine sulfate (0.50 g, 2.09 mmol) was mixed with morpholinium $^{13}$C-

formate and heated at 100 °C in an oil bath for 1.5 h. The temperature was then raised to 200 °C and heating continued for a further 2.5 h. Excess formyl reagent was recovered by refluxing the reaction mixture with absolute ethanol. 8-$^{13}$C-Guanine was filtered from the ethanol solution (0.39 g, 75% yield): $^1$H NMR ($D_2O$ and NaOD) $\delta$ 7.62, 7.22 (1H, C8−H, $J_{C-H}$ = 195.9 Hz); $^{13}$C NMR (DMSO-$d_6$, $\delta$ 39.40 ppm) $\delta$ 149.52.

**Preparation of *N*$^2$-Palmitoyl-8-$^{13}$C-Guanine.** To a suspension of 8-$^{13}$C guanine (0.30 g, 1.97 mmol) in 15 mL of dry pyridine was added palmitoyl chloride (1.20 g, 4.36 mmol), and the reaction mixture refluxed overnight. Pyridine was evaporated under reduced pressure. The residue was put in absolute ethanol (100 mL) and refluxed for 2 h. The solution was filtered while hot. The filtrate was washed with hot ethanol and dried to give *N*$^2$-palmitoyl 8-$^{13}$C-guanine (0.44 g, 57%).

**Preparation of 2′,3′,5′-*O*-*tert*-Butyldimethylsilyl-*N*-*tert*-butyldimethylsilyl-8-$^{13}$C-guanosine.** *N*$^2$-Palmitoyl-8-$^{13}$C-guanine (0.585 g, 1.50 mmol), 1,2,3,5-tetra-*O*-acetyl-$\beta$-D-ribofuranose (0.382 g, 1.20 mmol), and $AlCl_3$ (200 mg, 1.50 mmol) was refluxed in dry chlorobenzene (40 mL) for 4.5 h. The reaction mixture was evaporated to dryness under reduced pressure. The residue was stirred in 80 mL of $H_2O$ overnight. The water solution was filtered and water was evaporated. To the residue were added DMF (4 mL) and TBDMSCl. The solution was filtered into a 10-mL round-bottom flask. Imidazole (0.90 g, 13.2 mmol) and additional TBDMSCl (combined weight of 0.99 g, 6.6 mmol) were added. The reaction mixture was stirred under Ar for 4 days. The resulting mixture was poured into EtOAc and water. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated. Column chromatography (2:1 $CH_2Cl_2$ and $CH_3CN$) gave 2′,3′,5′-*O*-*tert*-butyldimethylsilyl-*N*-*tert*-butyldimethylsilyl-8-$^{13}$C-guanosine (0.110 g, 10%): $^1$H NMR ($CDCl_3$) $\delta$ 12.37 (1H, s, N1−H), 8.07 and 7.68 (1H, d, C8−H, $J_{C-H}$ = 212.3 Hz), 6.00 (1H, dd, $J_1$ = 5.2 Hz, $J_2$ = 7.8 Hz, C1′−H), 5.21 (1H, s, C2−NH), 4.37 (1H, dd, $J$ = 4.2 Hz, 4.3 Hz, C2′−H), 4.14 (1H, d, $J$ = 4.2 Hz, C3′−H), 4.01 (s, C4′−H), 3.76 (2H, dd, $J$ = 8.0 Hz, 2.4 Hz, C5′−H), 0.98, 0.94, 0.93 (s, 36 H, 4*tert*-butyl), 0.12, 0.11, 0.09, 0.08, 0.06, 0.05 (24H, s, 6Me); $^{13}$C NMR ($CDCl_3$) $\delta$ 158.13 (s, C6), 154.66 (s, C2), 152.41 (s, C4), 134.68 (s, C8, $J_{C-H}$ = 212.0 Hz), 116.94 (s, C5), 86.22 (s, C1′), 84.67 (s, C4′), 76.81 (C2′), 73.46 (s, C3′), 63.70 (s, C5′), 26.41, 25.79, 25.52, 25.38 (3*C*($CH_3$)$_3$), 18.30, 17.78 (s, 3*C*($CH_3$)$_3$), −5.01, 5.80 (6$CH_3$); FAB/MS *m*/*z* 741.5 [M + H]$^+$. The $^{13}$C abundance estimated from MS is almost 96%.

**Photosensitized Oxidation of 2′,3′,5′-*O*-*tert*-Butyldimethylsilyl-*N*-*tert*-butyldimethylsilyl-8-$^{13}$C-guanosine.** A total of ∼20 mg (0.027 mmol) of 2′,3′,5′-*O*-*tert*-butyldimethylsilyl-*N*-*tert*-butyldimethylsilyl-8-$^{13}$C-guanosine in 0.5 mL of $CBr_2F_2$ in a 5-mm NMR tube was irradiated below −100 °C (in a slush of 1:1 THF, MeOH, and liquid $N_2$) with ∼5 × 10$^{-5}$ M 2,9,16,23-tetra-*tert*-butyl-19*H*,31*H*-phthalocyanine as sensitizer and a Cermax 300-W xenon lamp as the light source. A chromium glass filter was used to cut off wavelengths below 547 nm. Dry $O_2$ was bubbled into the solution for 1 min every 0.5 h. After 6 h of photolysis, the reaction mixture was transferred to the precooled NMR probe. Low-temperature NMR spectra were taken on the Bruker ARX 500. The temperature range is −100 °C to room temperature. See text for detailed information about $^{13}$C NMR spectra of the reaction mixture. The $^1$H NMR spectrum of the reaction mixture at 0 °C and room temperature is the same as those previously reported[29] except for the $^{13}$C splitting of the 8-H for unreacted guanosine. FAB/MS: *m*/*z* 729.4, 12 mass units less than that of the starting material. Quantitative analysis of the $^{13}$C NMR spectrum was carried out by integrating $^1$H-decoupled $^{13}$C NMR peaks with a pulse delay of 2 s. A control experiment showed that the inverse gating acquisition method gave almost the same integration as the $^1$H-decoupling method. These experiments also showed that the intensity of $CO_2$ did not change from −90 to −20 °C, which suggests that $CO_2$ was still dissolved in the solvent. Since we were following the same carbons in the reaction mixture, integrating $^1$H-decoupled $^{13}$C NMR peaks can provide reliable information about concentration changes in the reaction mixture. Quantitative studies using $^1$H-decoupled $^{13}$C NMR spectra have also been reported in other cases.[46,47]

**$CO_2$ Distribution and an $^{18}O_2$ Tracer Study.** Photosensitized oxidation was carried out in the same manner as above. After the solution in a 5-mm NMR tube was purged with argon, $O_2$ gas was introduced into the solution by a gastight syringe every 0.5 h. The NMR tube was capped with a septum rubber cap. After completion of photolysis, the NMR tube was slowly warmed to the desired temperature. A gas sample above the solution was taken with a 25-$\mu$L gastight syringe and analyzed by GC/MS. The data shown in Table 1 are the mean values of four to five determinations that are within ±5% from the mean value. The measurements of the $^{45}CO_2/^{44}CO_2$ ratio were carried out at room temperature using the 8-$^{13}$C-guanosine derivative and normal oxygen. The $CO_2$ isotopic distribution was measured at room temperature using unlabeled guanosine derivative and $^{18}O_2$-enriched $O_2$ gas ($^{32}O_2$:$^{34}O_2$:$^{36}O_2$ = 100:0.98:5.40). The $CO_2$ distribution was calculated as follows.

(1) 2-O: Assuming that the two O atoms of the resulting $CO_2$ come from one $O_2$ molecule and using natural carbon distribution $^{12}$C:$^{13}$C = 100:1.10, the $CO_2$ distribution is $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{48}CO_2$ = 100:1.10:0.98:5.40. If $^{48}CO_2$ is derived solely from the 8-C, then the ratio of $CO_2$ derived from 8-C to $CO_2$ derived from other carbons is equal to the ratio of $^{45}CO_2$ to $^{44}CO_2$ which is 1:1.37 obtained from the photooxidation of 8-$^{13}$C-guanosine at −100 °C and warmed to room temperature. Assuming the distribution of $CO_2$ derived from carbons other than 8-C is the same as the natural $CO_2$ distribution $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{48}CO_2$ = 100:1.18:0.4:0, then the corrected $CO_2$ distribution is $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{48}CO_2$ = 100:1.15:0.64:2.28.

(2) 1-O: Assuming that the two O atoms of the resulting $CO_2$ come from two $O_2$ molecules and using natural carbon distribution $^{12}$C:$^{13}$C = 100:1.10, the $CO_2$ distribution is $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{47}CO_2$:$^{48}CO_2$ = 100:3.06:10.83:0.22:0.29. After the correction for $CO_2$ derived from carbons other than 8-C, the $CO_2$ distribution is $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{47}CO_2$:$^{48}CO_2$ = 100:2.09:4.80:0.09:0.12.

In the above calculations, we assume that $^{34}O_2$ consists of two $^{17}$O. $^{34}O_2$ could also consist of one $^{16}$O and one $^{18}$O. In that case, the 2-O result is the same $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{48}CO_2$ = 100:1.15:0.64:2.28; 1-O is $^{44}CO_2$:$^{45}CO_2$ :$^{46}CO_2$ :$^{48}CO_2$ = 100:1.15:5.17:0.15, which is not much different from the above calculation.

**Acknowledgment.** We thank the NSF for support from Grant CHE-9730386. The Avance 500 NMR is supported by NSF CHE-9974928.

**Supporting Information Available:** $^1$H NMR, $^{13}$C NMR, $^1$H−$^{13}$C HMQC, and $^1$H−$^{13}$C HMBC spectra of 8-$^{13}$C-guanosine; $^{13}$C NMR, $^1$H−$^{13}$C HMQC, and $^1$H−$^{13}$C HMBC spectra of the reaction mixture at −70 °C (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA012038X

(46) Mareci, T. H.; Scott, K. N. *Anal. Chem.* **1977**, *49*, 2130.
(47) Allerhand, A.; Doddrell, D.; Komoroski, R. *J. Chem. Phys.* **1971**, *55*, 189.