Photosensitized Oxygenation of a 7,8-Dihydro-8-oxoguanosine Derivative. Formation of Dioxetane and Hydroperoxide Intermediates

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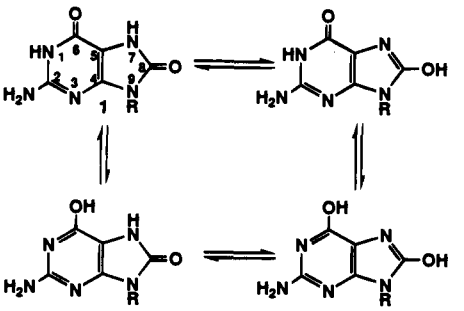
Abstract: Low-temperature NMR studies of the dye-sensitized photooxygenation of an 8-oxoguanosine derivative provide direct evidence for dioxetanes as primary intermediates and their unprecedented rearrangement to hydroperoxides. These dioxetanes and hydroperoxides are stable only at low temperatures. Upon warming, they rearrange to a variety of products.

Introduction

There has been much interest in mechanisms of oxidative damage to DNA and its biological consequences in living cells.[1−7] Oxidation of DNA decreases its transformation efficiency, inhibits DNA replication, and causes G to T tranversions.[4,8−11] These effects are generally believed to be related to human problems such as cancer and aging.[2−4,8,12,13] Photosensitized oxidation plays an important role in oxidative damage because environmental chemicals or natural cell constituents such as porphyrins or flavins can act as photosensitizers for this damage. Singlet oxygen is one of the major species that can cause these effects.[4,6,9,14−16]

Several products of the photooxidation of nucleic acid derivatives have been reported and intermediates such as endoperoxides and dioxetanes have been proposed to account for them.[17−19] However, the mechanisms and intermediates involved are still unclear. In a previous paper, we reported that a methyl group at the C-8 position of a guanine derivative stabilizes the primary endoperoxide from [4 + 2] cycloaddition of singlet oxygen and allowed its characterization by low-temperature NMR spectroscopy.[20] Another proposed intermediate, the dioxetane product of [2 + 2] cycloaddition, has not been observed experimentally so far.

Scheme 1



The 8-oxo derivative (**1**) is an important product of oxidative attack on guanosine.[21] This compound is usually thought to be a marker for hydroxyl radical attack, but it can also be formed in singlet oxygen reactions.[14,22−24] Although several tautomeric forms are possible (Scheme 1), structural studies and NMR spectroscopy clearly indicate that the 6,8-diketo form predominates.[25−27]

Since the predominant tautomer of **1** lacks a N7−C8 double bond, [2 + 4] cycloaddition of singlet oxygen to the 5-membered ring should be less likely. We now report direct observation of both diastereomeric dioxetanes and hydroperoxide intermediates in the low-temperature photosensitized oxidation of 2′,3′,5′-tri((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (**2**), an organic-soluble derivative of **1**.

Results

The photosensitized oxygenation of **2** at −80 °C was carried out with tetraphenylporphine (TPP) or Rose Bengal (RB) as

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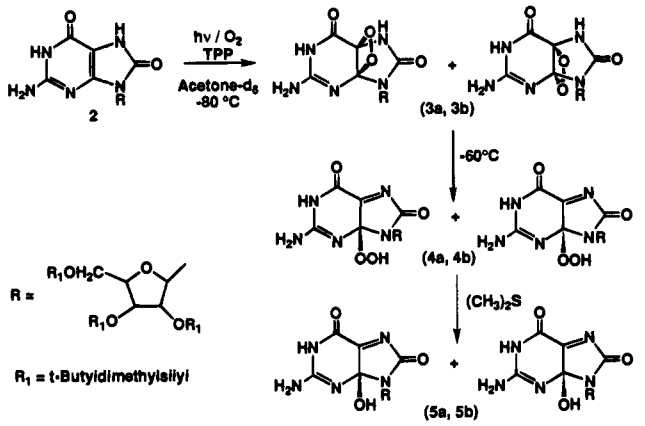
Scheme 2



sensitizer in methylene chloride-d_2 or acetone-d_6, using a 1% potassium dichromate filter solution (cut-off < 500 nm) with a Cermax 300 W Xenon lamp as the light source. The reaction was complete within 1 h and the mixture was kept in liquid N_2 for NMR analysis (−80 °C). The reaction mixture contains two sets of two pairs of resonances, assigned to diastereomeric dioxetanes (**3a, 3b**) and diastereomeric hydroperoxides (**4a, 4b**) with relative yields of 6%, 15%, 46%, and 33%, respectively (Scheme 2; see below for assignments).[28] When the ^{13}C NMR spectrum of the reaction mixture was taken at −60 °C, the resonances assigned to the dioxetanes disappeared and only those assigned to the hydroperoxides remained.

The ^{13}C NMR spectrum of the reaction mixture at −80 °C is consistent with the assigned structures. Five pairs of two singlets from the oxidized guanine residue are visible at −80 °C and disappear at −60 °C. These resonances were assigned to the diastereomeric dioxetanes (**3a, 3b**); two sets of quaternary sp^3 carbons appear at δ 81.4, 81.5 and 103.5, 103.5 ppm (the latter are not resolved but the double intensity and the fact that all other lines are doubled imply two peaks) and three quaternary sp^2 carbons between 166 and 172 ppm. These peaks disappear on warming to −60 °C and those remaining were assigned to hydroperoxides **4a, 4b**. In contrast to the dioxetanes, the hydroperoxides showed only one pair of sp^3 quaternary carbons at 82.4 and 82.6 ppm. These peaks disappeared upon addition of dimethyl sulfide and new peaks at 69.0 and 69.1 ppm appeared, assigned to the corresponding alcohols (**5a, 5b**). This behavior is similar to the unstable hydroperoxide products of photooxidation of ascorbic acid, where the hydroperoxide carbon also shifts upfield on reduction to the alcohol.[29,30] The ^{13}C NMR spectra for the guanine part of these intermediates are summarized in Table 1.

^1H NMR also provides some help in characterizing these intermediates. Although the proton spectra at −80 °C were partly obscured by line-broadening, multiple products, and overlapping absorptions of the sugar moieties, those taken at −60 °C were quite clean. Only the two diastereomeric hydroperoxides (**4a** and **4b**, about 1:1) and no other products were observed. The two characteristic hydroperoxide peaks at 9.37 and 9.33 ppm gradually disappeared upon adding dimethyl sulfide and a new broad OH peak appeared around 3.9 ppm. The sugar residues remained intact during the photolysis, and their resonances were similar to those of starting material **2** (Table 2).

(28) Relative yields were estimated by their ^{13}C NMR peak heights at the C(4) posltion.

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Table 1. ^{13}C NMR Chemical Shifts of Starting Material and Photooxygenation Products of **2**[a]

compd	^{13}C NMR chemical shifts[f]				
2[b]	100.5	149.2	153.1	153.2	153.4
3a[c]	81.4	103.5	166.5	170.1	171.7
3b[c]	81.5	103.5	166.6	170.3	171.9
4a[d]	82.4	155.2	166.7	170.4	172.0
4b[d]	82.6	155.6	166.8	170.6	172.8
5a[e]	69.0	155.0	166.5	174.5	175.6
5b[e]	69.1	155.4	166.6	175.5	175.8

[a]Chemical shifts (Bruker AM 360, 90 MHz for ^{13}C NMR) are in ppm downfield from internal Me_4Si; peaks in the 60−100 ppm region were assigned using DEPT. [b]Solvent: methylene chloride-d_2 at room temperature. [c]Solvent: acetone-d_6 at −80 °C. [d]Solvent: acetone-d_6 at −60 °C. [e]Solvent: acetone-d_6 at −40 °C. [f]Only the guanine resonances are shown.

Table 2. ^1H NMR Chemical Shifts of Starting Material and Photooxygenation Products of **2**[a]

compd	^1H NMR chemical shifts[e]				
	C(1′)−H	C(2′)−H	C(3′)−H	C(4′)−H	C(5′)−H
2[b]	5.76	5.16	4.44	4.00	3.90; 3.75
4a[c]	5.58	5.28	4.31	3.85	3.65
4b[c]	5.58	5.15	4.37	3.85	3.65
5a[d]	5.61	5.25	4.34	3.86	3.66
5b[d]	5.59	5.16	4.43	3.86	3.66

[a]Chemical shifts (Bruker AM 360, 360.136 MHz for ^1H NMR) are in ppm downfield from internal Me_4Si. [b]Solvent: methylene chloride-d_2 at room temperature. [c]Solvent: acetone-d_6 at −60 °C. [d]Solvent: acetone-d_6 at −20 °C. [e]Only the sugar resonances are shown.

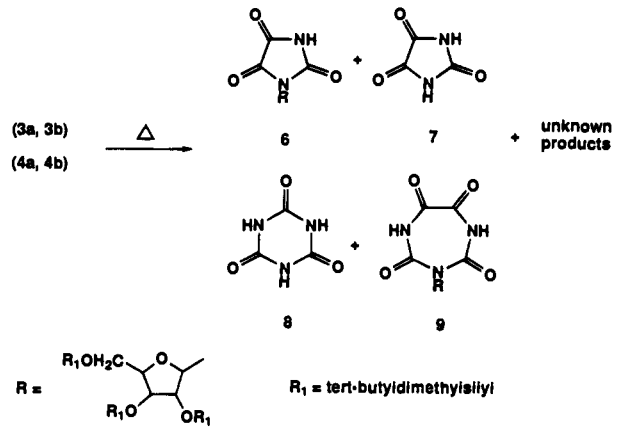
Low-temperature FAB mass spectra of the reaction products at −78 °C provide supporting evidence for the structures, although they do not distinguish between dioxetanes and hydroperoxides. Characteristic peaks at *m/e* 674.4 (16% of base, M + 1), 658.4 (15%, M + 1 − 16), and 642.3 (14%, M + 1 − 32) are strongly indicative of the incorporation of molecular oxygen and the loss of one and two oxygens in the mass spectrum. Hydroxyl products **5a, 5b** have an ion at *m/e* 658.4 (100%, M + 1), 16 au less than hydroperoxides **4a, 4b**.

The hydroperoxide intermediates (**4a, 4b**) decompose to a complicated product mixture above −20 °C. Several unstable intermediates in the decomposition are observed in the proton NMR. Since the lifetimes of these intermediates are too short to be detected by ^{13}C NMR, their structures cannot yet be established. Compounds **6** and **9** were the two major stable decomposition products; their structures were determined by spectroscopic data (^1H-NMR, ^{13}C-NMR, and FAB MS, see Experimental Section). Compounds **7** and **8** were minor products, identified by comparison with authentic samples. Yields of recovered products were less than 30%. Thus, the bulk of the material must decompose during chromatographic purification, presumably to uncharacterized small fragments such as NH_3, CO_2, urea, guanidine, etc. The reduced hydroxyl products (**5a, 5b**) are more stable and survive at −40 °C for 12 h without significant decomposition. At room temperature, they decompose to several products within 2 h. The isolable decomposition products from these hydroxyl reduction products (**5a, 5b**) are only parabanic acid (**7**) and its ribose derivative (**6**) as shown in Scheme 4.

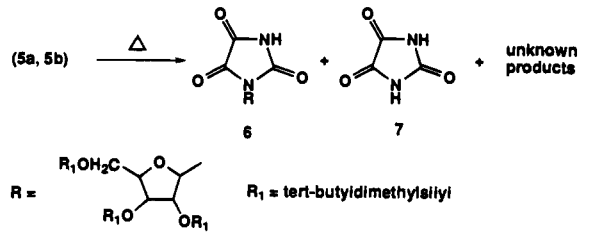
Discussion

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 have been suggested.[17−19] The formation of an endoperoxide was observed in the photo-

Scheme 3

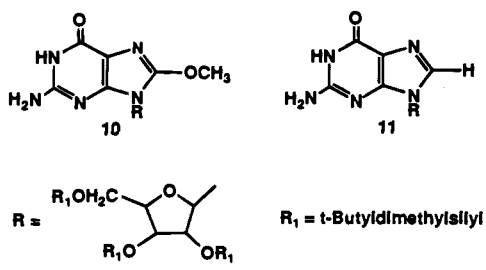


Scheme 4



sensitized oxidation of an 8-methylguanosine derivative,[20] which suggests that [4 + 2] cycloaddition is more favorable than [2 + 2] in the native system.

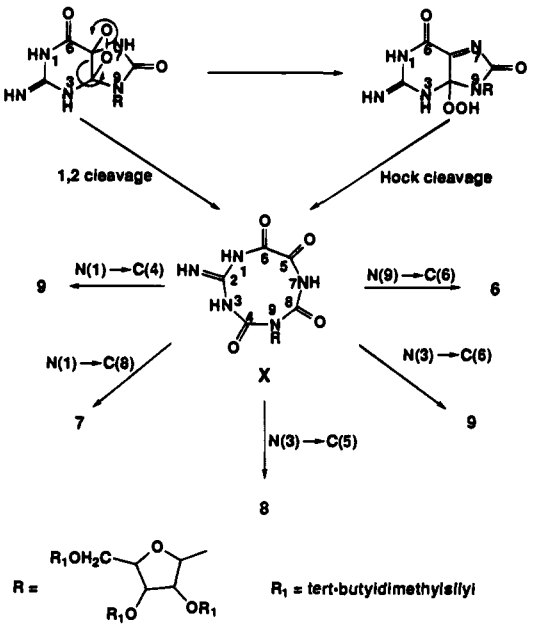
The 8-hydroxyguanosine derivative is known to exist predominantly in the more stable keto form.[25-27] The lack of a N7−C8 double bond in the keto form permits only formation of the [2 + 2] cycloadduct. However, the enol form makes up a small fraction of the mixture at equilibrium. This tautomer would be expected to give efficient [4 + 2] cycloaddition, since the k_r (chemical reaction) value toward singlet oxygen for the closely analogous 8-methoxyguanosine derivative (**10**) is 11.56 × 10^5, about 9 times that of the unsubstituted guanosine derivative (**11**).[31] However, the k_r value is about 17 times less for **10** than for the 7,8-dihydro-8-oxoguanosine derivative **2**, the keto form.[31] If we assume that 10% of the enol form is present in solution, the relative amount of [4 + 2] product formed should be no more than 0.5%, and the remainder should react by [2 + 2] cycloaddition. The products isolated on warming the reaction mixtures to room temperature were those of substantial degradation, and no starting material was recovered. This result contrasts with that of the endoperoxide from the 8-methylguanosine derivative **11**, where loss of oxygen to regenerate starting material was observed.[20].



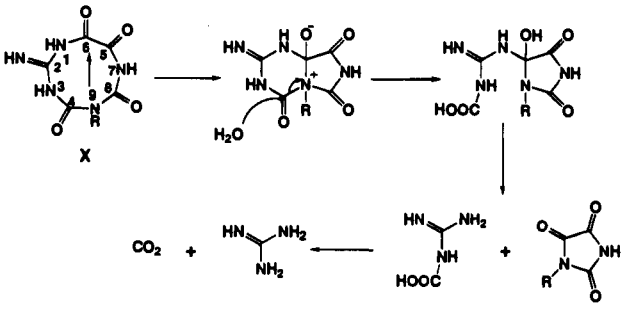
The mechanism of the formation of the unstable dioxetane and hydroperoxide is still unclear. In principle, in compounds with an activated double bond and an adjacent hydrogen, both

(31) Sheu, C.; Foote, C. S., manuscript in preparation.

Scheme 5



Scheme 6



[2 + 2] cycloaddition to give dioxetanes **4** and ene reaction to allylic hydroperoxides **5** could occur independently. Since the proton at N7 is labile, proton transfer from N7 to the dioxetane oxygen would be expected to give the hydroperoxide. The ratio of (**3a** + **4a**):(**3b** + **4b**) at −80 °C is about 1:1, the same as the **4a**:**4b** ratio at −60 °C. This result and the absence of other products at −60 °C suggest that the dioxetanes react exclusively to give the hydroperoxides on warming.

The formation of products **6**−**9** from **3** and **4** may occur via 1,2 bond cleavage of the dioxetane (**3**) or Hock cleavage[32] of the hydroperoxide (**4**) as shown in Scheme 5. The 9-membered ring of cleavage product **X** has many functional groups, so that subsequent cyclization or hydrolysis would be expected to give a complex product mixture. In Scheme 5, the symbol N(a) → C(b) means cyclization of compound **X** between the N atom at position a and the C atom at position b. An example of cyclization of compound **X** between the N atom at position 9 and the C atom at position 6 to form product **6** is shown in Scheme 6. Formation of **6** and **7** from hydroxyl reduction products (**5a**, **5b**) is shown in Scheme 7.

The mechanism proposed in Scheme 6 is consistent with the results of Sussenbach and Berends,[33,34] who showed that carbon dioxide was formed mainly from the C-6 position and guanidine from the C-2 position, whereas the parabanic acid was derived from the C-8 position of the original guanine.

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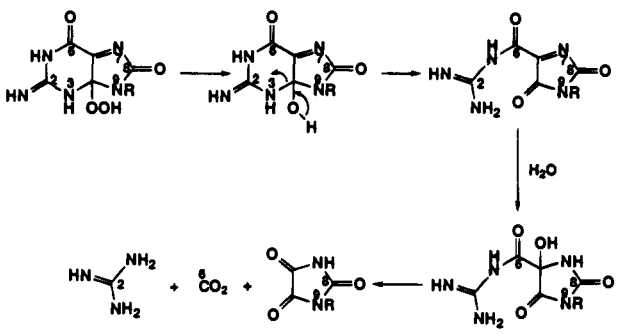
(33) Sussenbach, J. S.; Berends, W. *Biochem. Biophys. Res. Commun.* **1964**, *16*, 263−266.
(34) Sussenbach, J. S.; Berends, W. *Biochim. Biophys. Acta* **1965**, *95*, 184−185.

Scheme 7



Oxo radicals have been shown to react with the sugar residues in 2′-deoxyguanosine to induce DNA strand breakage.[35,36] The intact sugar residues and the fact that the addition of 2,6-di-*tert*-butylphenol, a radical scavenger, does not inhibit the photooxidation strongly suggests that a radical chain mechainism is not involved in the oxidation. The reaction is also faster in deuterated solvent and is inhibited by adding singlet oxygen quenchers, which indicates that singlet oxygen is the reactive intermediate.

Conclusion

Reaction of singlet oxygen with 2′,3′,5′-tri((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (**2**) gives both diastereomeric dioxetanes and hydroperoxides as reactive intermediates that can be directly observed by low-temperature NMR spectroscopy. Upon warming to room temperature, the dioxetanes open to produce more of the hydroperoxides, then at higher temperature, the latter rearrange to several products. To our knowledge, this is the first direct observation of dioxetane and hydroperoxide intermediates in the guanine system.

Experimental Section

General. Low-temperature ^{1}H-NMR and ^{13}C-NMR and DEPT spectra were recorded on Bruker AM-360 and AM-500 spectrometers. ^{13}C-NMR spectra were taken with the solvent peak as reference. Chemical shift values are in ppm downfield from internal tetramethylsilane. Low-temperature FAB mass spectra were obtained by transferring the sample into a precooled probe on a VG ZAB-SE instrument. Thin-layer chromatograms were obtained using either DC-Fertigplatten Kieselgel 60 F_{254} or DC-Plastikfolien Kieselgel 60 F_{254} from E. Merck. Column chromatography was performed on silica gel 60, 70−230 mesh or 230−400 mesh (flash column) from E. Merck.

Materials. Commercial solvents were Fisher AR, used without further purification. Deuterated solvents were from Cambridge Isotope Laboratory dried over 4 Å molecular sieves. 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine (TPP) and rose bengal were used as received from Aldrich. 2′,3′,5′-Tri((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine was synthesized by modified literature procedures that will be reported elsewhere.[31]

General Photolysis Procedure. Photolyses were carried out in 5 mm NMR tubes with 0.1−0.05 M substrates in 0.5 mL deuterated solvents. A Cermax 300 W Xenon lamp powered at 14 V, 20 A was the light source. A 1% potassium dichromate filter solution was used to cutoff wavelengths below 500 nm. The tubes were immersed in a dry ice/acetone bath at −78 °C, and an 18 cm running water filter was put in front of the sample to eliminate heating. TPP was used as the sensitizer in methylene chloride-d_2 or acetone-d_6, while rose bengal was used in acetone-d_6. Oxygen was passed through a drying tube containing anhydrous $CaCl_2$ and molecular sieves and was continuously bubbled through the solution via a Teflon tube. The reaction progress was monitored by TLC or NMR spectroscopy.

Low-Temperature Photooxygenation of 2′,3′,5′-Tri((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (2). **2** (12.88 mg, 0.02 mmol) was dissolved in ca. 0.4 mL of acetone-d_6 in a 5-mm NMR tube containing ca. 5 × 10^{-5} M 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (TPP) as sensitizer. Similar results were obtained in methylene chloride-d_2. Photooxygenation at −78 °C was complete within 1 h. The NMR tube was transferred quickly to the precooled and preshimmed probe of the Bruker AM 360 NMR instrument. For good resolution of ^{1}H NMR and ^{13}C NMR spectra, acetone-d_6 was used as solvent because of the viscosity and low solubility of products in methylene chloride-d_2 at low temperature.

The ^{1}H NMR spectra taken after irradiation at −80 °C showed two major and two minor products . ^{13}C NMR taken at −80 °C showed four sets of purine carbons. ^{13}C NMR (δ, ppm, −80 °C, acetone-d_6): 81.4, 103.5, 166.5, 170.1, 171.7; 81.5, 103.5, 166.6, 170.3, 171.9); (82.4, 155.2, 166.7, 170.4, 172.0; 82.6, 155.6, 166.8, 170.6, 172.8. Identification of carbon resonances of purine bases in the sugar region (100−60 ppm) was aided by DEPT experiments.

Spectra after warming to −60 °C showed only two sets of products in a 1:1 ratio. ^{1}H NMR (δ, ppm, −60 °C, acetone-d_6) for product A: 5.58 (1H, d, J = 6.4 Hz, 1′-H), 5.28 (1H, dd, J = 2.2, 6.4 Hz, 2′-H), 4.37 (1H, d, J = 2.2 Hz, 3′-H), 3.85 (1H, m, 4′-H), 3.80 (1H, m, 5′-H), 3.63 (1H, m, 5′-H), 8.56 (1H, s, br, NH), 9.37 (1H, s, OOH), 9.74 (1H, s, NH), 12.64 (1H, s, N(1)-H). ^{1}H NMR (δ, ppm, −60 °C, acetone-d_6) for product B: 5.58 (1H, d, J = 6.4 Hz, 1′-H), 5.16 (1H, dd, J = 4.0, 6.4 Hz, 2′-H), 4.33 (1H, d, J = 4.0 Hz, 3′-H), 3.85 (1H, m, 4′-H), 3.80 (1H, m, 5′-H), 3.63 (1H, m, 5′-H), 8.44 (1H, s, br, NH), 9.33 (1H, s, OOH), 9.53 (1H, s, NH), 12.64 (1H, s, N(1)-H). ^{13}C NMR (δ, ppm, −60 °C, acetone-d_6): 173.0 (s), 171.5 (s), 170.55 (s), 170.45 (s), 166.79 (s), 166.73 (s), 155.61 (s), 155.20 (s), 82.58 (s), 82.44 (s), 86.12 (d), 85.81 (d), 85.52 (d), 85.37 (d), 73.07 (d), 72.8 (d), 70.93 (d), 70.10 (d), 62.80 (t), 62.77 (t). Silyl protecting groups on the sugar moiety also showed two sets of carbon peaks, confirming the formation of two products: 25.96 (q), 25.92 (q), 25.92 (q), 25.90 (q), 25.81 (q), 25.71 (q); 18.52 (s), 18.48 (s), 18.33 (s), 18.33 (s), 18.11 (s), 18.02 (s); −4.24 (q), −4.58 (q), −4.60 (q), −4.66 (q), −4.68 (q), −4.68 (q), −5.15 (q), −5.53 (q), −5.56 (q), −5.59 (q), −5.63 (q), −5.63 (q).

Upon warming the reaction mixture from −60 °C to room temperature, several intermediates were detected by ^{1}H-NMR spectroscopy. Since these intermediates are very unstable, attempts to isolate them failed. At room temperature, these intermediates convert to two major products, isolated by column chromatography (1:1 CH_2Cl_2/CH_3CN) to give the major product, **6**. ^{1}H NMR (δ, ppm, 0 °C, acetone-d_6): 5.56 (1H, d, J = 2.9 Hz, 1′-H), 4.86 (1H, dd, J = 2.9, 3.1 Hz, 2′-H), 4.56 (1H, dd, J = 3.1, 3.9 Hz, 3′-H), 3.94 (1H, ddd, J = 3.3, 3.4, 3.8 Hz, 4′-H), 3.86 (1H, dd, J = 3.8, 8.2 Hz, 5′-H), 3.72 (1H, dd, J = 3.4, 8.2 Hz, 5′-H), 0.95, 0.90, 0.89 (s, 27 H, 3 × *tert*-butyl), 0.17, 0.15, 0.09, 0.07, 0.06, 0.05 (s, 18 H, 6 × CH_3). ^{13}C NMR (δ, ppm, −20 °C, acetone-d_6): 158.07, (s, 2 × C=O), 153.95 (s, C=O), 85.95 (d, C(1′)), 73.16 (d, C(2′)), 72.5 (d, C(3′)), 85.06 (d, C(4′)), 63.22 (t, C(5′), 26.29, 26.24, 26.14 (q, 3 × C(CH_3)$_3$), 18.89, 18.61, 18.49 (s, 3 × C(CH_3)$_3$), −4.14, −4.36, −4.46, −4.62, −5.24, −5.25 (q, 6 × Me). MS (FAB): 590 (M + 1), 574, 531, 492, 473, 459, 445, 433, 417.

The second major product (**9**) was obtained by using pure acetonitrile as eluent. ^{1}H NMR (δ, ppm, 0 °C, acetone-d_6): 9.4 (1H, s, br, NH), 7.8 (1H, s, br, NH), 5.59 (1H, d, J = 3.7 Hz, 1′-H), 5.01 (1H, dd, J = 3.7, 3.5 Hz, 2′-H), 4.44 (1H, dd, J = 2.9, 3.5 Hz, 3′-H), 3.90 (1H, m, 4′-H), 3.86 (1H, m, 5′-H′), 3.67 (1H, dd, J = 4.1, 7.7 Hz, 5′-H″), 0.94, 0.90, 0.89 (s, 27 H, 3 × *tert*-butyl), 0.16, 0.14, 0.09, 0.07, 0.06, 0.05 (s, 18 H, 6 × CH_3). MS (FAB): 633 (M + 1).

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Supplementary Material Available: Low-temperature ^{1}H NMR and ^{13}C NMR spectra for compounds **2**−**5** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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