Reactivity toward Singlet Oxygen of a 7,8-Dihydro-8-oxoguanosine ("8-Hydroxyguanosine") Formed by Photooxidation of a Guanosine Derivative

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Abstract: Total quenching ($k_r + k_q$) and chemical reaction rates (k_r) for the removal of singlet oxygen by 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)guanosine (**1**) and its oxidation product, 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (**2**), were determined by the time-resolved infrared luminescence technique and competition experiments, respectively. Compound **2** is two orders of magnitude more reactive with singlet oxygen than **1**. A mechanism for the formation of **2** from **1** with singlet oxygen is proposed.

7,8-Dihydro-8-oxoguanine (often called 8-hydroxyguanine) is an important product of oxidative attack on nucleic acids. This compound has been detected in various systems in which hydroxyl radicals are formed, such as ionizing radiation, metal ions−O_2, polyphenol−H_2O_2−Fe^{2+}, asbestos−H_2O_2, the photo-Fenton reagent, etc.[1−6] This compound is generally agreed to be formed by attack of a hydroxyl radical at the C-8 position of the guanine to form 8-hydroxyguanine, which is known to tautomerize to the more stable 8-keto form (7,8-dihydro-8-oxoguanine) in aqueous solution.[7−11] Recently, the formation of 7,8-dihydro-8-oxoguanosine or deoxyguanosine by hydration of the guanine radical cation within DNA[12] or by singlet oxygen attack on the corresponding guanosine has also been reported.[13−15]

Since many of these oxidizing agents are suspected mutagens or carcinogens, questions about the role of 8-oxygenated guanosine derivatives in genetic damage have arisen. Several studies of the mutagenic consequences of oxidative DNA damage involving the formation of the 8-oxo derivative both *in vitro* and *in vivo* have been reported.[15−19]

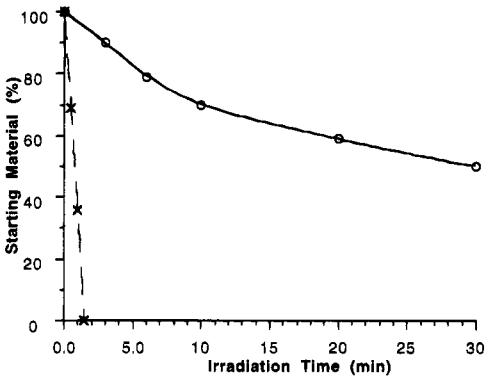

Figure 1. Disappearance of guanosine derivative **1** (○) and 8-oxo derivative **2** (×) in sensitized photooxygenation. Initial concentrations: **1**, 5.0 × 10^{-3} M; **2**, 5.1 × 10^{-3} M, 6.1 × 10^{-5} M TPP as sensitizer in acetone-d_6.

In studies of photosensitized oxygenation of 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)guanosine (**1**), we found that a small amount of the corresponding product, 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (**2**), was formed.[20] Similar observations were reported by Ravanat et al.,[21] who detected less than 1% of 7,8-dihydro-8-oxo-2′-deoxyguanosine (**3**) in phthalocyanine and naphthalocyanine photosensitized oxidation of 2′-deoxyguanosine. Recently, Kasai et al.[12] also reported that less than 5% of **3** was formed through a type I (non-singlet oxygen) mechanism in the riboflavine-photosensitized reaction of DNA.

Since the photoreactivity of this 8-oxygenated guanosine toward singlet oxygen has not yet been established, we measured ($k_r + k_q$), the rate constant for total removal of $^1O_2$, by 1270 nm luminescence decay, and k_r (the rate constant for chemical reaction) by competition experiments. Figure 1 shows the disappearance of the two substrates under comparable conditions. It is clear from Figure 1 that **2** is far more reactive than **1**.

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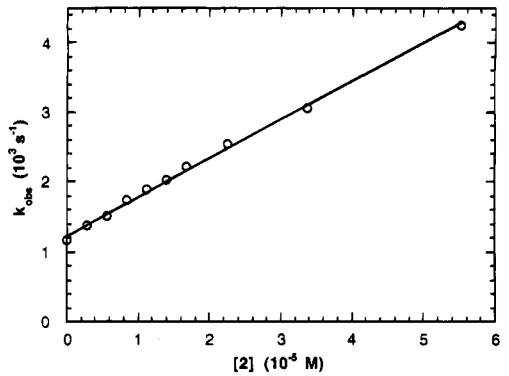


Figure 2. Luminescence quenching of singlet oxygen by 8-oxo derivative **2** in acetone-d_6; k_{obs} is the observed rate constant for the decay of singlet oxygen in the presence of a quencher. The plot of k_{obs} vs [**2**] has a slope of $(k_r + k_q)$ for the reaction of **2** with singlet oxygen and an intercept of k_d.

Table 1. Rates of Total Quenching $(k_r + k_q)$ and Chemical Reaction (k_r) of Guanosine Derivative **1** and 8-Oxo Derivative **2** with $^1O_2$ in Acetone-d_6

substrate	$(k_r + k_q)$, M^{-1} s^{-1}	k_r, M^{-1} s^{-1}	$k_r/(k_r + k_q) \times 100$
1	6.33×10^6	1.36×10^5	2.1
2	5.54×10^7	1.92×10^7	35
TME	3.00×10^7		
2M2P	7.59×10^5		

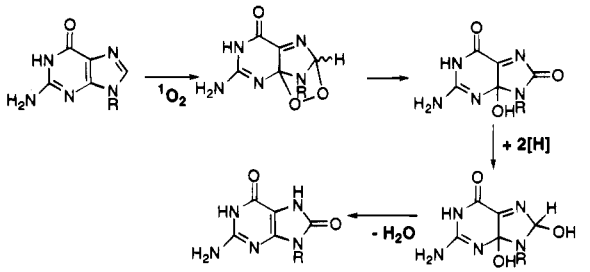
Direct measurements of $(k_r + k_q)$ for the two compounds were made using the decay of singlet oxygen luminescence at 1270 nm as previously described.[22] Typical results for **2** are shown in Figure 2; the rate constant determined is 5.54×10^7 M^{-1} s^{-1}, an order of magnitude larger than that for compound **1** (data not shown).

The value of chemical quenching (k_r) for compound **2** was determined separately by a competition experiment with tetramethylethylene (TME). TME is known to quench $^1O_2$ only chemically, $(k_r + k_q) \sim k_r = 3.00 \times 10^7$ M^{-1} s^{-1}.[23] The disappearance of **2** and TME was monitored by ^1H NMR spectroscopy and the results were fit to the equation of Higgins et al.[24] The value of $k_r^{8\text{-OH-G}}/k_r^{TME}$ determined by this method is 0.64, which gives a k_r value of 1.92×10^7 M^{-1} s^{-1} for **2**. A similar experiment for guanosine **1** was done by competition with 2M2P[22] and the $k_r^{8\text{-H-G}}/k_r^{2M2P}$ value is 0.18, which gives a k_r value of 1.36×10^5 M^{-1} s^{-1} for **1**, two orders of magnitude lower than the value for **2**. The kinetic parameters determined by these techniques are summarized in Table 1.

Although $k_r + k_q$ for compound **2** is only 9 times that of **1**, the relative rate of chemical reaction is about 130 times larger. The 8-oxo compound is thus extremely reactive toward singlet oxygen. Also the ratio of $k_r/(k_r + k_q)$ for **2** is about 17 times larger than **1**; indeed, only 2% of the interaction of singlet oxygen with the guanosine derivative leads to chemical reaction; the major route is quenching.

Both [4 + 2] and [2 + 2] cycloadditions have been observed in purine systems.[25,26] The 6,8-diketo form is the most stable form of 8-hydroxyguanine, at least in water,[7-11] and compound **2** can only undergo [2 + 2] cycloaddition from this tautomer. However, compound **1** probably undergoes [4 + 2] cycloaddition (as conclusively shown in the 8-methyl case by characterization of the endoperoxide).[25] The large amount of physical quenching (98%) in compound **1** suggests that the retro-Diels–Alder reaction is probably facile, as in the case of the 8-methylguanosine derivative.[25]

Scheme 1



Since **2** is much more reactive than **1** toward singlet oxygenation, it would not be expected to build up in solution during photooxidation of **1**, consistent with the small amount detected.[20] The high reactivity of **2** also implies that it could be a major product of the photooxygenation of **1**. In addition, the low yield and complex product mixture in the photosensitized oxidation of guanosine derivatives may be partly due to further oxidation of this **2**, which we have shown to give a complex product mixture at room temperature.[25,26]

The oxidation potentials of compounds **1** and **2** were measured by cyclic voltammetry in dry dimethylformamide with 0.1 M tetraethylammonium perchlorate as electrolyte. In both cases, the scans were irreversible and results are expressed as "half peak" potentials, $E_{p/2}$. $E_{p/2}$ (vs AgCl) is 0.85 V for **2** and 1.28 V for **1**. Thus the 8-oxo derivative is far more easily oxidized than the parent guanosine derivative, in agreement with its higher reactivity toward singlet oxygen.

The mechanism for the formation of compound **2** by singlet oxygenation is still unclear. A pathway has been postulated by Devasagayam et al.[15] via an unstable endoperoxide as an intermediate (Scheme 1). In this case, two reducing equivalents have to be supplied, which is not easy to rationalize.

Another pathway proposed by Boiteux et al.[27] is that an electron transfer from the guanine base to singlet oxygen occurs, resulting in the formation of a guanine radical cation and a superoxide radical anion. Further reaction of the guanine radical cation may result in the formation of a C-8 hydroxylated guanine radical, which on subsequent one-electron oxidation would produce 8-hydroxyguanine.[28] However, if the energetics of electron transfer from guanine to singlet oxygen are evaluated using the Weller equation[29,30] this electron transfer is endothermic by ~1.1 V. We propose an alternative pathway for the formation of this 8-oxopurine (Scheme 2).

In this mechanism, a very reactive 8-hydroperoxy derivative is formed by rearrangement of the initial endoperoxide. This hydroperoxide should be as strong an oxidant as a peracid (it is, in fact, an iminoperacid) and would be expected to donate an oxygen atom to guanosine or other oxidizable molecules in the system. Support for this mechanism comes from the fact that compound **1** 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) (Scheme 3). The structures of compounds **4** and **5** are confirmed by MS, ^1H-NMR, ^{13}C-NMR, and X-ray crystal structures (in the case of **4**).[20] Direct

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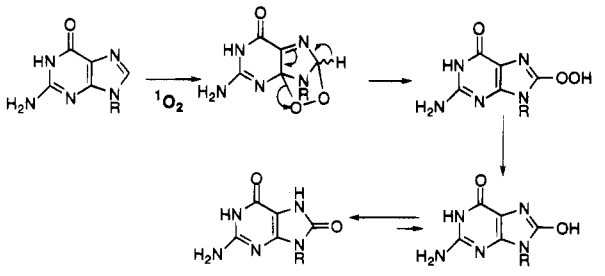
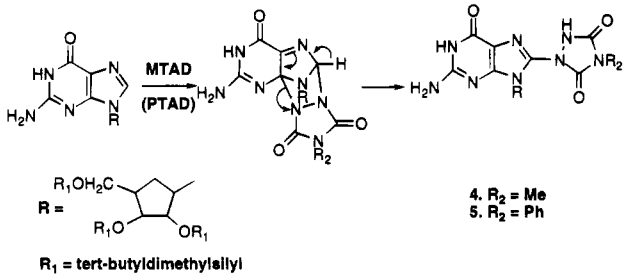
Scheme 2



Scheme 3



observation of endoperoxides from the photosensitized oxidation of the 8-methylguanosine derivative also supports this mechanism.[26]

Experimental Section

General. Melting points (uncorrected) were obtained on a Büchi capillary melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AF-200, 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 in the indicated deuterated solvent. ^{13}C-NMR peak multiplicities were determined by DEPT experiments. EI mass spectra were recorded on an AEI MS-902 instrument. Ultraviolet−visible spectra were recorded on a Beckman Model 25 spectrophotometer. Infrared spectra were taken on a Perkin-Elmer PE 580 instrument and FT-IR on a Perkin-Elmer 1600 instrument. Samples were prepared either neat on NaCl plates or as KBr pellets. Thin-layer chromatograms were obtained 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 (flash) from E. Merck, and on neutral alumina, activity I, from Fisher.

Materials. Commercial solvents were Fisher AR, used without further purification. Deuterated solvents were from Cambridge Isotope Laboratory dried over 4 Å Molecular Sieves before use. Anhydrous *N,N*-dimethylformamide was obtained by stirring DMF with calcium hydride (5 g/L) overnight at room temperature and distilled under reduced pressure (bp 70−80 °C, 20−30 mmHg). Guanosine hydrate, imidazole, and 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (TPP) were from Aldrich and were used without further purification. Tetraethylammonium perchlorate was recrystallized from water and dried under reduced pressure.

General Photolysis Procedure. Photolyses were carried out in 5-mm NMR tubes with 0.1− 0.05 M substrates in 0.5 mL of deuterated solvents. A Cermax 300 W xenon lamp was the light source. A 1% potassium dichromate filter solution was used to cutoff wavelengths below 500 nm. The NMR tubes were immersed in a bath at various temperatures and an 18 cm running water filter was used to eliminate heating. TPP (6×10^{-5} M) was the sensitizer in acetone-d_6. Oxygen was continuously bubbled through the solution during irradiation and reaction progress was monitored by NMR.

Determination of Oxidation Potentials. Oxidation potentials for compounds **1** and **2** were determined by cyclic voltammetry (Model CV-1B, Bioanalytical Systems Inc.), using a platinum button as electrode and a Ag/AgCl electrode as reference. The solvent was dry dimethylformamide with 0.10 M tetraethylammonium perchlorate as electrolyte; the solution was deoxygenated by purging with helium; substrate concentration was 5 mM. The anodic and cathodic limits were 1.9 and 1.0 V, respectively, and the scan rate was 300 mV/s. The scans were irreversible in all cases, and thus the results are expressed as "half peak" potentials, $E_{p/2}$.

Direct Determination of Singlet Oxygen Quenching Rate (k_r + k_q) by Substrates. The rates of interaction ($k_r + k_q$) for 2′,3′,5′-tris-((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (**2**) and 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)guanosine (**1**) with singlet oxygen were determined by time-resolved measurement of singlet oxygen luminescence decay at 1270 nm. The apparatus was a modification of the one previously described.[23] Sensitizing dyes were excited with either the second (532 nm) or third (355 nm) harmonic of a Quanta-Ray (DCR-2) Nd:YAG laser. The laser pulse was filtered to remove any fundamental from the laser using a 355/532 nm pass/1060 nm reflecting mirror, followed with a Schott KG-3 infrared absorbing filter to remove any residual fundamental radiation at 1064 nm ($T = 10^{-4}$ at 1064 nm, 0.9 at 532 nm, and 0.8 at 355 nm). The 355-nm pulse was also filtered with a 355 nm pass/532 nm reflecting mirror. The near-infrared emission from $^1O_2$ was monitored at right angles to the laser beam and filtered with a Schott RG-850 and Silicon 1100 nm (Infrared Optics) cutoff filters. The detector was a liquid nitrogen-cooled germanium photodiode (Model EO-817P, North Coast Scientific Corp.). The signal was averaged (10−12 shots) in a transient digitizer (LeCroy 9410), then transferred to a Macintosh IIci computer using Labview software. The data were analyzed by fitting with a first-order exponential fit, using Igor graphics software and using a macro written by Dr. R. Kanner.

Determination of the Reaction Rate (k_r) of the Substrates with Singlet Oxygen. The value of k_r for 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)-7,8-dihydro-8-oxoguanosine (**2**) was determined by competition with tetramethylethylene (TME) and for the less reactive 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)guanosine (**1**) by competition with 2-methyl-2-pentene (2M2P). In both cases, the relative rate constants were determined by measuring the relative disappearance of the starting material or the product formation at low conversion (<20%). The appearance of the products or the disappearance of the starting material was monitored by NMR spectroscopy. A small amount of dimethylformamide (DMF) was added as internal reference for integrations. Zero-order and first-order baseline corrections were applied before peak integration. Since $k_q \ll k_r$ for TME and 2M2P, $(k_r + k_q) \sim k_r$ values of TME and 2M2P were determined by direct measurements with the laser apparatus.

Preparation of 2′,3′,5′-Tris((*tert*-butyldimethylsilyl)oxy)guanosine. To a solution of guanosine (dried by heating over phosphorus pentoxide in vacuo at 56 °C for 2 h before use, 5.6 g, 20 mmol) and imidazole (13.6 g, 200 mmol) in 50 mL of anhydrous dimethylformamide (DMF) was added 15.0 g (100 mmol) of *tert*-butyldimethylsilyl chloride (TBDMSCl). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 26 h. The resulting mixture was poured into EtOAc−H_2O. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. Column chromatographic purification using 1:1 methylene chloride and acetonitrile gave 11.4 g (91%) of 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)guanosine, which was recrystallized from 95% EtOH to give white needle-like crystals; mp > 300 °C dec. Parent *m/e* (FAB) 626.35893, calcd for $C_{28}H_{56}N_5O_5Si_3$ 626.35890.

^{1}H NMR (δ, ppm, CD_2Cl_2) 3.82 (dd, J = 11.6 Hz, 2.3 Hz, 1H, C(5′)-H′), 4.02 (dd, J = 11.6 Hz, 3.3 Hz, 1H, C(5′)-H″), 4.11 (ddd, J = 4.3 Hz, 3.3 Hz, 2.3 Hz, 1H, C(4′)-H), 4.32 (dd, J = 4.3 Hz, 4.0 Hz, 1H, C(3′)-H), 4.44 (dd, J = 4.0 Hz, 3.9 Hz, C(2′)-H), 5.83 (d, J = 3.9 Hz, 1H, C(1′)-H), 6.63 (s, br, 2H, C(2)-NH_2), 7.97 (s, 1H, C(8)-H), 12.3 (s, br, 1H, N(1)-H), 0.99, 0.94, 0.89 (s, 27H, 3 × *tert*-butyl), 0.17, 0.16, 0.13, 0.11, 0.06, 0.03 (s, 18H, 6 × Me); ^{13}C NMR (δ, ppm, CD_2Cl_2) 154.4 (s, C(2)), 151.6 (s, C(4)), 117.6 (s, C(5)), 159.5 (s, C(6)), 135.9 (d, C(8)), 88.7 (d, C(1′)), 76.9 (d, C(2′)), 71.5 (d, C(3′)), 85.0 (d, C(4′)), 62.5 (t, C(5′), 26.3, 26.0, 25.9 (q, 3 × C(CH_3)$_3$), 18.8, 18.3, 18.2 (s, 3 × C(CH$_3$)$_3$), −4.11, −4.52, −4.61,−4.65, −5.25, −5.28 (q, 6 × Me); IR (KBr, cm^{-1}) 3320, 3200, 2912, 2720, 1728, 1685, 1650, 1570, 1488, 1420, 1392, 1364, 1340, 1177 , 1121, 1100, 1083, 1052, 777, 681.

Preparation of 8-Bromoguanosine. Preparation of 8-bromoguanosine followed Long's procedure.[31] A 300-mL solution of saturated

bromine−water was added to a suspension of guanosine hydrate (10.0 g, 35 mmol) in 50 mL of water with a rate such that the yellow color of the reaction mixture disappeared between each addition. The resulting white precipitate was filtered and washed with 50 mL of cold water and 50 mL of cold acetone, then recrystallized from water and dried at 100 °C/0.05 mmHg to give 9.7 g of needle-like crystals: yield 77%; mp 200−203 °C dec (lit.[31] mp 201−203 °C dec).

^{1}H NMR (δ, ppm, DMSO-d_6) 4.92 (t, J = 5.5Hz, 1H, C(5′)-OH), 5.09 (d, J = 5.1 Hz, 1H, C(3′)-OH), 5.45 (d, J = 6.2 Hz, 1H, C(2′)-OH), 3.50, 3.63 (m, 2H, C(5′)-H, ABX), 3.85 (m, 1H, C(4′)-H), 4.13 (m, 1H, C(3′)-H), 5.00 (ddd, J = 6.2, 6.2, 6.3 Hz, C(2′)-H), 5.68 (d, J = 6.3 Hz, 1H, C(1′)-H), 6.50 (s, br, 2H, C(2)-NH$_2$), 10.82 (s, br, 1H, N(1)-H) [lit.[32] (δ, ppm, DMSO-d_6) 3.46−3.75 (m, 2H, 5′-H), 3.75−4.01 (m, 1H, 4′-H), 4.01−4.31 (m, 1H, 3′-H), 4.82−5.38 (m 4H, 2′-H; 2′-, 3′-, and 5′-OH, D$_2$O exchangeable), 5.75 (d, 1H, 1′-H), 6.48 (s, 2H, 2-NH2, D$_2$O exchangeable), 10.81 (br, s, 1-NH, D$_2$O exchangeable)]; ^{13}C NMR (δ, ppm, DMSO-d_6) 153.5 (s, C(2)), 152.1 (s, C(4)), 117.5 (s, C(5)), 155.5 (s, C(6)), 121.2 (d, C(8)), 89.7 (d, C(1′)), 70.6 (d, C(2′)), 70.3 (d, C(3′)), 85.9 (d, C(4′)), 62.1 (t, C(5′)) [lit.[11] (δ, ppm, DMSO-d_6) 153.5, 152.1, 117.7, 155.5, 121.2, 89.9, 70.6, 70.5, 86.0, 62.2]; IR (KBr, cm^{-1}) 1698, 1633, 1586, 1531, 1518, 1470, 1377, 1349, 1294, 1111, 1083, 1059, 1049, 1029, 981, 858, 748, 695, 623, 609.

Preparation of 8-Benzyloxyguanosine. Anhydrous 8-bromoguanosine (dried by heating over phosphorus pentoxide in vacuo at 56 °C for 2 h before use, 5.4 g, 15 mmol) in 200 mL of anhydrous dimethyl sulfoxide was added to a sodium benzyloxide solution, which was generated and maintained under nitrogen atmosphere by dissolving 1.9 g of sodium in 200 mL of anhydrous benzyl alcohol. The reaction mixture was stirred at 65−70 °C for 42 h and then cooled to room temperature. The solution was neutralized by adding glacial acetic acid and then slowly poured into 3 L of diethyl ether. The resulting white precipitates were collected by filtration and washed with acetone and water, then recrystallized from water and dried over phosphorus pentoxide in vacuo at 56 °C to give the pure product: mp 169−171 °C (lit.[32] mp 170−171 °C).

^{1}H NMR (δ, ppm, DMSO-d_6) 5.39 (s, 2H, OCH$_2$Ph), 4.83 (t, J = 5.8 Hz, 1H, C(5′)-OH), 4.99 (d, J = 5.3 Hz, 1H, C(3′)-OH), 5.33 (d, J = 6.1 Hz, 1H, C(2′)-OH), 3.45, 3.56 (m, 2H, C(5′)-H, ABX), 3.78 (m, 1H, C(4′)-H), 4.05 (m, 1H, C(3′)-H), 4.71 (m, 1H, C(2′)-H), 5.59 (d, J = 6.1 Hz, 1H, C(1′)-H), 6.33 (s, br, 2H, C(2)-NH$_2$), 10.59 (s, br, 1H, N(1)-H) [lit.[32] (δ, ppm, DMSO-d_6) 3.36−3.65 (m, 2H, 5′-H), 3.66−3.88 (m, 1H, 4′-H), 3.88−4.04 (m, 1H, 3′-H), 4.60−4.94 (m, 4H, 2′-H; 2′-, 3′-, and 5′-OH, D$_2$O exchangeable), 5.41 (s, 2H, 8-OCH$_2$Ar), 5.64 (d, 1H, 1′-H), 6.26 (s, 2H, 2-NH$_2$, D$_2$O exchangeable),7.21−7.53 (m, 5H, 8-OC$_6$H$_5$)]; ^{13}C NMR (δ, ppm, DMSO-d_6) 153.1 (s, C(2)), 151.7 (s, C(4)), 110.9 (s, C(5)), 155.8 (s, C(6)), 150.7 (d, C(8)), 86.2 (d, C(1′)), 70.6 (d, C(2′)), 70.5 (d, C(3′)), 85.1 (d, C(4′)), 62.1 (t, C(5′)); 56.5 (q, OCH$_3$); IR (KBr, cm^{-1}) 3320, 3208, 2930, 2850, 1685, 1637, 1593, 1566, 1450, 1358, 1121, 1093, 1075, 1055, 1042, 989, 772, 728, 697, 676, 658.

Preparation of 2′,3′,5′-Tris((*tert*-butyldimethylsilyl)oxy)-8-benzyloxyguanosine. To a solution of anhydrous 8-benzyloxyguanosine (dried by heating over phosphorus pentoxide in vacuo at 56 °C for 2 h before use, 1.95 g, 5.02 mmol) and imidazole (3.40 g, 50.0 mmol) in 25 mL of anhydrous dimethylformamide was added 3.73 g (24.9 mmol) of *tert*-butyldimethylsilyl chloride (TBDMSCl). The reaction mixture was stirred at room temperature under nitrogen for 3 days. The reaction mixture was poured slowly into water and the resulting white precipitate was filtered. Column chromatographic purification using 1:1 methylene chloride and acetonitrile gave 3.07 g (84%) of 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)-8-benzyloxyguanosine, which was recrystallized from EtOH−H$_2$O to give needle-like crystals, mp 130−132 °C. Parent *m/e* (FAB) 732.40079, calcd for $C_{35}H_{62}N_5O_6Si_3$ 732.40076.

^{1}H NMR (δ, ppm, CD_2Cl_2) 3.66 (m, 2H, C(5′)-H), 3.93 (m, 1H, C(4′)-H), 4.32 (dd, J = 4.8, 3.1 Hz, 1H, C(3′)-H), 4.98 (dd, J = 6.1, 4.8 Hz, 1H, C(2′)-H), 5.43 (s, 3H, OCH$_2$Ar), 5.54 (s, br, 2H, C(2)-NH$_2$), 5.80 (d, J = 6.1 Hz, 1H, C(1′)-H), 7.35−7.50 (m, 5H, OC$_6$H$_5$), 12.05 (s, br, 1H, N(1)-H), 0.92, 0.84, 0.80 (s, 27H, 3 × *tert*-butyl), 0.09 (s, 6H, 2 × CH$_3$'s), −0.01, −0.03, −0.04, −0.18 (s, 18H, 4 × Me); ^{13}C NMR (δ, ppm, CD_2Cl_2) 153.2 (s, C(2)), 152.8 (s, C(4)), 112.2 (s, C(5)), 158.3 (s, C(6)), 151.7 (d, C(8)), 86.9 (d, C(1′)), 72.8 (d, C(2′)), 72.2 (d, C(3′)), 85.3 (d, C(4′)), 63.3 (t, C(5′), 72.0 (q, OCH$_2$Ar), 26.02, 25.90, 25.85 (q, 3 × C(*C*H$_3$)$_3$), 18.52, 18.31, 18.17, (s, 3 × *C*(CH$_3$)$_3$), −4.30, −4.50, −4.93, −5.28, −5.34, −5.40 (q, 6 × Me); IR (KBr, cm^{-1}) 3500, 3320, 3133, 2954, 2929, 2858, 1885, 1653, 1627, 1593, 1565, 1473, 1464, 1361, 1348, 1258, 1167, 1140, 1080, 837, 777.

Preparation of 2′,3′,5′-Tris((*tert*-butyldimethylsilyl)oxy)-8-oxo-7,8-dihydroguanosine. A solution of 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)-8-benzyloxyguanosine (1.46 g, 2.0 mmol) dissolved in 10 mL of 95% EtOH was added to 0.2 g of 20% palladium hydroxide on carbon (Pearlman's catalyst) suspended in 10 mL of 95% EtOH. The mixture was hydrogenated at 50 psi at room temperature. The hydrogenation was complete within 2 h and the catalyst was removed by filtration and washed twice with 95% EtOH and chloroform. The filtrate was concentrated and column chromatographic purification using 1:1 methylene chloride and acetonitrile gave 1.12 g (87%) of 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy)-8-oxo-7,8-dihydroguanosine, which was recrystallized from hot water to give needle-like crystals, mp 204−206 °C. Parent *m/e* (FAB) 642.35384, calcd for $C_{28}H_{56}N_5O_6Si_3$ 642.35382.

^{1}H NMR (δ, ppm, CD_2Cl_2) 3.75 (m, 1H, C(5′)-H′), 3.90 (m, 1H, C(5′)-H″), 4.00 (m, 1H, C(4′)-H), 4.44 (m, 1H, C(3′)-H), 5.16 (m, 1H, C(2′)-H), 5.76 (d, J = 4.9 Hz, 1H, C(1′) H), 5.45 (s, br, 2H, C(2)-NH$_2$), 11.14 (s, br, 1H, N(7)-H), 12.38 (s, br, 1H, N(1)-H), 0.95, 0.88, 0.84 (s, 27H, 3 × *tert*-butyl), 0.15, 0.14, 0.07, 0.06, 0.02, −0.07 (s, 18H, 6 × Me); ^{13}C NMR (δ, ppm, CD_2Cl_2) 153.2 (s, C(2)), 149.4 (s, C(4)), 100.5 (s, C(5)), 153.4 (s, C(6)), 153.2 (d, C(8)), 85.8 (d, C(1′)), 72.9 (d, C(2′)), 71.1 (d, C(3′)), 85.2 (d, C(4′)), 62.5 (t, C(5′), 26.1, 26.0, 25.9 (q, 3 × C(*C*H$_3$)$_3$), 18.6, 18.4, 18.3, (s, 3 × *C*(CH$_3$)$_3$), −4.18, −4.34, −4.46, −4.66, −5.23, −5.27 (q, 6 × Me); IR (KBr, cm^{-1}) 3350, 3214, 3169, 2955, 2930, 2897, 2886, 2859, 1718, 1706, 1684, 1624, 1597, 1473, 1464, 1440, 1361, 1257, 1164, 1138, 1077, 872, 837, 815, 777.

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Supplementary Material Available: ^{1}H NMR and ^{13}C NMR spectra for compounds **1** and **2** and their precursors (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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