N^2,3-Etheno-2'-deoxyguanosine [8,9-Dihydro-9-oxo-2'-deoxy-3-β-D-ribofuranosylimidazo[2,1-*b*]purine]: A Practical Synthesis and Characterization

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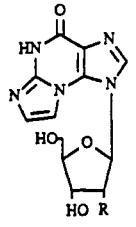
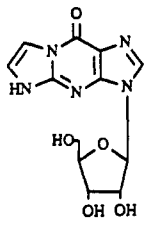
The literature methods for the syntheses of N^2,3-etheno-2'-deoxyguanosine (**1a**) and its 5'-*O*-phosphate are associated with very low overall yields. We now describe a route starting in the riboside series that permits the production of **1a** in practical quantities and has allowed its complete chemical characterization for the first time. O^6-Benzylguanosine (**6b**) when treated with bromoacetaldehyde under conditions of continuous buffering gives the N^2,3-etheno derivative **7b** in 48% yield. The 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl) derivative (**8b**, 89% yield) of **7b** when allowed to react with phenyl chlorothionoformate led to the corresponding 2' ester (**10**, 50%). 2'-Deoxygenation of **10** by the Barton procedure then afforded 65% of **11**, and deprotection of the latter ($Bu_4N^+F^-$) gave **12** quantitatively. Catalytic hydrogenation of **12** then produced pure **1a** in 86% yield. Stability studies on **1a** have confirmed its exquisite sensitivity to acid and demonstrated its relative stability to alkali and the other reagents used in automated DNA synthesis (phosphoramidate method).

Vinyl chloride is produced industrially in very large amounts for the production of commercial polymers. It is now established that exposure of humans to the vapor of the monomer can lead to hemangiosarcoma.[1,2] Both of the hepatic metabolites of this simple monomer, namely chloroethylene oxide and chloroacetaldehyde, not only interact with DNA forming exocyclic etheno derivatives with all of the heterocyclic bases except thymine[3] but introduce interstrand cross-links also.[4] The interaction with 2'-deoxyguanosine residues produces angular N^2,3-etheno-2'-deoxyguanosine (**1a**) lesions in the liver DNA of



1a, R=H
1b, R=OH



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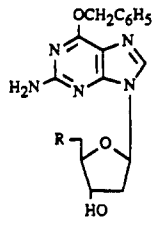

3a, R=OH
3b, R=PO_3H_2

rodents exposed[3,5] to vinyl chloride, as evidenced by the detection of the free base, N^2,3-ethenoguanine, after enzymatic hydrolysis. Singer et al.[7] have shown that the angular isomer **1a**, when present in DNA, has the potential to cause mispairings that lead to mutagenic events. During in vitro DNA synthesis both mammalian and bacterial polymerases pair both 2'-deoxycytidine and 2'-deoxythymidine opposite **1a** thus leading to normal DNA replication in the former case but mutated (G → A transitions) DNA in the latter case.[8,11] Recent evidence suggests that this etheno lesion is not easily repaired and appears to persist in tissue for many months.[12,13] Interestingly, the linear 1,N^2-etheno isomer **2** has not been detected in double-stranded DNA, despite the fact that chloroacetaldehyde reacts predominantly with guanosine to produce this type of structure.[6] Its absence may indicate efficient cellular repair of this lesion.

Our interest in **1a** is related to our ongoing program[14-17] of incorporating modified 2'-deoxynucleosides (defined as mutagenic or carcinogenic) site-specifically into oligomeric DNA. These oligomers are then employed in a variety of physicochemical and biological studies in an attempt to determine the impact of such lesions on living systems.

Although the synthesis of **1a** (and its 5'-*O*-phosphate) is already recorded in the literature,[18] the published method gives very low overall yields, and although it may be suitable for studies at the biological level,[8] it cannot afford the macroscopic quantities needed for total synthesis studies. The published route basically relies on the condensation of O^6-benzyl-2'-deoxyguanosine (**3a**) or its 5'-*O*-phosphate (**3b**) with bromoacetaldehyde in aqueous sodium bicarbonate. The acidic pH (4.0-4.5) range within which optimal results might be expected is precluded,

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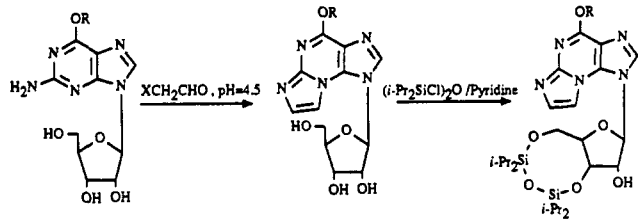
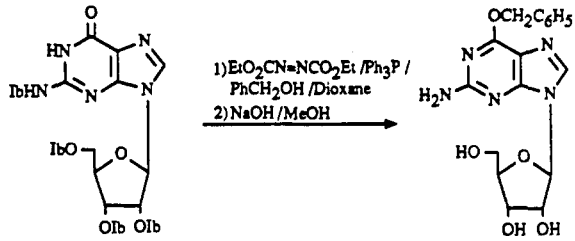
Scheme I



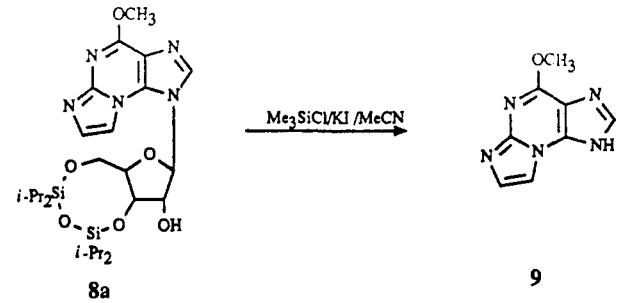
because of the sensitivity of the product to acid-catalyzed depurination. By contrast in the more acid-stable ribonucleoside series, guanosine withstands such conditions and in this case Kusmierek et al.[19] found that **6b** could be



converted to **7b** in 25% yield (Scheme I). This on catalytic hydrogenation afforded **1b** in 80% yield. It occurred to us that if this type of ribonucleoside would withstand the 2'-deoxygenation reaction sequence[27] then a potential route for the synthesis of larger quantities of **1a** might become available.

In a first attempt to utilize such an approach route we started (Scheme I) with the more easily available[20] O^6-methylguanosine (**6a**). This, when treated with aqueous 50% chloroacetaldehyde in the presence of catalytic amounts of potassium iodide, gave N^2,3-etheno-O^6-methylguanosine (**7a**) in 35% yield. Unfortunately, direct demethylation of **7a** was not possible because of its insolubility in the solvents[22] that are normally used for this reaction. In an attempt to avoid this problem **7a** was converted to the more soluble disiloxane derivative **8a** by treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in the presence of imidazole. Nevertheless, when demethylation of **8a** was attempted by means of trimethylsilyl chloride and potassium iodide[23] under a variety of mild conditions (including the presence of an organic base), only the insoluble N^2,3-etheno-O^6-methylguanine (**9**) was formed. By contrast, in a model experiment O^6-methylguanosine (**6a**) was easily converted quantitatively to guanosine under the same conditions.

Given the apparent instability of the glycosidic linkage in **8a** to even traces of acid it was decided to proceed with the O^6-benzyl derivative **7b** which, as noted previously, can be deprotected cleanly by catalytic hydrogenation under strictly neutral conditions.[19] However, rather than



use the low-yielding (23%) phenyldiazomethane method[19] for the preparation of **6b** we employed the Mitsunobu reaction.[24,25] Accordingly, when N^2,2',3',5'-tetraisobutyrylguanosine (**4**) was treated with benzyl alcohol, diethyl azodicarboxylate, and Ph_3P in boiling dioxane, its O^6-benzyl derivative **5** could be isolated crystalline in 48% yield. Deacylation of **5** to **6b** was then accomplished quantitatively by dilute methanolic sodium hydroxide. In the conversion of **6b** to the N^2,3-etheno derivative **7b** we utilized the reaction conditions of Kusmierek et al.,[19] but by modifying the workup procedure significantly (see Experimental Section) we were able to improve the yield from 25% to 48% (pure recrystallized). This material after protection of the 3' and 5' oxygen atoms served well for the 2'-deoxygenation procedure, which basically was accomplished by the Barton method,[26] as modified by Robins et al.[27] for nucleosides (Scheme II).

Thus, regioselective protection of **7b** with 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane in the presence of dry pyridine gave **8b** in quantitative yield (Scheme I). Subsequent reaction of **8b** with phenyl chlorothionoformate in the presence of DMAP led to the 2'-phenoxythiocarbonyl ester (**10**, 50% yield, Scheme II). This compound was then deoxygenated by treating it with tri-*n*-butyltin hydride and a catalytic amount of 2,2'-azobisisobutyronitrile (AIBN). The product after purification by flash column chromatography gave a 65% yield (pure) of the desired 2'-deoxyribonucleoside **11**. Removal of the disiloxane protecting group was then easily achieved by treatment with 2 molar equiv of tetrabutylammonium fluoride in tetrahydrofuran for 15 min at room temperature. This afforded O^6-benzyl-N^2,3-etheno-2'-deoxyguanosine (**12**) in quantitative yield. Finally, the benzyl group at the O^6 position was eliminated by hydrogenation over a 20% Pd-on-charcoal catalyst. This led to the desired N^2,3-etheno-2'-deoxyguanosine (**1a**) as a pure crystalline substance in 86% yield. Full characterization of this compound is described in the Experimental Section. All physical data confirm the structure.

Although the overall yield (7.5%) of **1a** from guanosine (or 13.5% from the easily available O^6-benzylguanosine) cannot be regarded as outstanding, the methods presented are reliable, all intermediates can easily be purified, and the target compound (**1a**) can be obtained pure in practical quantities.

Our aim originally was to determine if sufficient quantities of this material could be obtained for incorporation into oligomeric DNA by solid-phase synthesis.

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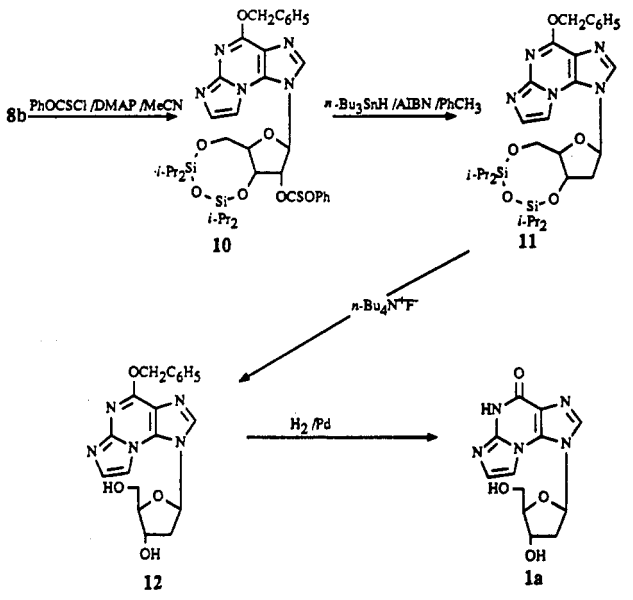
Scheme II



With the synthetic problem solved we now examined the stability of **1a** to the conditions of DNA synthesis.

We found that **1a** will survive concd aqueous ammonia for 30 min at room temperature (the conditions used to remove phenoxyacetyl protecting groups from the nucleoside bases). It is also stable to the activating conditions (tetrazole/CH_3CN, 1.5 min), capping conditions (*N*-methylimidazole, Ac_2O, 2,6-lutidine/THF, 1.5 min), and phosphite–phosphate oxidation conditions (I_2–H_2O–2,6-lutidine/THF, 1 min). However, its exquisite sensitivity toward 3% dichloroacetic acid in CH_2Cl_2 (detritylation conditions) precludes its use in normal DNA synthesis. Other attempts to achieve this goal, using methods which avoid acid conditions in the synthesis of DNA, will be reported later.

Experimental Section

All organic solvents used in the synthetic and chromatographic procedures were of HPLC grade purchased from Aldrich and were used as such unless otherwise specified. Dimethylformamide, purchased from Fisher Scientific Co., was purified by drying over neutral alumina (super acitivity) for 16 h followed by decantation and distillation at 59–60 °C/1 mm. It was then stored over activated molecular sieves (4 Å). Pyridine was purified by distillation from powdered calcium hydride. Guanosine was purchased from Sigma Chemical Co. Acetonitrile, dioxane, and tetrahydrofuran were dried according to literature procedure.[28]

Thin-layer chromatography (TLC) was performed on silica-coated aluminum plates obtained from Merck Inc. Flash column chromatography was performed using silica gel (40–63 μm grade) purchased from Krackler Scientific Co. (Albany, NY). Melting points are uncorrected. UV spectra were recorded on Perkin-Elmer Lambda-5 spectrometer. ^1H NMR and ^{13}C NMR spectra were obtained from a General Electric QE-300 spectrometer using tetramethylsilane as internal standard. Fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS -890/DS-90 instrument using thioglycerol as the matrix.

***O*⁶-Methylguanosine (6a).** Guanosine (4.6 g, 16 mmol), dried by heating in an air oven at 110 °C for 48 h and then by coevaporating with dry pyridine (30 mL), was suspended in 80 mL of dry pyridine. To the suspension was added dropwise trifluoroacetic anhydride (20 mL) with cooling in an ice bath. After 15 min a dilute solution of sodium methoxide (prepared by dissolving 14.6 g of sodium in 2400 mL of methanol) was added first in 30-mL portions over a period of 40 min (600 mL) and the remainder (1800 mL) in one portion. The reaction mixture was stirred at room temperature for 60 h and then neutralized with 96 mL of pyridine in 32 mL of concd hydrochloric acid. The excess acid was neutralized by adding sodium bicarbonate, and the mixture was evaporated to dryness. The residue was warmed with dry *n*-propanol (200 mL) and filtered while hot to remove the insoluble sodium chloride. The filtrate on cooling gave a crude solid which on isolation and recrystallization from ethyl acetate (200 mL) gave *O*⁶-methylguanosine (**6a**) as cream-colored crystals (4 g, 83% yield): mp 134–35 °C (lit.[29] mp 133–35 °C); R_f 0.7 (CH_3OH–$CHCl_3$ (1:4)); ^1H NMR (DMSO-d_6) δ (ppm) 8.11 (s, 1 H, H-8), 6.46 (br s, 2 H, NH_2), 5.78 (d, J = 5 Hz, 1 H, H-1′), 5.42 (d, 1 H, 2′-OH), 5.16 and 5.12 (2 H, 3′- and 5′-OH), 4.46–4.48 (m, 1 H, H-2′), 4.16 (1 H, H-3′), 4.08 (1 H, H-4′), 3.96 (s, 3 H, OCH_3), 3.61 and 3.53 (2 H, H-5′, -5″).

***N*²,3-Etheno-*O*⁶-methylguanosine (7a).** *O*⁶-Methylguanosine (**6a**, 2.97 g, 10 mmol) was dissolved in a mixture of methanol (10 mL) and acetate buffer (60 mL, pH 4.5; prepared by mixing 15 mL of 0.2 M sodium acetate, 15 mL of 0.2 M acetic acid, and 30 mL of water) and treated with 10.5 mL of 50% aqueous chloroacetaldehyde solution and a catalytic amount of potassium iodide (166 mg). The pH of the reaction mixture which fell to 3 after the addition of chloroacetaldehyde was adjusted to 4.5 by the addition of sodium acetate solution (0.2 M, 20 mL) and the reaction mixture stirred at 37 °C for 36 h after which it was neutralized with sodium bicarbonate. The neutral solution was extracted with a mixture of ethyl acetate and 2-methyl-2-propanol (1:1, 70 mL × 3). The combined organic layers were dried over $MgSO_4$ and evaporated to dryness, and the residue was purified by flash chromatography on silica gel using a 21.5-cm × 4-cm column. Elution with CH_3OH–$CHCl_3$ (17:83) gave **7a** which could be crystallized from ethyl acetate as shiny needles (0.9 g, 35% yield): mp 162–164 °C dec; R_f 0.28 (CH_3OH–$CHCl_3$ (17:83)), 0.45 (CH_3OH–CH_2Cl_2 (1:4)); ^1H NMR (DMSO-d_6) δ 8.40 (s, 1 H, H-8), 7.90 and 7.48 (d, J = 1.5 Hz, 2 H, etheno protons), 6.21 (d, J = 4.2 Hz, 1 H, H-1′), 5.80 (1 H, 2′-OH), 5.40 (1 H, 3′-OH), 5.20 (1 H, 5′-OH), 4.5 (1 H, H-2′), 4.20 (1 H, H-3′), 4.10 (s, 3 H, OCH_3), 3.5–3.7 (m, 2 H, H-5′, -5″); ^{13}C NMR (DMSO-d_6) δ 146.77, 139.53, 134.69, 131.10, 117.20, 108.31, 89.11, 85.92, 74.80, 69.42, 60.32, and 53.47. Anal. Calcd for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.92; H, 4.54; N, 22.11.

***N*²-3-Etheno-*O*⁶-methyl-3′,5′-*O*-(tetraisopropyldisiloxa-1,3-diyl)guanosine (8a).** A solution of *N*²,3-etheno-*O*⁶-methylguanosine (**7a**, 240 mg, 0.75 mmol) in dry DMF (1 mL) was treated with imidazole (224 mg, 3.3 mmol) followed by 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.24 g, 82 mmol). The reaction mixture was stirred at 24 °C under nitrogen for 24 h after which it was poured into ice-cold water (50 mL) with stirring. The solid which separated was filtered, washed with water, and dried to give crude **8a** as a colorless solid (0.4 g; 80% yield). It was purified by preparative TLC on silica gel using a CH_3OH–$CHCl_3$ (5:95) mixture as the developing solvent. Isolation of the major component afforded pure *N*²,3-etheno-*O*⁶-methyl-3′,5′-*O*-(tetraisopropyldisiloxa-1,3-diyl)guanosine (**8a**) as a glassy solid: R_f 0.78 (CH_3OH–$CHCl_3$ (17:83)), 0.7 (CH_3OH–CH_2Cl_2 (1:4)); ^1H NMR ($CDCl_3$) δ 8.12 (s, 1 H, H-8), 7.84, 7.51 (d, J = 1 Hz, 1 H each, etheno protons), 6.36 (d, J = 4.2 Hz, 1 H, H-1′), 6.13 (1 H, 2′-OH), 4.51 (1 H, H-2′), 4.33 (1 H, H-3′), 4.21 (1 H, H-4′), 4.06 (s, 3 H, OCH_3), 3.95–4.04 (m, 2 H, H-5′, -5″), 0.95–1.12 (m, 28 H, isopropyl protons); FAB-MS 564 (M + 1), 190 (M – ribose unit). Anal. Calcd for $C_{25}H_{41}N_5O_6Si_2$: C, 53.26; H, 7.33; N, 12.42. Found: C, 53.58; H, 7.64; N, 12.63.

Attempted Demethylation of *N*²,3-Etheno-*O*⁶-methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)guanosine. Compound **8a** (44 mg, 0.78 mmol) was dissolved in dry acetonitrile (5 mL), and to it was added potassium iodide (16.8 mg) and trimethylsilyl chloride (0.013 mL). The reaction mixture was stirred at 24 °C until TLC showed the complete absence of the starting material and its replacement by a more polar product (6 h). The solid which separated during the course of the reaction was filtered off, washed with ether, and dried. Its ^1H NMR spectrum was consistent with *N*²,3-etheno-*O*⁶-methylguanine[6]

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(9). Evaporation of the filtrate and trituration of the residue with dry ether afforded additional **9** (total yield 12 mg): ^{1}H NMR (DMSO-d_6) δ 8.68 (s, 1 H, H-8) 8.38 and 8.06 (d, J = 2.4 Hz, 2 H, etheno protons), 4.24 (s, 3 H, OCH_3).

O^6-Benzylguanosine (6b). A solution of N^2,2′,3′,5′-tetraisobutyrylguanosine[30] (**4**, 2.81 g, 5 mmol), triphenylphosphine (1.97 g, 7.5 mmol), and diethyl azodicarboxylate (1.31 g, 7.5 mmol) in dry dioxane (100 mL) was treated with benzyl alcohol (0.81 g, 7.5 mmol). The mixture was stirred at 24 °C for 48 h, after which the solvent was evaporated under reduced pressure and the resulting oil was then subjected to column chromatography on silica gel. Elution with CH_3OH–$CHCl_3$ (1:49) gave the desired O^6-benzyl-N^2,2′,3′,5′-tetraisobutyrylguanosine (**5**, 1.58 g, 48.3% yield): mp 106–108 °C; R_f 0.95 (CH_3OH–$CHCl_3$ (5:95)); ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1 H, H-8), 7.93 (1 H, NH), 7.5 and 7.3 (m, 5 H, ArH) 6.07 (d, 1 H, H-1′), 5.88 (1 H, H-2′), 5.79 (1 H, H-3′), 5.64 (s, 2 H, OCH_2), 4.44 (m, 3 H, H-4′, -5′, -5″), 2.56 (m, 4 H, 4 × CH), 1.26–1.30 (m, 24 H, 8 × CH_3).

Compound **5** (1.5 g) was dissolved in a methanolic solution of sodium hydroxide (0.5%, 300 mL) and stirred at rt for 48 h after which it was neutralized with acetic acid (50%, 2 mL). The solvents were evaporated under reduced pressure and cold water added to the residue. O^6-Benzylguanosine (**6b**) precipitated as a white fluffy solid. It was recrystallized from methanol (0.82 g, 93% yield): mp 158–60 °C (lit.[31] mp 158–60 °C); ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1 H, H8), 7.47 and 7.38 (m, 5 H, ArH), 6.50 (s, 2 H, NH_2), 5.79 (d, 1 H, H-1′), 5.49 (s, 2 H, $ArOCH_2$), 5.40 (d, 1 H, 2′-OH), 5.14 and 5.10 (2 H, 3′-OH and 5′-OH), 4.46 (1 H, H-2′), 4.10 (1 H, H-3′), 3.88 (1 H, H-4′), 3.60, 3.43 (m, 2 H, H-5′ and H-5″).

O^6-Benzyl-N^2,3-ethenoguanosine (9-(Benzyloxy)-3-β-D-ribofuranosylimidazo[2,1-*b*]purine) (7b). O^6-Benzylguanosine (**6b**, 3.73 g, 10 mmol) was dissolved in ethanol (125 mL) and acetate buffer (pH 4.5, 200 mL; prepared by mixing 50 mL of 0.2 M aqueous sodium acetate with 50 mL of 0.2 M aqueous acetic acid and making the volume up to 200 mL with water). To this solution was added 45 mL of aqueous bromoacetaldehyde solution (prepared by stirring 10 mL of bromoacetaldehyde diethyl acetal with 30 mL of 1 N hydrochloric acid and 5 mL of ethanol for 72 h and using the resulting solution) in portions (9 × 5 mL). The pH of the reaction mixture which fell after the addition of each portion of bromoacetaldehyde was adjusted to 4.5 by adding 0.2 M sodium acetate solution. After the addition was complete (15 min) the reaction mixture was stirred at 37–40 °C for 30 h. It was then cooled, and solid sodium bicarbonate was then added with stirring to pH 8. The colorless solid which separated was filtered, washed with cold water, and recrystallized from methanol–water (9:1) to give O^6-benzyl-N^2,3-ethenoguanosine (**7b**, 1.9 g, 47.9% yield) as shiny needles: mp 184–85 °C (lit.[19] mp 179–81 °C); R_f 0.45 (CH_3OH–$CHCl_3$ (1:4)); ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1 H, H-8), 7.97 and 7.49 (d, J = 2 Hz, 1 H each, etheno protons), 7.55 and 7.41 (m, 5 H, ArH), 6.23 (d, 1 H, H-1′), 5.81 (d, 1 H, 2′-OH), 5.58 (s, 2 H, OCH_2), 5.35 (1 H, 3′-OH), 5.10 (1 H, 2′-OH), 4.43 (1 H, H-2′), 4.11 (1 H, H-3′), 4.01 (1 H, H-4′), 3.67 and 3.56 (2 H, H-5′ and -5″); FAB-MS (background subtracted) 398 (M + 1, 100), 266 (heterocyclic base + H, 64.2).

N^2,3-Ethenoguanosine (8,9-Dihydro-9-oxo-3-β-D-ribofuranosylimidazo[2,1-*b*]purine) (1b). A solution of O^6-benzyl-N^2,3-ethenoguanosine (**7b**; 397 mg, 1 mmol) in ethanol (180 mL) and water (15 mL) was hydrogenated (2 atm) at rt in the presence of a 10% Pd-on-C catalyst (150 mg). After 48 h, the reaction mixture was filtered through Celite and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in hot methanol (100 mL) and kept overnight. The colorless crystals which separated were filtered, washed with cold methanol, and dried to give N^2,3-ethenoguanosine (**1b**; 261 mg, 85% yield); R_f 0.22 (i-C_3H_7OH–H_2O–concd NH_3 (7:1:1)); UV λ_{max} (CH_3OH) 228 and 260 nm; ^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1 H, H-8), 7.69 and 7.18 (2 H, etheno protons), 6.10 (1 H, H-1′), 4.46 (1 H, H-2′), 4.17 (1 H, H-3′), 4.06 (1 H, H-4′), 3.73 and 3.60 (2 H, H-5′, 5″); FAB-MS 308 (M + H) 176 (heterocyclic base + H). Anal. Calcd for $C_{12}H_{13}N_5O_5$: C, 46.91; H, 4.26; N, 22.79. Found: C, 47.08; H, 4.34; N, 22.82.

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O^6-Benzyl-N^2,3-etheno-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)guanosine (8b). To a suspension of O^6-benzyl-N^2,3-ethenoguanosine (**7b**; 1.19 g, 3 mmol) in dry pyridine (24 mL) contained in a two-necked flask equipped with septum-capped inlets was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.2 mL, 3.3 mmol) by means of a syringe and the mixture stirred under nitrogen for 12 h. The pyridine was removed under reduced pressure, and the residue, after trituration with cold water, was filtered, dried in vacuum desiccator over P_2O_5, and purified by flash chromatography on a 21.5-cm × 4-cm column using CH_3OH–$CHCl_3$ (0.3:99.7) as the eluent. Isolation of the major component afforded O^6-benzyl-N^2,3-etheno-3′,5′-O-(tetraisopropyldisiloxa-1,3-diyl)guanosine as a glassy solid (**8b**, 1.70 g, 89% yield): R_f 0.6 (CH_3OH–$CHCl_3$ (5:95)), 0.2 (hexane–EtAc (1:4)); ^{1}H NMR (DMSO-d_6) δ 8.16 (s, 1 H, H-8), 7.88 (1 H, etheno proton), 7.42–7.58 (m, 6 H, etheno + ArH), 6.42 (1 H, H-1′), 6.15 (1 H, 2′-OH), 5.61 (s, 2 H, OCH_2), 4.53 (1 H, H-2′), 4.32 (1 H, H-3′) 4.22 (1 H, H-4′), 4.02 (2 H, H-5′, -5″), 1.07 (m, 28 H, isopropyl protons); ^{13}C NMR ($CDCl_3$) δ 136.12, 133.88, 132.52, 128.87, 128.37, 128.09, 117.83, 107.10, 90.45, 82.66, 76.21, 68.67, 69.15, 60.29, 17.39, 17.30, 17.23, 16.98, 16.85, 13.41, 13.02, 12.88, and 12.70; FAB-MS 640 (M + H), 266 (heterocyclic base + H). Anal. Calcd for $C_{31}H_{45}N_5O_6Si_2$: C, 58.19; H, 7.09; N, 10.94. Found: C, 57.99; H, 7.22; N, 10.98.

O^6-Benzyl-N^2,3-etheno-2′-O-[phenoxy(thiocarbonyl)]-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)guanosine (10). A suspension of compound **8b** (703 mg, 1.1 mmol) and 4-(dimethylamino)pyridine (375 mg) in dry acetonitrile (20 mL) was treated with phenyl chlorothionoformate (208.7 mg, 220 μL). The suspended solid slowly went into solution when stirred at 24 °C under nitrogen. After 12 h, the acetonitrile was removed by evaporation, the residue was dissolved in methylene chloride (50 mL), and the solution was washed with water (3 × 40 mL), dried over $MgSO_4$, and evaporated to dryness. The residual solid was subjected to column chromatography over silica gel on a 21.5-cm × 4-cm column with CH_3OH–$CHCl_3$ (1:99) as the eluent. Fractions of 30 mL were collected, and the required compound was found in fractions 12–32. Evaporation of the solvents gave **10** as a glassy solid (420 mg, 50% yield): R_f 0.38 (CH_3OH–$CHCl_3$ (1:99)), 0.6 (hexane–EtAc (1:4)); ^{1}H NMR (DMSO-d_6) δ 8.09 (s, 1 H, H-8), 7.00–7.57 (m, 12 H, 2 ArH + etheno protons), 6.47 (1 H, H-1′), 6.24 (1 H, H-2′), 5.68 (s, 2 H, OCH_2), 4.81 (1 H, H-3′), 4.31 (1 H, H-4′), 4.14 and 4.28 (2 H, H-5′, -5″), 0.98–1.20 (m, 28 H, isopropyl protons); ^{13}C NMR ($CDCl_3$) δ 193.61, 153.41, 133.78, 132.72, 129.74, 128.56, 128.39, 128.14, 126.93, 121.48, 106.53, 87.88, 83.75, 83.33, 68.57, 68.78, 59.89, 17.40, 17.34, 17.23, 16.98, 13.43, and 12.96; FAB-MS 776 (M + H), 266 (heterocyclic base + H). Anal. Calcd for $C_{38}H_{49}N_5O_7Si_2S$: C, 58.81; H, 6.36; N, 9.02. Found: C, 58.62; H, 6.60; N, 8.95.

O^6-Benzyl-N^2,3-etheno-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)guanosine (11). Compound **10** (800 mg) was dissolved in dry toluene (25 mL). After degassing with oxygen-free argon for 20 min, tributyltin hydride (585 μL) and azobisisobutyronitrile (60 mg) were added. The reaction mixture under nitrogen was heated at reflux for 3 h. The solvent was evaporated, and the residue was subjected to column chromatography on silica gel on a 21.5-cm × 4-cm column using CH_3OH–$CHCl_3$–Et_3N (3:96.5:0.5) as the eluting solvent. Fractions of 30 mL were collected, and the desired material was found in fractions 7–20. These were pooled and evaporated to give **11** as a colorless glassy solid (410 mg, 65% yield): R_f 0.24 (CH_3OH–$CHCl_3$–Et_3N (3:96.5:0.5)), 0.36 (CH_3OH–$CHCl_3$ (3:97)); ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1 H, H-8), 7.28–7.54 (m, 7 H, ArH + etheno protons), 6.43 (t, 1 H, H-1′), 5.70 (s, 2 H, CH_2), 4.74 (1 H, H-3′), 4.01 (1 H, H-4′), 3.95 (2 H, H-5′, -5″), 2.70–2.72 (2 H, H-2′, -2″), 0.92–1.20 (m, 28 H, isopropyl protons); ^{13}C NMR ($CDCl_3$) δ 147.77, 136.19, 134.33, 133.62, 132.65, 128.85, 128.78, 128.36, 128.05, 117.74, 106.92, 86.14, 84.14, 69.59, 68.58, 61.75, 40.39, 17.46, 17.20, 17.01, 16.94, 13.51, 13.45, 13.12, 12.92 and 12.61; FAB-MS 624 (M + H), 266 (heterocyclic base + H). Anal. Calcd for $C_{31}H_{45}N_5O_5Si_2$: C, 59.68; H, 7.27; N, 11.23. Found: C, 60.03; H, 7.29; N, 11.26.

O^6-Benzyl-N^2,3-etheno-2′-deoxyguanosine (12). A solution of O^6-benzyl-N^2,3-etheno-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)guanosine (11; 245 mg) in dry tetrahydrofuran (2.5 mL) was treated with tetrabutylammonium fluoride (0.78

mL). The reaction mixture was stirred at rt for 30 min after which the volatiles were removed under reduced pressure. The residue was washed with ether (2 × 5 mL) and triturated with cold water. The resulting colorless solid was filtered, dried, and then recrystallized from ethanol to give O^6-benzyl-N^2,3-etheno-2′-deoxyguanosine (12; 147 mg, 98% yield): mp 175–177 °C; R_f 0.5 (CH_3OH–$CHCl_3$ (1:4)), 0.72 (n-C_3H_7OH–H_2O–NH_3 (7:1:2)); ^1H NMR (DMSO-d_6) δ 8.36 (s, 1 H, H-8), 8.01 and 7.58 (d, J = 1.2 Hz, 2 H, etheno protons), 7.38–7.50 (m, 5 H, ArH) 6.62 (t, J = 5.7 Hz, 1 H, H-1′), 5.57 (s, 2 H, OCH_2), 5.46 (1 H, 3′-OH), 4.87 (1 H, 5′-OH), 4.41 (1 H, H-3′), 3.96 (1 H, H-4′), 3.46 (m, 2 H, H-5′, -5″), 2.90–2.96 (m, 1 H, H-2′), 2.46 (m, 1 H, H-2″); ^{13}C NMR (DMSO-d_6) δ 146.59, 136.56, 135.86, 131.55, 128.50, 128.33, 128.11, 116.21, 108.70, 88.56, 85.08, 69.85, 67.55, 60.93 and 38.94; FAB-MS 382 (M + H), 266 (heterocyclic base + H). Anal. Calcd for $C_{19}H_{19}N_5O_4$: C, 59.84; H. 5.02; N, 18.36. Found: C, 59.48; H, 4.92; N, 17.98.

N^2,3-Etheno-2′-deoxyguanosine (8,9-Dihydro-9-oxo-2′-deoxy-β-D-ribofuranosylimidazo[2,1-*b*]purine) (1a). O^6-Benzyl-N^2,3-etheno-2′-deoxyguanosine (12, 130 mg) was dissolved in a mixture of ethanol (50 mL), water (3 mL), and concd NH_3 (4 mL) and hydrogenated over a 20% Pd-on-C catalyst (45 mg) at 25 °C for 48 h. The catalyst was removed by filtration through Celite, and the solvents were evaporated. When the residue was dissolved in methanol and kept overnight, N^2,3-ethno-2′-deoxyguanosine (1a) crystallized as a colorless solid (55 mg). Additional compound (25 mg) could be obtained from the mother liquor by preparative thin-layer chromatography using n-C_3H_7OH–H_2O–concd NH_3 (7:2:1) as the developing solvent; mp softens at 210 °C and decomposes at 252 °C: R_f 0.5 (n-C_3H_7OH–H_2O–concd NH_3 (7:2:1)), 0.38 (i-C_3H_7OH–H_2O–concd NH_3 (7:1:1)); UV λ_{max} (CH_3OH) 228 nm (ε 25 052) and 262 nm (ε 8894); ^1H NMR (DMSO-d_6) δ 12.35 (br s, 1 H, NH), 8.21 (s, 1 H, H-8), 7.71 and 7.13 (2 s, 2 H, etheno protons), 6.44 (t, 1 H, H-1′), 5.43 (br s, 1 H, 3′-OH), 4.89 (1 H, 5′-OH), 4.40 (1 H, H-3′), 3.91 (1 H, H-4′), 3.48 (2 H, H-5′, 5′), 2.78 and 2.42 (m, 2 H, H-2′, -2″); ^{13}C NMR (DMSO-d_6) δ 156.12 (C-6), 142.72 (C-2), 135.55 (C-8), 133.63 (C-4), 128.62 (etheno 1″), 119.6 (C-5), 109.53 (etheno 2″), 88.4 (C-4′), 84.9 (C-1′), 69.5 (C-3′), 60.9 (C-5′), 39.5 (C-2′), FAB-MS (background subtracted) 292 (M + H, 39), 176 (heterocyclic base + H, 100). Anal. Calcd for $C_{12}H_{13}N_5O_4$: C, 49.48; H, 4.50; N, 24.04. Found: C, 49.09; H, 4.43; N, 24.03.

Stability of 1a to Conditions of Automated DNA Synthesis. (a) Detritylation Conditions. Compound 1a (2 mg) was added to an Eppendorf tube containing 2 mL of dichloroacetic acid/CH_2Cl_2 (3:97) at 25 °C. After 1 min the solution was neutralized with $(C_2H_5)_3N$ to pH 7 and analyzed on TLC using n-C_3H_7OH–H_2O–concd NH_3 (7:2:1) as the developing solvent. This showed a complete absence of 1a and the presence of a new product which was identified as N^2,3-ethenoguanine by comparison with an authentic sample.[6]

(b) Deacylation Conditions. Compound 1a (1 mg) was added to a concd NH_3 solution (1 mL) in an Eppendorf tube. The tube was sealed and kept at 25 °C for 30 min, after which its contents were neutralized to pH 7 with dilute acetic acid. Its TLC analysis showed no change in 1a.

The stability of 1a under activating, capping, and phosphite–phosphate oxidizing conditions was studied in a similar manner. None of these reagents affected 1a at normal temperatures.

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Supplementary Material Available: ^1H NMR spectra of 1a, 1b, 7a, 8a, 8b, 10, 11, and 12 (9 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.