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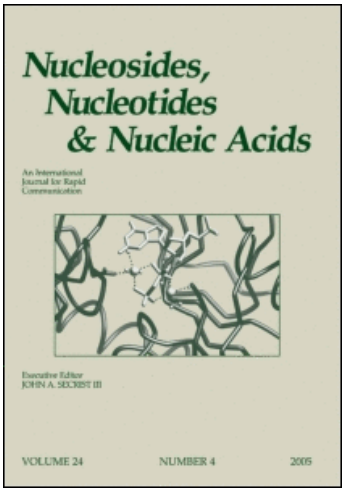

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REGIOSELECTIVE 1-ALKYLATION OF 2′-DEOXYGUANOSINE

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ABSTRACT

A method has been found for the regioselective alkylation of the nitrogen at the 1-position of 2′-deoxyguanosine. This consists in the reaction, in tetrahydrofuran solution, of a fully protected form of dG, namely the 3′5′-*O*-bis(*tert*-butyldimethylsilyl)-N^2-dimethylaminomethylene derivative, with an alkyl halide in the presence of cesium carbonate. The yields of these previously unavailable derivatives of 2′-deoxyguanosine range from good to excellent. Confirmation of the structure of these substances comes from a comparison of their spectroscopic properties with those of the known 1-methyl homologue. In particular, the UV spectra of these new derivatives and the known 1-methyl homologue are essentially identical.

INTRODUCTION

Damage to cellular DNA is considered to be a key step in the initiation of chemical carcinogenesis. Although carcinogens can react at a variety of sites on DNA bases and may attack several bases simultaneously, it is evident that some base sequences are more prone to attack than others. Nevertheless, it is difficult to evaluate how any one lesion alters the structure or coding

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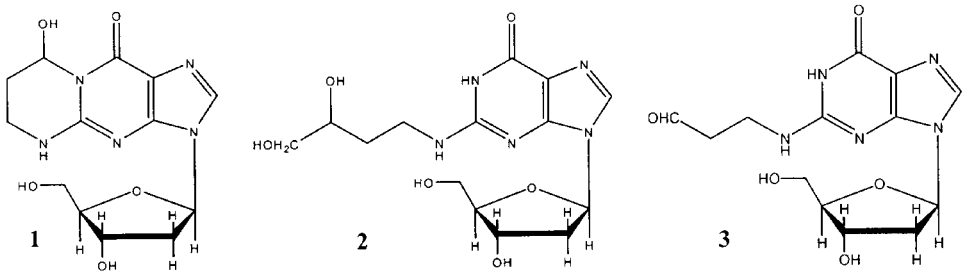
properties of the damaged DNA. One approach that has been pioneered[1] to solve this problem is the method of total synthesis. This involves the preparation, via automated methods, of completely homogeneous DNA oligomers having a modified (damaged) base located site-specifically within the sequence. This then permits studies on the way in which the lesion alters DNA structure and on its mutagenic spectrum and potency. Of the four DNA nucleosides, 2′-deoxyguanosine (dG) is of special interest because of its high reactivity towards electrophiles. The latter, depending on their reactivity, or perhaps polarizing ability, may attack at the 1, N^2, O^6, C7 or C8 positions of the guanine moiety. Bipolar electrophiles such as unsaturated or α-halo-carbonyl compounds may give exocyclic adducts. The electrophiles themselves may have their origins in the environment or may occur endogenously in the mammalian system. Where the formation of such adducts is known to occur, it is important to study the possible mutagenic/carcinogenic consequences of such base modifications in DNA.

One of our specific interests has been the damage induced in DNA by acrolein (acraldehyde or prop-2-enal), a substance that is ubiquitous in the environment because of its origin principally in burning organic matter. Previously, we had succeeded[2] in introducing into oligomeric DNA, the nucleoside (**1**) that represents the major acrolein lesion[3] in natural DNA. The key to the generation of this alkali-sensitive lesion in oligomeric DNA was the introduction of the precursor diol **2**, which is completely stable to the ammonia deprotection step of DNA synthesis. Once introduced, cleavage of the diol by sodium metaperiodate, a reagent that does not affect normal DNA, then allowed the generation of the desired lesion **1** in either single- or double stranded DNA. Subsequently, this strategy was adopted by Harris and his associates[4] but using a post-synthetic approach for the introduction of the diol **2** into oligomeric DNA. Although **1** exists as a single cyclic diastereomer (due to H-bonding with the C6 carbonyl oxygen) in both the monomeric nucleoside and single-stranded DNA, surprisingly, it adopts almost exclusively the open-chain form (**3**) when annealed with the complementary strand.[5] Thus the energy loss associated with the normal dG/dC hydrogen bonding, possible with **3**, must be significantly greater than that associated



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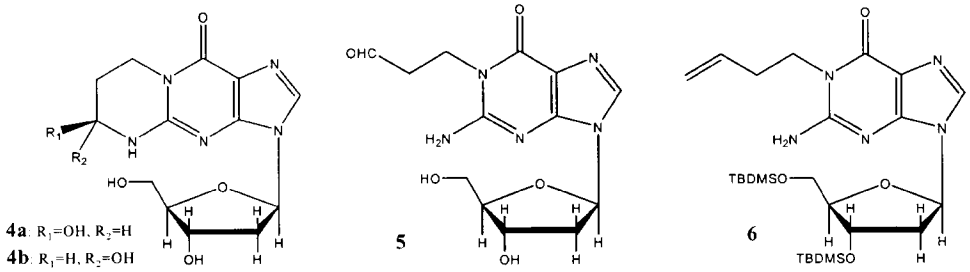

with the system when it is in the cyclic form and in which significant perturbation of the normal DNA structure must occur. Perhaps not surprisingly, **1** proved to be only a weak mutagen in a bacterial system[6] in keeping with the findings for the simple N^2-monoalkylation products of dG, derived from butadiene monoxide. These also showed only weak mutagenic activity in a bacterial system.[7] The question as to whether or not this type of adduct is mutagenic in mammalian system is still open.

Continuing our research in this area, we elected to attempt to synthesize the isomeric lesions **4ab**, which are known to be formed in modest quantity when either synthetic DNA or dG is exposed to acrolein. Although these adducts have not been isolated from natural DNA that had been treated with acrolein, it seems likely that they should be produced in small amounts. A second point of interest is that probably these isomeric adducts will exist in DNA largely in the cyclic forms rather than as the open-chain isomer **5**. Here no significant energy advantage would be gained by ring-opening because unlike the situation of **1** vs. **3**, little difference could be expected between the energy value for complementary base-pairing of **4ab** and that of **5**. Both forms necessarily will severely compromise the Watson-Crick base-pairing of the dG/dC dyad. Thus, one might speculate that these more minor adducts could be the true mutagenic culprits.

RESULTS AND DISCUSSION

In attempting to introduce lesions **4ab** into oligomeric DNA, we elected to follow a strategy similar to that used[2] for the more common lesion **2**. This required the synthesis of **6** as the key intermediate and initially we had thought to do this by a direct Wittig methylenation of **4ab**, which recently became available in bulk.[8] Nevertheless, the direct 1-alkylation of dG by 4-bromobut-1-ene also appeared to be an attractive route. A review of the literature, however, concerning the alkylation of dG at the 1-position was not encouraging. In a comprehensive paper on the alkylation of purines and purine nucleosides Broom et al.[9] had shown that 2′-deoxyguanosine could be methylated at the 1-position by methyl iodide in DMSO using potassium

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carbonate as the base. However the yield of the purified product was only 21%, although in the more stable guanosine series, it rose to 53%. Alkylation studies[10] with benzyl bromide and NaOH in CF_3CH_2OH gave at best 45% of the 1-benzyl derivative, whereas, in *N*,*N*-dimethylacetamide under neutral conditions, only a 6% yield was obtained.[11] In previous unpublished work, we had examined the use of the easily prepared thallium salt of dG. Although this had reacted smoothly with allyl bromide to give a good yield of the expected 1-substituted product, we now found that 4-bromobut-1-ene failed to give anything but traces of **6**. In a different approach Badet et al.[12] reported that diphenylalkyl sulfonium salts alkylate dG efficiently at the 1-position and in the case of diphenyl methyl sulfonium fluoroborate they claimed a 94% yield of the 1-methyl derivative together with 4% of O^6-methyl-dG. However we failed completely to reproduce the results despite varying the reaction condition markedly. The 1-methylation of 2′-deoxyguanosine[13] and other related purines[14] by Meerwein's acetal $[(CH_3)_2NC(OCH_3)_2]$ is well-documented. However higher homologues of this acetal are not available or are difficult to prepare, thus precluding this reaction as a general approach to 1-alkylation of dG. More encouraging was the direct alkylation of the O^3, O^5-bis-TBDMS derivative (7) of dG. When this compound was treated in tetrahydrofuran (THF) with 4-bromobut-1-ene using tetrabutylammonium hydroxide as the base, the desired 1-alkylated product **6** was obtained (27%) but the dominant compound formed was the isomeric N^2-(3-butenyl)-dG (**8**)(47%). At this point it came to our attention that when the peripherally protected form of dG, namely **9**, was subjected to the Mitsunobu reaction, alkylation at the 1-position became the dominant reaction.[15] This encouraged us to attempt a simple direct alkylation of **9**. Much to our satisfaction, when **9** was heated in THF with cesium carbonate and 4-bromobut-1-ene, the desired product **10a** was obtained in $\sim$94% yield. It should be noted that when a stronger base is used the yield drops significantly. Potassium *t*-butoxide, for example, gave only 16.5% of **10a**. The general and high-yielding nature of the procedure using cesium carbonate in THF for the alkylation of **9** at the 1-position is clearly seen in the results in Table 1. Even in the case of the highly promiscuous benzyl bromide,[11] a 63% yield of the 1-substitution product (**10e**) was obtained. Nevertheless, despite the advantages of the reaction conditions that we have utilized, the real key to the high yields appears to be the amidine protection of the 2-amino group. This seems to suppress alkylation at other positions in the purine nucleosides.

Proof that this alkylation procedure proceeds regioselectively at the 1-position of dG comes from the NMR, IR and UV spectral data. The latter were particularly informative. The graphs and λ_{max} values for **10a** and the known 1-methyl derivative[9,13] **11** were found to be essentially identical (Fig. 1).

In their infrared spectra both **10a** and the 1-methyl derivative **11** show carbonyl absorption bands at 1689 cm^{-1} and 1691 cm^{-1}, respectively,

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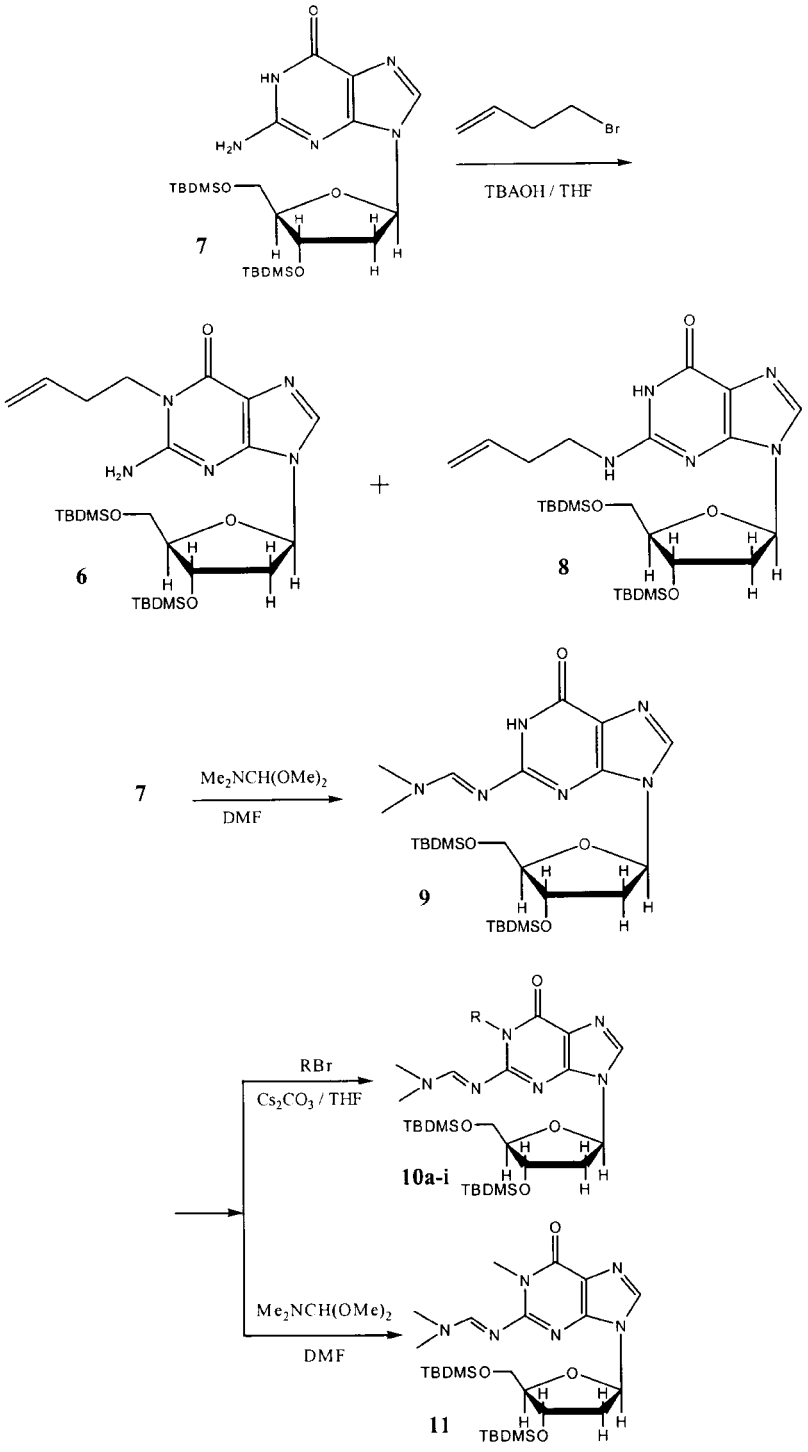

Chart 1.

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Table 1. 1-Alkylation on 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**9**)

Alkylating Agent	% Yield of 1-Alkylated Product (10a–i)
4-Bromo-1-butene (a)	93.7
3-Bromo-1-propene (b)	81.0
1-Bromohexane (c)	92.0
6-Bromo-1-hexene (d)	94.0
Benzyl bromide (e)	63.0
3-Bromopropionaldehyde dimethyl acetal (f)	85.0
5-Bromo-2-methyl-2-pentene (g)	93.0
Phenylethyl Bromide (h)	91.0
2-Bromopentane (i)	75.0

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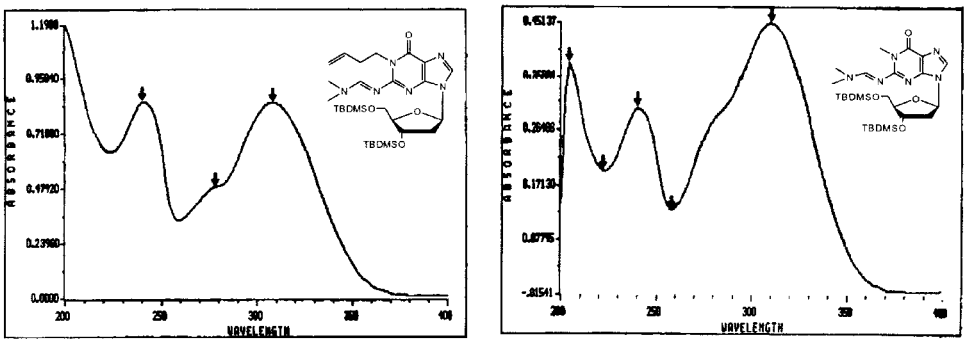

Figure 1. UV spectra of compounds **10a** and **11** in methanol.

whereas the O^6-methyl derivative shows no absorption in this region. ^{1}H NMR studies on **10a** also revealed that the 1-methylene group of the butenyl side chain shows absorption at δ; 4.378 (2H, *t*, J = 7.2 Hz) a position more characteristic of attachment to amide nitrogen, rather than to an oxygen atom.[16]

Further studies on the chemistry of these new derivatives of 2′-deoxyguanosine and the successful use of **10a** for the introduction of **4ab** into oligomeric DNA will be reported in a separate publication.[17]

EXPERIMENTAL PROCEDURES

All reagents and solvents were of commercial grade and were used as such unless otherwise specified. The ^{1}H NMR spectra were recorded on a

Bruker AC-250 or a General Electric QE-300 spectromer. Fast Atom Bombardment (FAB) Mass Spectra were recorded on a MicroMass Trio 2000, and glycerol or nitrobenzene was used as the matrix. Flash column chromatographic separations were carried out on 230–400 mesh silica gel (TSI Chemical Company, Cambridge, MA). The purity of all new products was checked by TLC analysis in two solvent systems (EtOAc:Hexane (2:1)) and (CH_2Cl_2:MeOH (10:1)). In all cases only a single spot was observed. In addition the high resolution mass spectra of these compounds showed no other parent ion except the expected molecular ion.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(but-3-enyl)-2′-deoxyguanosine (6) and 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-N^2-(but-3-enyl)-2′-deoxyguanosine (8). To a suspension of 3′,5′-***O***-bis(tert-butyldimethylsilyl)-2′-deoxyguanosine (**7**) (50 mg, 0.1 mmol) and tetrabutylammonium hydroxide (TBAOH)(40%, 65 μL, 0.11 mmol) in THF (1 mL) was added 4-bromo-1-butene (20 μL, 0.22 mmol). The mixture was stirred and heated at 45°C under nitrogen overnight. The solution was evaporated under reduced pressure and the residue was diluted with CH_2Cl_2 (200 mL). The organic phase was washed with water and brine then dried over anhydrous $MgSO_4$, filtered, evaporated. The resulting material was purified by silica gel chromatography with CH_2Cl_2/MeOH/concentrated ammonia in water (20/1/0.1–10/1/0.1) as the eluent, to afford two products **6** and **8**. Compound (**6**): 15 mg, 27% yield; ^{1}H NMR (DMSO-d_6, 300 MHz) δ; ppm: 0.019 (6H, s, Si-$(CH_3)_2$), 0.090 (6H, s, Si-$(CH_3)_2$), 0.847 (9H, s, C-$(CH_3)_3$), 0.875(9H, s, C-$(CH_3)_3$), 2.230 (1H, m, H_2'), 2.283 (2H, m, C=C-C**H$_2$**-C-N), 2.715 (1H, m, H_2'), 3.677 (2H, m, H_5'), 3.800 (1H, m, H_4'), 4.438 (2H, t, J=6.7 Hz, C=C-C-C**H$_2$**-N), 4.492 (1H, m, H_3'), 5.066 (1H, dd, J = 1.4, 10.2 Hz, **H**C=C-C-C-N), 5.146 (1H, dd, J = 1.8, 16.5 Hz, **H**C=C-C-C-N), 5.852 (1H, m, C=C**H**-C–C-N), 6.183 (1H, t, J = 6.7 Hz, H_1), 6.408 (2H, bs, 2-N**H$_2$**), 8.031 (1H, s, H_8). FAB-MS: m/z 550(M+H^+). Compound (**8**): 26 mg, Y. 46.9%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ; ppm: 0.022 (6H, s, Si-$(CH_3)_2$), 0.088 (6H, s, Si-$(CH_3)_2$), 0.850 (9H, s, C-$(CH_3)_3$), 0.872 (9H, s, C-$(CH_3)_3$), 2.223 (1H, m, H_2'), 2.291 (2H, m, C=C-C**H$_2$**-C-N), 2.644 (1H, m, H_2'), 3.650 (2H, m, H_5'), 3.795 (1H, m, H_4'), 3.996 (2H, dt, J = 0.9, 7.2 Hz, C=C-C-C**H$_2$**-N), 4.464 (1H, m, H_3'), 4.966 (1H, dd, J = 1.0, 10.4 Hz, **H**C=C-C-C-N), 5.008 (1H, dd, J = 1.8, 16.9 Hz, **H**C=C-C-C-N), 5.812 (1H, m, C=C**H**-C-C-N), 6.089 (1H, t, J = 6.9 Hz, $H_{1'}$), 7.087 (H, s, 2-N**H**), 7.886 (1H, s, H_8) 9.869 (1H, s, H_1). FAB-MS: m/z 572(M+Na^+), 550(M+H^+).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (9). To a suspension of previously dried 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine(**7**) (1.980 g, 4 mmol) in anhydrous dimethylformamide (DMF, 20 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (2.66 mL, 20 mmol). The reaction mixture was stirred at room

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temperature until the starting material disappeared (about 3 h, monitored by TLC). The reaction was quenched with water (1 mL), then the solvents were evaporated under reduced pressure to give a white solid, which was chromatographed over silica gel. Elution with CH_2Cl_2/methanol (20/1) gave the desired product **9** as a white powder (2.09 g, 95% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ; ppm: 0.051 (6H, s, $Si(CH_3)_2$), 0.078 (6H, s, $Si(CH_3)_2$), 0.882 (9H, s, C-$(CH_3)_3$), 0.887 (9H, s, C-$(CH_3)_3$), 2.372 (2H, m, H_2'), 3.078 (3H, s, NCH_3), 3.153 (3H, s, NCH_3), 3.740 (2H, d, J = 3.6 Hz, H_5'), 3.943 (**1**H, m, H_4'), 4.545 (1H, m, H_3'), 6.329 (**1**H, t, J = 6.6 Hz, H_1'), 7.842 (1H, s, H_8), 8.585 (1H, s, 2-N=C**H**N), 9.653 (1H, s, H_1). FAB-MS: m/z 551 ($M+H^+$). IR (KBr, λ, cm^{-1}): 1629.75 (T0.86%), 1672.18 (T1.48%); UV (MeOH): λ_{max}, 236, 276 (shoulder), 306; λ_{min}, 218, 255.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(but-3-enyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10a). To a suspension of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**9**)(2.36 g, 4.28 mmol) in THF (80 mL) was added Cs_2CO_3 (3.486 g, 10.7 mmol). The mixture was stirred at room temperature for 10 min, then 4-bromo-1-butene (1.72 mL, 17.14 mmol) was added and the mixture was heated at 70°C under nitrogen overnight. The reaction mixture was evaporated under reduced pressure to remove volatiles and the residue was diluted with CH_2Cl_2 (200 mL). The methylene chloride solution was separated, washed with water, then brine, and finally dried over anhydrous $MgSO_4$, filtered and evaporated. The residual solid was purified by silica gel chromatography (eluent: CH_2Cl_2/MeOH/concentrated ammonia in water: 20/1/0.1–10/1/0.1) to afford desired product as a white solid (2.34 g, 93.7%). MP: 84–85°C; ^{1}H NMR (CD_3OD, 300 MHz) δ; ppm: 0.072 (6H, s, Si-$(CH_3)_2$), 0.102 (6H, s, Si-$(CH_3)_2$), 0.904 (9H, s, C-$(CH_3)_3$), 0.911 (9H, s, C-$(CH_3)_3$), 2.340 (1H, m, H_2'), 2.446 (3H, m, C=C-C**H$_2$**-C-N, H_2'), 3.118 (3H, s, NCH_3), 3.188 (3H, s, NCH_3), 3.750 (2H, d, J = 3.6 Hz, H_5'), 3.962 (1H, dd, J = 3.6, 6.6 Hz, H_4'), 4.378 (2H, t, J = 7.2 Hz, C=C-C-C**H$_2$**-N), 4.564 (1H, m, H_3'), 4.963 (1H, d, J = 10.2 Hz, **H**C=C-C-C-N), 5.024 (1H, dd, J = 1.8, 13.5 Hz, **H**C=C-C-C-N), 5.860 (**1**H, m, C=C**H**-C-C-N), 6.344 (**1**H, t, J = 6.7 Hz, $H_{1'}$), 7.834 (1H, s, H_8), 8.563 (1H, s, 2-N=C**H**N). HRMS (FAB+) (m/e) Calcd for $C_{29}H_{53}N_6O_4Si_2$ $(M+1)^+$ 605.9402; found 605.9404; IR (KBr, λ, cm^{-1}): 1626.86 (T21.85%), 1689.54 (T23.04%); UV (MeOH): λ_{max}, 240(ε = 13,897), 278 (shoulder) (ε = 7720), 308(ε = 13,868); λ_{min}, 222(ε = 10,230), 258(ε = 5404).

Similar methods were used to make the following series of 1-alkylated derivatives of 2′-deoxyguanosine:

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(prop-2-enyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10b) (81% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ; ppm: 0.056 (6H, s, $Si(CH_3)_2$), 0.085 (6H, s, $Si(CH_3)_2$), 0.887 (9H, s, C-$(CH_3)_3$), 0.894 (9H, s, C-$(CH_3)_3$), 2.341 (1H, m, H_2'), 2.408 (1H, m, H_2'), 3.087 (3H, s,

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NC=CNCH$_3$), 3.158 (3H, s, NC=CNCH$_3$), 3.739 (2H, d, J = 3.6 Hz, H$_5$′), 3.946 (**1**H, m, H$_4$′), 4.549 (1H, m, H$_3$′), 4.925 (2H, d, J = 5.7 Hz, C=C-C**H$_2$**-N), 5.049 (1H, dd, J = 1.2, 10.5 Hz, **H**C=C-C-N), 5.108 (1H, dd, J = 1.8, 17.4 Hz, **H**C=C-C-N), 5.886 (1H, m, C=C**H**-C-N), 6.329 (**1**H, t, J = 6.6 Hz, H$_1$′), 7.817 (1H, s, H$_8$), 8.523 (1H, s, 2-N=C**H**N); HRMS(FAB+) (m/e) Calcd for $C_{28}H_{51}N_6O_4Si_2$ $(M+1)^+$ 591.3510; found 591.3508; UV (MeOH): λ_{max}, 208(ε = 19,800), 234(ε = 16,286), 270 (shoulder) (ε = 8768), 304(ε = 18,779); λ_{min}, 224(ε = 15,230), 256(ε = 7734).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-hexyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (10c) (92% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ; ppm: 0.056 (6H, s, Si(CH$_3$)$_2$), 0.087 (6H, s, Si(CH$_3$)$_2$), 0.838 (3H, t, J = 7.2 Hz, C**H**$_3$(CH$_2$)$_6$N), 0.888 (9H, s, C-(CH$_3$)$_3$), 0.896 (9H, s, C-(CH$_3$)$_3$), 1.279 (6H, m, CH$_3$(C**H**$_2$)$_3$(CH$_2$)$_2$N), 1.650 (2H, m, CH$_3$(CH$_2$)$_3$(C**H$_2$**)(CH$_2$)N), 2.325 (1H, m, H$_2$′), 2.418 (1H, m, H$_2$′), 3.093 (3H, s, NC=CNCH$_3$), 3.167 (3H, s, NC=CNCH$_3$), 3.736 (2H, d, J = 3.9 Hz, H$_5$′), 3.945 (1H, m, H$_4$′), 4.268 (2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_4$(C**H$_2$**)N), 4.549 (1H, m, H$_3$′), 6.325 (1H, t, J = 6.6 Hz, H$_1$′), 7.811 (**1**H, s, H$_8$), 8.535 (1H, s, 2-N=C**H**N); HRMS (FAB+) (m/e) Calcd for $C_{31}H_{59}N_6O_4Si_2$ $(M+1)^+$ 635.4136; found 635.4135; UV (MeOH): λ_{max}, 208(ε = 14,714), 240(ε = 14,078), 282 (shoulder) (ε = 10,456), 306(ε = 16,042); λ_{min}, 224(ε = 10,807), 258(ε = 5562).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(hex-5-enyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10d) (yield: 94%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ; ppm: 0.022 (6H, s, Si(CH$_3$)$_2$), 0.051 (6H, s, Si(CH$_3$)$_2$), 0.854 (9H, s, C-(CH$_3$)$_3$), 0.859 (9H, s, C-(CH$_3$)$_3$), 1.385 (2H, m, CH$_2$=CHCH$_2$C**H$_2$** (CH$_2$)$_2$N), 1.646 (2H, m, CH$_2$=CHCH$_2$ CH$_2$C**H$_2$** CH$_2$N),), 2.013 (2H, m, CH$_2$=CHC**H$_2$**CH$_2$CH$_2$CH$_2$N), 2.308 (2H, m, H$_2$′), 3.050 (3H, s, NC=CNCH$_3$), 3.133 (3H, s, NC=CNCH$_3$), 3.705 (2H, d, J = 3.5 Hz, H$_5$′), 3.915 (1H, m, H$_4$′), 4.244 (2H, t, J = 7.25 Hz, CH$_2$=CHCH$_2$CH$_2$CH$_2$C**H$_2$**N), 4.515 (1H, m, H$_3$′), 4.947 (1H, dd, J = 1.6, 10.6 Hz, CH**H**=CH-CHCH$_2$CH$_2$CH$_2$CH$_2$N), 4.903 (1H, dd, J = 1.8, 17.5 Hz, CH**H** = CHCH$_2$CH$_2$CH$_2$CH$_2$N), 5.746 (1H, m, CH$_2$=C**H**CH$_2$CH$_2$CH$_2$CH$_2$N), 6.286 (1H, t, J = 6.6 Hz, H$_1$′), 7.775 (1H, s, H$_8$), 8.496 (1H, s, 2-N=C**H**N); HRMS (FAB+) (m/e) Calcd for $C_{31}H_{57}N_6O_4Si_2$ $(M+1)^+$ 633.3980; found 633.3981; UV (MeOH): λ_{max}, 208(ε = 11,620), 240(ε = 11,500), 280 (shoulder) (ε = 8312), 304(ε = 13,154); λ_{min}, 224(ε = 8886), 258(ε = 4607).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-benzyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (10e) (yield: 63%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ; ppm: 0.035 (6H, s, Si(CH$_3$)$_2$), 0.063 (6H, s, Si(CH$_3$)$_2$), 0.903 (9H, s, C-(CH$_3$)$_3$), 0.949 (9H, s, C-(CH$_3$)$_3$), 2.355 (1H, m, H$_2$′), 2.438 (1H, m, H$_2$′), 3.061 (3H, s, NC=CNC**H**$_3$), 3.127 (3H, s, NC=CNC**H**$_3$), 3.752 (2H, d, J = 3.6 Hz, H$_5$′), 3.959 (1H, m, H$_4$′), 4.564 (1H, m, H$_3$′), 5.540 (2H, m, 1-PhC**H$_2$**-N), 6.337

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(1H, t, J = 6.7 Hz, H_1'), 7.230 (3H, m, $H_{3''}$, $H_{4''}$, $H_{5''}$ in C_6H_5), 7.330 (2H, m, $H_{2''}$, $H_{6''}$ in C_6H_5), 7.856 (1H, s, H_8), 8.491 (1H, s, 2-N=C**H**N); HRMS (FAB+) (m/e) Calcd for $C_{32}H_{53}N_6O_4Si_2$ $(M+1)^+$ 641.3667; found 641.3670; UV (MeOH): λ_{max}, 214(ε = 18,723), 240(ε = 14,572), 282 (shoulder) (ε = 10,407), 304(ε = 16,674); λ_{min}, 228(ε = 12,654), 258(ε = 6472).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(3,3-dimethoxylpropyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10f) (yield: 85%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ; ppm: 0.031 (6H, s, $Si(CH_3)_2$), 0.061 (6H, s, $Si(CH_3)_2$), 0.863 (9H, s, C-$(CH_3)_3$), 0.869 (9H, s, C-$(CH_3)_3$), 1.961 (2H, m, 1-C-C**H$_2$**-C-N, H_2'), 2.327 (2H, m, H_2'), 3.082 (3H, s, NC=CNC**H**$_3$), 3.141 (3H, s, NC=CNC**H**$_3$), 3.278 (6H, s, OC**H**$_3$), 3.711 (2H, d, J = 3.6 Hz, H_5'), 3.920 (1H, m, H_4'), 4.333 (2H, t, J = 7.5 Hz, 1-C-C**H$_2$**-N), 4.479 (2H, t, J = 5.65 Hz, 1-C**H$_2$**C-C-N), 4.525 (1H, m, H_3'), 6.295 (1H, t, J = 6.7 Hz, H_1'), 7.787 (1H, s, H_8), 8.509 (1H, s, **2**-N=C**H**N); HRMS (FAB+) (m/e) Calcd for $C_{30}H_{57}N_6O_6Si_2$ $(M+1)^+$ 653.3878, found 653.3876; UV (MeOH): λ_{max}, 210(ε = 9770), 240 (ε = 10,680), 280 (shoulder) (ε = 7563), 306(ε = 12,187); λ_{min}, 224(ε = 8165), 258(ε = 4323).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(4-methylpent-3-enyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10g) (yield: 93%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 0.018 (6H, s, $Si(CH_3)_2$), 0.048 (6H, s, $Si(CH_3)_2$), 0.850 (9H, s, C-$(CH_3)_3$), 0.856 (9H, s, C-$(CH_3)_3$), 1.534 (3H, s, (CH_3)(C**H**$_3$)-C=CHCH$_2$CH$_2$N), 1.601 (3H, s, (CH_3)(C**H**$_3$)C=CHCH$_2$CH$_2$N), 2.280 (2H, m, $(CH_3)_2$C=CHC**H$_2$**CH$_2$N),), 2.343 (2H, m, H_2'), 3.053 (3H, s, NC=CN-CNC**H**$_3$), 3.129 (3H, s, NC=CNC**H**$_3$), 3.704 (2H, d, J = 3.7 Hz, H_5'), 3.907 (1H, m, H_4'), 4.210 (2H, t, J = 7.75 Hz, $(CH_3)_2$C=CHCH$_2$C**H$_2$**N), 4.513 (1H, m, H_3'), 5.135 (1H, t, J = 14.3 Hz, $(CH_3)_2$C=C**H**CH$_2$CH$_2$N), 6.285 (1H, t, J = 6.6 Hz, H_1'), 7.770 (1H, s, H_8), 8.479 (1H, s, 2-N=C**H**N); HRMS (FAB+) (m/e) Calcd for $C_{31}H_{57}N_6O_4Si_2$ $(M+1)^+$ 633.3980; found 633.3981; UV (MeOH): λ_{max}, 208(ε = 17,690), 240(ε = 15,266), 282 (shoulder) (ε = 11,294), 306(ε = 17,315); λ_{min}, 226(ε = 12,610), 258(ε = 6412).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(2-phenylethyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10h) (yield: 91%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 0.075 (6H, s, $Si(CH_3)_2$), 0.098 (6H, s, $Si(CH_3)_2$), 0.907 (18H, s, C-$(CH_3)_3$), 2.394 (2H, m, H_2'),), 2.971 (2H, t, J = 7.6 Hz, PhC**H$_2$**CH$_2$N), 3.002 (3H, s, NC=CNC**H**$_3$), 3.119 (3H, s, NC=CNC**H**$_3$), 3.753 (2H, d, J = 3.7 Hz, H_5'), 3.959 (1H, m, H_4'), 4.540 (1H, m, H_3'), 4.546 (2H, t, J = 7.5 Hz, PhCH$_2$C**H$_2$**N), 6.334 (1H, t, J = 6.67 Hz, H_1'), 7.196 (5H, m, C$_6$**H**$_5$), 7.840 (1H, s, H_8), 8.290 (1H, s, 2-N=C**H**N); HRMS (FAB+)(m/e) Calcd for $C_{33}H_{55}N_6O_4Si_2$ $(M+1)^+$ 655.3823; found 655.3822; UV (MeOH): λ_{max}, 214(ε = 18,568), 238(ε = 13,888), 282 (shoulder) (ε = 11,139), 304(ε = 16,280); λ_{min}, 230(ε = 12,858), 258(ε = 6418).

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3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(1-methylbutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10i) (yield: 75%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 0.058 (6H, s, $Si(CH_3)_2$), 0.089 (6H, s, $Si(CH_3)_2$), 0.891 (9H, s, C-$(CH_3)_3$), 0.833 (3H, t, J = 7.2 Hz, C**H**$_3$CH$_2$CH$_2$(CH$_3$)CHN), 0.898 (9H, s, C-$(CH_3)_3$), 1.234 (3H, m, CH$_3$CH$_2$CH$_2$(C**H**$_3$)CHN), 1.493 (2H, m, CH$_3$C**H**$_2$CH$_2$(CH$_3$)CHN), 1.772 (1H, m, CH$_3$CH$_2$CH**H**(CH$_3$)CHN),), 2.094 (1H, m, CH$_3$CH$_2$CH**H**(CH$_3$)CHN), 2.355 (2H, m, H_2'), 3.104 (3H, s, NC=CNCH$_3$), 3.162 (3H, s, NC=CNCH$_3$), 3.733 (2H, d, J=3.2 Hz, H_5'), 3.939 (1H, m, H_4'), 4.548 (1H, m, H_3'), 5.574 (1H, m, CH$_3$ CH$_2$CH$_2$(CH$_3$)C**H**N), 6.309 (1H, t, J=6.7 Hz, H_1'), 7.787 (1H, s, H_8), 8.493 (1H, s, 2-N=C**H**N); HRMS (FAB+) (m/e) Calcd for $C_{30}H_{57}N_6O_4Si_2$ $(M+1)^+$ 621.3980; found 621.3981; UV (MeOH): λ_{max}, 210(ε = 12,710), 240(ε = 12,643), 282 (shoulder) (ε = 9186), 306(ε = 13,822); λ_{min}, 224(ε = 9680), 258(ε = 5267).

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3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-methyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (11).[13,14] To a suspension of previously dried 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-N^2-dimethylamino-methylene-2′-deoxyguanosine (110 mg, 0.2 mmol) in anhydrous dimethylformamide (DMF, 1 mL) was added *N,N*-dimethylformamide dimethyl acetal (133 μL, 1 mmol). The reaction mixture was stirred at 70°C overnight, then the reaction was quenched with water (0.1 mL), and the solvents were removed *in vacuum* to give a white solid, which was purified by silica gel. Elution with CH_2Cl_2 and methanol (20:1) afforded **11** as a white powder (53 mg, 47%). MP: 130–132°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 0.073 (6H, s, $Si(CH_3)_2$), 0.103 (6H, s, $Si(CH_3)_2$), 0.905 (9H, s, C-$(CH_3)_3$), 0.912 (9H, s, C-$(CH_3)_3$), 2.347 (1H, m, H_2'), 2.443 (1H, m, H_2'), 3.128 (3H, s, NC=CNCH$_3$), 3.186 (3H, s, NC=CNCH$_3$), 3.643 (3H, s, 1-NCH$_3$), 3.753 (2H, d, J = 3.6 Hz, H_5'), 3.963 (1H, m, H_4'), 4.569 (1H, m, H_3'), 6.350 (1H, t, J=6.6 Hz, H_1'), 7.853 (1H, s, H_8), 8.541 (1H, s, 2-N=C**H**N); FAB-MS: m/z 587 $(M+Na^+)$, 565$(M+H^+)$; IR (KBr, λ, cm^{-1}): 1627.82 (T57.83%), 1691.47(T67.48%); UV (MeOH): λ_{max}, 206(ε = 18,380), 240(ε = 15,954), 282 (shoulder) (ε = 14,382), 308(ε = 21,560); λ_{min}, 224(ε = 12,456), 258(ε = 7257).

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