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Synthesis of the minor acrolein adducts of 2′-deoxyguanosine and their generation in oligomeric DNA☆

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Abstract

Acrolein, a known mutagen, undergoes reaction in vitro under physiological conditions with both 2′-deoxyguanosine and native DNA to give rise to exocyclic adducts of the 5,6,7,8-tetrahydropyrimido[1,2-*a*]purine-10(3*H*)-one class having an hydroxy group at either the 6 or the 8 position. Previously we have shown that the 8-hydroxy derivative in a bacterial system has very low mutagenicity probably because in double-stranded DNA this residue exists in the open-chain aldehydic form [N^2-(3-oxopropyl)-2′-deoxyguanosine] (**3**). To continue our investigation in this area, we needed ample supplies of the 6-hydroxy isomers. This current paper describes high-yield simple methods for the synthesis in bulk of the 6-hydroxy adduct **1** and its incorporation into DNA oligomers. The basic methods for the synthesis of the adduct **1**, involve 1-substitution of dG derivatives with a 3-butenyl group, dihydroxylation of the olefin with osmium tetroxide and *N*-methylmorpholine *N*-oxide, then diol cleavage with periodate ion after incorporation of the 1-(3,4-diacetoxybutyl)-2′-deoxyguanosine into oligomeric DNA.
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Keywords: Acrolein; Mutagenicity; 2′-Deoxyguanosine adducts; 1-Alkylation; Osmium tetroxide; Oligomeric synthesis; Diol cleavage; Xenodeoxynucleotides

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1. Introduction

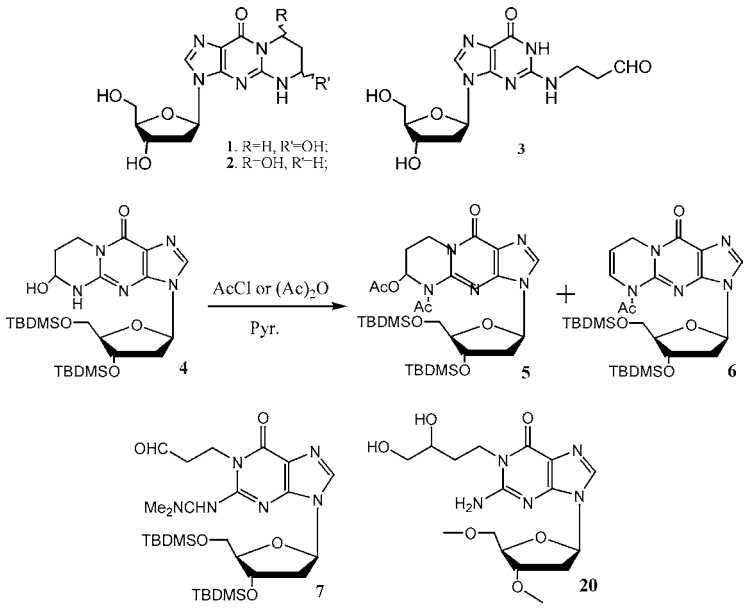
Acrolein is a substance ubiquitous in the human environment [1]. It is regarded as genotoxic and mutagenic because it reacts readily with nucleophilic biological macromolecules. However, evaluation of the possible carcinogenicity of acrolein has been complicated by its irritant properties, toxicity, and especially the difficulty of introducing its nucleoside adducts, site-specifically, into DNA [2].

The major reaction products of acrolein with 2′-deoxyguanosine[1] are adducts, which are formed through a pair of regio-isomeric Michael additions with initial bond formation happening either at the 1 or N^2 position, followed by ring closure to form three exocyclic hydroxy-isomeric adducts. These have been characterized by Chung et al. [3] as the 6- and 8-hydroxy-tetrahydropyrimido-[1,2-*a*]purine-10(3*H*)-one nucleosides adducts **1** and **2** (Scheme 1). Although it has been shown that the 8-hydroxy adduct is formed when DNA is treated acrolein, the 6-hydroxy adducts (**1**) have not been reported [3]. Nevertheless, given the chemical reactivity of acrolein it seems likely that **1** is also generated in DNA in small amounts. Such exocyclic nucleic acid adducts, because they have the potential to disrupt base-pairing in DNA, probably play an important role in mutagenesis and possibly carcinogenesis [4,5]. However, it is difficult to evaluate how any one adduct alters the structure or coding properties of the damaged DNA.

Recently we devised [6] an original strategy for the generation in DNA of alkali-labile adducts derived from α,β-unsaturated aldehydes. This uses an alkali-resistant terminal homo-1,2-diol as a masked form of the aldehyde. The diol is easily protected as its diacetyl derivative during oligomer synthesis but is regenerated during the subsequent ammonia deprotection step. Treatment with periodate then generates the desired aldehyde adduct in essentially quantitative yield together with one equivalent of formaldehyde. From mass special evidence it does not appear that the formaldehyde interferes further with the DNA. This method was applied by our group to the synthesis of several oligomers containing **2** as a nucleotide residue [6] and was later adopted by Nechev et al. [7] for the same purpose and for the generation in DNA of the closely related crotonaldehyde adducts [8]. More recently the method has been applied by Wang and Rizzo [9] to the synthesis of the 1,N^2-deoxyguanosine adducts of the lipid peroxidation product, *trans*-4-hydroxynonenal.

In our own investigations [10] of the structural aspects of the DNA lesion derived from **2**, we found that it exists in both the monomeric nucleoside and in single-stranded DNA as the closed-ring form depicted in **2**, but in double-stranded DNA it exists dominantly, if not exclusively, in the ring-opened form **3** (or its hydrate). A similar observation was made by Marnett and his associates [11] in the case of the exocyclic adduct (M_1G) derived from malondialdehyde and 2′-deoxyguanosine. It was also found [12,13] that this lesion is non-mutagenic in bacterial systems, a finding that is in accord with previous work by Lloyd [14] who demonstrated that the N^2-monoalkyl adducts of 2′-deoxyguanosine derived from butadiene

[1] *Abbreviations used:* dG, 2′-deoxyguanosine.



Scheme 1.

mono-epoxide also were only marginally mutagenic. This may be a general rule in prokaryotic systems for relatively small N^2-alkyl substituents. However in a mammalian system (COS-7 cells) this lesion has been shown to be significantly mutagenic [15]. Nevertheless it is not clear if the mutagenicity of acrolein is entirely due to this adduct (i.e. **2**) or if other adducts are involved.

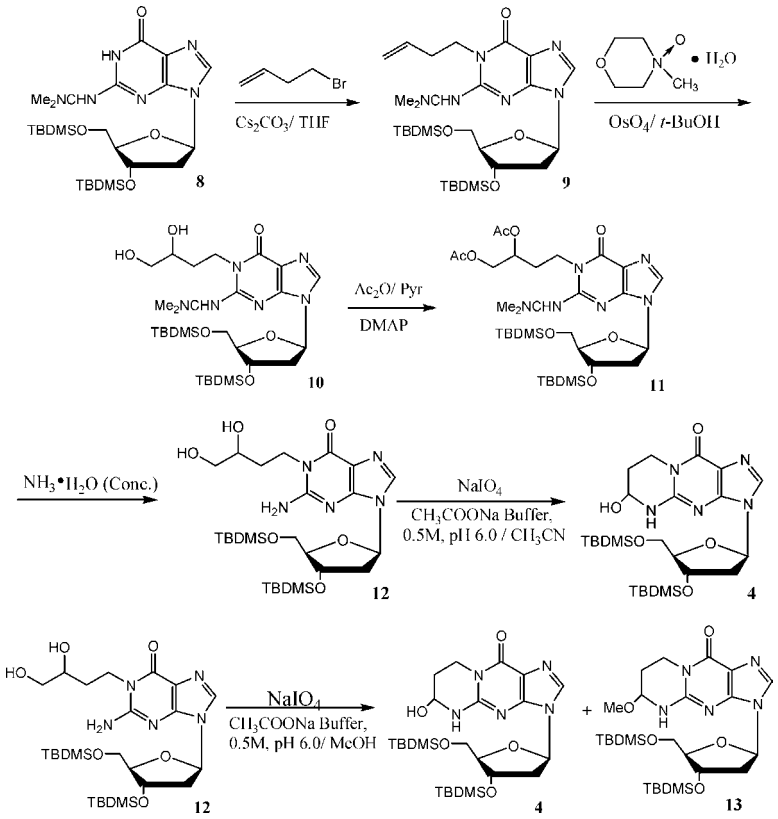
In the light of these uncertainties we decided that it would be important to study the isomeric adduct **1** and its incorporation into DNA oligomers. We therefore turned our attention to preparative studies to try to develop a high yield method that would allow the synthesis of **1** in bulk and thereafter the preparation of homogeneous oligomeric DNA containing **1**, located site-specifically within the linear array.

2. Results and discussion

In a previous publication [16], we described a method for the synthesis of 3-(3′, 5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxy-β-D-erythro-pentofuranosyl)-6-hydroxy-5, 6,7,8-tetrahydropyrimido[1,2-*a*]purine-10(3*H*)-one **(4)** in high yield. It was important to test the stability of compound **4** under the conditions needed for deprotection and release of synthetic oligodeoxynucleotides from the control pore glass (CPG) support [11], since our aim was the site-specific incorporation of this monomer into oligomers. Compound **4** was treated with 28% NH_4OH containing a small amount of MeOH for 20 h at room temperature in sealed condition. The analysis by HPLC

showed that about 13% of the starting material **4** decomposed. After treatment with 80% HOAc for 40 min at room temperature, about 18% of starting material **4** hydrolyzed. It was clear that monomer **4** is not stable to the conditions needed to obtain a completely deprotected oligomer. Attempts to protect the OH group of **4** by acetylation led principally to **6** and only a small amount of the desired **5** (Scheme 1).

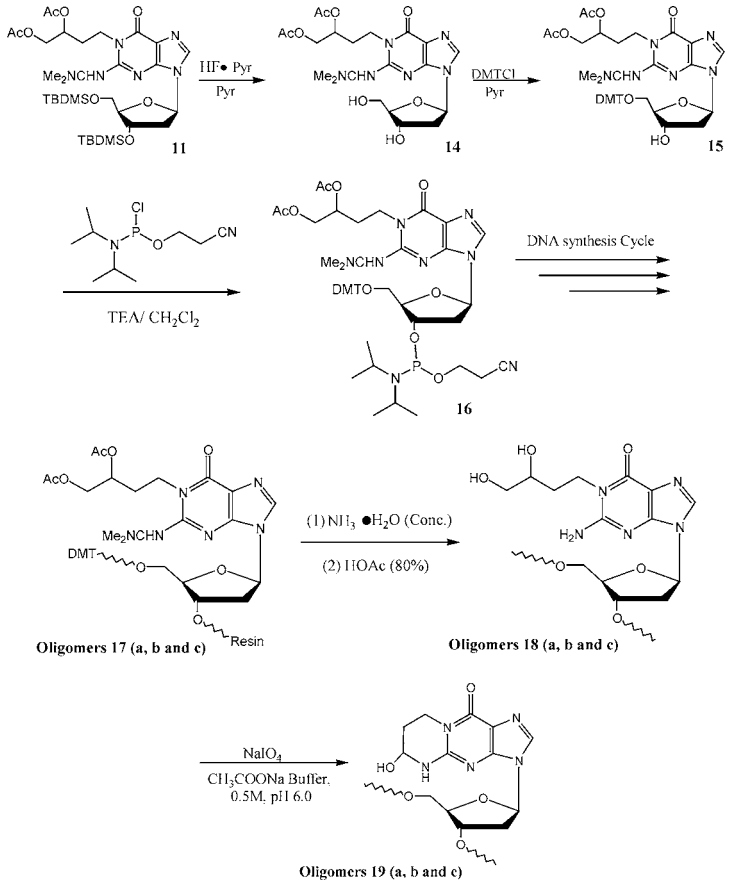
Further work on this approach was abandoned when it was found that a key intermediate **9** could be prepared directly (Scheme 2) and in exceptionally high yield (94%) by the reaction in THF solution of N^2-dimethylaminomethylene-3′, 5′-di-*t*-butyldimethylsilyl-2′-deoxyguanosine (**8**) with 4-bromobutene in the presence of the mild base Cs_2CO_3 [17]. That substitution at the 1-position of the dG derivative had occurred was confirmed chemically. Oxidation of **9** with *N*-methylmorpholine-*N*-oxide in the presence of a catalytic quality of osmium tetroxide at room temperature in *t*-BuOH gave 3′, 5′-*O*-bis(*tert*-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**10**). The free hydroxy groups in diol **10** were then protected by acetylation to give **11** using acetic anhydride in pyridine. The ^{1}H NMR spectrum of **11** showed the presence of two new peaks at δ 1.961 and 1.965 each representing an acetyl group thus substantiating structure (**11**).



Scheme 2.

The diacetyl compound **11** was then treated with methanolic ammonia solution which led to 3′, 5′-*O*-bis(*tert*-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-2′-deoxyguanosine (**12**). When the latter was oxidized by sodium periodate in aqueous CH_3CN, the known [16] cyclic 6-hydroxy derivative **4** was obtained. When the oxidation was run in aqueous MeOH, two products namely cyclic 6-hydroxy and 6-methoxy acrolein adducts of dG (**4** and **13**) were formed as expected. Both adducts showed ^{1}H NMR, mass and UV spectra identical with previously synthesized [16] samples. In addition their HPLC behavior was identical.

The DMT-phosphoramidite **16** (Scheme 3), the building block required for the introduction of the nucleoside lesion into DNA, was then synthesized as follows from



Oligomers: X=modified dG

a. 7-mer: 5'-T-T-C-X-A-T-T-3'

b. 13-mer: 5'-C-T-C-C-T-C-X-A-T-A-C-C-T-3'

c. 28-mer: 5'-C-T-G-C-T-C-C-T-C-X-A-T-A-C-C-T-A-C-A-C-G-C-T-A-G-A-A-C-3'

Scheme 3.

the diacetyl compound **11**. Selective removal of the silyl protecting groups to give compound (**14**) was accomplished with HF in excess pyridine, a reaction that leaves intact both the nucleoside linkage and the N^2-dimethylaminomethylene moiety. The conversion of compound **14** into the corresponding 5′-*O*-dimethoxytrityl derivative was then achieved by treatment with 4, 4′-dimethoxytrityl chloride in pyridine under standard conditions. The resulting 5′-*O*-(4,4′-dimethoxytrityl-1-(3,4-diacetylbutyl))-N^2-dimethylaminomethylene-2′-deoxyguanosine (**15**) was then allowed to react with 3.3 eq of 2-cyanoethyl diisopropylchlorophosphoramidite in the presence of 6 eq of triethylamine to obtain the required product **16**, whose structure was confirmed by mass spectral, ^{1}H NMR and ^{31}P NMR data. Having shown that the cyclic acrolein adducts can be formed by cleavage of the precursor the diol under conditions mentioned above, we now directed our attention to the generation of lesion **14** in DNA.

The experimental heptamer **17a** [5′-TTCXATT-3′, where X = 1-(3,4-diacetoxybufyl)-N^2-(dimethylaminomethylene) dG] was synthesized using standard phosphoramidite chemistry on an Applied Biosystems 394 synthesizer (Foster City, CA). The coupling time allowed for the modified base was 5 min which gave a coupling efficiency of 99%. The oligomer was treated with 28% NH_4OH at 55 °C overnight to cleave it from the (CPG) support and to remove the protecting groups. A Waters HPLC system (Milford, MA) consisting of a 600 E Multisolvent Delivery System, a U6K Injector, and a 996 Photodiode Array Detector was used to purify the modified oligomer. The dimethoxytrityl capped (DMT-on) oligonucleotide was purified on a Luna 5 μ Phenyl-Hexyl (250 × 10 mm) column (Phenomenex, Torrance, CA) run at 4 mL/min. A gradient of 16–36% acetonitrile (35 min) in 0.1 M TEAA buffer, pH 6.8, was used to collect the DMT-on oligomer. The DMT group was removed with 80% HOAc, and the pure DMT-off product (**18a**) was obtained using a gradient of 0–20% acetonitrile over 40 min. ESI-MS was used to confirm the mass of both, the DMT-on and the DMT-off oligomers. The observed masses were 2476.5 ± 0.9 and 2173.3 ± 0.4 Da, respectively (the corresponding calculated masses are 2476.4 and 2174.4 Da). Heptamer **18a** (5′-TTCXATT-3′ where X is the 1-(3,4-dihydroxybutyl) dG moiety (**20**), was treated with 100 mM sodium periodate in 0.5 M sodium acetate buffer, pH 6.0, for 5 min at room temperature [6] to generate the 6-hydroxy-1,N^2-propanodeoxyguanosine (6-OH-PdG) moiety. A final HPLC purification (Fig. 1a) was carried out under the same conditions described above for the DMT-off oligodeoxynucleotides. Two partially resolved peaks could be seen corresponding to the two possible diastereomers (6-hydroxy isomers). The peaks were collected as a single fraction and the mass determined by electrospray ionization mass spectrometry (ESI-MS). The observed mass for oligodeoxynucleotide (**19a**) was 2142.2 ± 0.1 (Fig. 1b, the calculated *m*/*z* is 2142.40 Da). In a separate experiment, each of the fractions corresponding to the two observed peaks was collected separately and dried under vacuum. Each fraction was redissolved in water and subjected to an additional HPLC pass. Two peaks were again observed for each fraction, indicating an active equilibrium between the two isomeric oligodeoxynucleotides.

The presence of the intact 6-hydroxy isomer (**1**) was confirmed by enzymatic analysis of the oligodeoxynucleotide **19a** using nuclease P_1 followed by treatment with snake venom phosphodiesterase, and bacterial alkaline phosphatase [18]. Subsequent

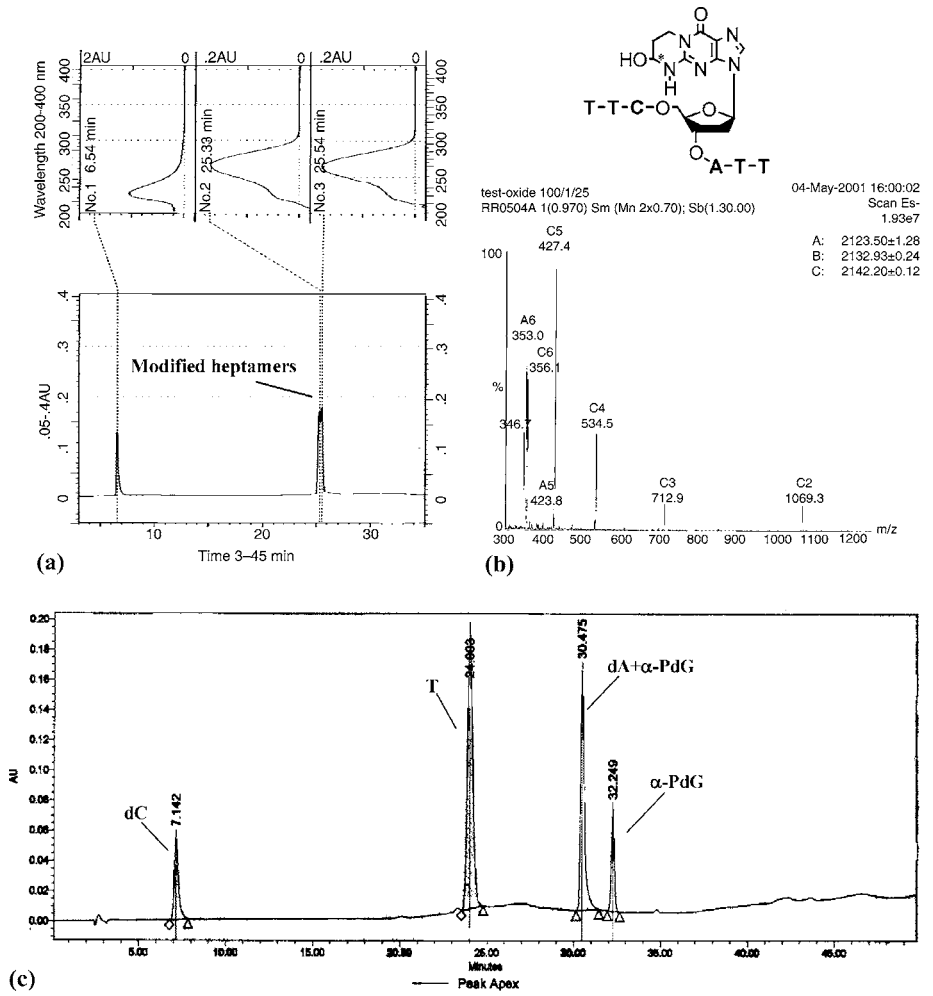


Fig. 1. Data related to the incorporation of 6-hydroxy-1,N^2-propanodeoxyguanosine (6-OH-PdG) into oligomeric DNA under normal DNA synthesizer condition: (a) the HPLC of the post-synthetically modified heptamers after oxidation with $NaIO_4$ and their UV spectra; (b) the electrospray mass spectrum of the purified heptamers; (c) the HPLC of the enzymatic digestion products of the site-specific modified heptamers.

HPLC analysis of the resulting nucleoside mixture and comparison with a prepared standard of 6-hydroxy isomer **1** [16] corroborated the structure of the desired oligomer. The 6-hydroxy isomer **1** gives two peaks, corresponding to the two possible diastereomers, one of which elutes at almost the same retention time as dA under the HPLC conditions used (Fig. 1c).

Modified nucleoside **14** was then introduced into a series of DNA oligomers. By way of examples, the 13-mer (**17b**) (5′-CTCCTC-**X**-ATACCT-3′) and the 28-mer

(**17c**) (5′-CTGCTCCTC-X-ATACCTACACGCTAGAAC-3′) were synthesized and purified as described for the heptamer above. When the purified oligomers (**18a** and **b**) containing the 1-(3,4-dihydroxybutyl) dG moiety were treated with an excess of sodium periodate in aqueous solution (0.1 M in CH_3COONa buffer, pH 6.8), until no starting material remained (15 min), they afforded the corresponding single-stranded DNA oligomers (**19a** and **b**) containing the desired adduct **1**. After a further HPLC purification (DMT-off conditions) both oligomers were characterized by ESI-MS. Observed m/z values for the **19a** and **19b**, respectively, were 3900.2 ± 0.7 (calcd. 3901.5) and 8508.0 ± 1.3 Da (calcd. 8509.6).

In conclusion, we have developed a synthetic method that allows the generation of the alkali-sensitive acrolein adduct **1** in DNA. We believe that this methodology has the general potential to allow the synthesis of DNA containing other alkali-sensitive lesions. Work is now in progress to determine the mutational spectrum of adduct **1** in both bacterial and mammalian cell systems, and to determine the effect of this lesion on DNA structure, using NMR methods.

After this work was completed, a publication by Nechev et al. [19] appeared describing a similar but not identical approach for the synthesis of DNA oligomers containing the 6-hydroxy acrolein adduct **1**. However our method has the advantage of using an alkylation procedure at the 1-position of 2′-deoxyguanosine that gives the desired products in high yield and in an excellent state of purity.

3. Experimental procedures

All reagents and solvents were of commercial grade and were used as such unless otherwise specified. The ^1H NMR spectra were recorded on a Bruker AC-250 or a General Electric QE-300 spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a micromass Trio 2000, glycerol or nitrobenzyl alcohol, being used as the matrix. Electrospray ionization mass spectra were obtained from a micromass Quattro LC. Flash column chromatographic separations were carried out on 230–400 mesh silica gel (TSI Chemical Company, Cambridge, MA). The purities of all new products were confirmed by TLC analysis in two solvent systems (EtOAc:hexane) and (CH_2Cl_2:MeOH). In all cases only a single spot was observed.

*3.1. 3′,5′-O-Bis(tert-butyldimethylsilyl)-1-(but-3-enyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**9**)*

To a suspension of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (2.205 g, 4 mmol) in THF (75 mL) was added Cs_2CO_3 (3.258 g, 10 mmol), and the mixture was stirred at room temperature for 10 min. 4-Bromo-1-butene (1.6 mL, 16 mmol) was then added and the mixture was heated at 70 °C under a nitrogen atmosphere overnight. The reaction mixture was then

evaporated under reduced pressure to remove solvent, and the residue was stirred with CH_2Cl_2 (200 mL). The organic extract was then washed with water and brine, dried with anhydrous Na_2SO_4, filtered, and evaporated. The residual material was purified by silica gel chromatography (CH_2Cl_2/MeOH/concentrated ammonia in water: 20/1/0.1–10/1/0.1) to afford **9** as a white solid (2.194 g, 94%). 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 (1H, m, C=C**H**–C–C–N), 6.344 (1H, t, $J = 6.7$ Hz, H'_1), 7.834 (1H, s, H_8), 8.563 (1H, s, 2-N=C**H**N); FAB-MS: m/z 627 ($M + Na^+$), 605 ($M + H^+$).

3.2. 3′,5′-O-Bis(tert-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**10**)

To a solution of osmium tetroxide (OsO_4, 2 mg) in 0.3 mL *tert*-butanol was added *N*-methylmorpholine *N*-oxide (1.38 mL, 50% in water, 6.66 mmol), water (280 μL), and acetone (1.7 mL), and the mixture was stirred at room temperature for 10 min. 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-1-(but-3-enyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (1.815 g, 3 mmol) was then dissolved in THF (4.5 mL) and the solution was cooled in an ice bath. The two solutions were mixed together and cooling was continued for a further 15 min, then the ice bath was removed and the reaction mixture was allowed to stand at room temperature for 20 h. The mixture was treated with 3 mL of 20% Na_2SO_3 and concentrated at reduced pressure.

The resulting residue was diluted with 50 mL ethyl acetate and the organic extract was washed with saturated $NaHCO_3$ solution, then water and brine, and finally dried over anhydrous Na_2SO_4. Concentration in vacuo afforded the desired product **10** as a white solid (1.815 g, 94.6%). MP: 90–92 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ, ppm: 0.067 (6H, s, Si–$(CH_3)_2$), 0.093 (6H, s, Si–$(CH_3)_2$), 0.895 (9H, s, C–$(CH_3)_3$), 0.900 (9H, s, C–$(CH_3)_3$), 1.676 (1H, m, 1-C–C–C**H**–C–N), 1.939 (1H, m, 1-C–C–C**H**–C–N), 2.380 (2H, m, H'_2), 3.118 (3H, s, NCH_3), 3.203 (3H, d, $J = 1.2$ Hz, NCH_3), 3.488 (3H, m, O–C**H**$_2$–C–C–C–N, O–C–C(O)**H**–C–C–N), 3.750 (2H, d, $J = 3.6$ Hz, H'_5), 3.956 (1H, dd, $J = 3.4$, 6.6 Hz, H'_4), 4.366 (H, m, C–C–C–C**H**–N), 4.563 (2H, m, H'_3, C–C–C–C**H**–N), 6.323 (1H, t, $J = 6.6$ Hz,$H_{1'}$), 7.878 (1H, s, H_8), 8.576 (1H, s, 2-N=C**H**N); FAB-MS: m/z 661.3 ($M + Na^+$), 639.3 ($M + H^+$).

3.3. 3′,5′-O-Bis(tert-butyldimethylsilyl)-1-(3,4-diacetoxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**11**)

To a solution of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**10**) (3.2 g, 5 mmol) and a catalytic amount of dimethylaminopyridine (DMAP) in pyridine (70 mL) was added acetic anhydride (Ac_2O, 3 mL, 29.5 mmol). The reaction mixture was stirred at room

temperature for 15 h, then poured over ice to produce a yellow solid. The solid was filtered and washed with water to obtain the desired product **11** as a yellow powder. This was dried under vacuum overnight (3.37 g, 93%). ^{1}H NMR ($CDC1_3$, 250 MHz) δ, ppm: 0.137 (12H, s, $Si(CH_3)_2$), 0.933 (18H, s, C–$(CH_3)_3$), 1.961 (3H, s, CH_3COO–), 1.965 (2H, m, 1-C–C–C**H$_2$**–C–N), 2.004 (3H, s, CH_3COO–), 2.346 (H, m, H'_2), 2.707 (H, m, H''_2), 3.129 (3H, s, NCH_3), 3.221 (3H, s, NCH_3), 3.717 (2H, m, H'_5), 3.962 (1H, m, H'_4), 4.070 (1H, m, O–C–C(O)**H**–C–C–N), 4.261 (1H, m, O–C–C–C–C**H**–N), 4.307 (2H, m, O–C**H$_2$**–C–C–C–N), 4.653 (1H, m, $H_{3'}$), 5.078 (1H, m, O–C–C–C–C**H**–N), 6.306 (1H, t, $J = 6.8$ Hz, $H_{1'}$), 8.020 (1H, s, H_8), 8.539 (1H, s, 2-N=C**H**N); FAB-MS: m/z 723.3 ($M + H^+$).

3.4. 1-(3,4-Diacetoxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**14**)

To a solution of 3′, 5′-*O*-bis(*tert*-butyldimethylsilyl)-1-(3,4-diacetoxybutyl)- N^2-dimethylaminomethylene-2′-deoxyguanosine (**11**, 1.464 g, 2 mmol) in pyridine (12 mL) cooled in an ice bath was added a solution (70%) of HF/pyridine (1.2 mL) and the ice bath was then withdrawn. The mixture was stirred at room temperature for 3 h, then was neutralized with $NaHCO_3$. An air stream was then used to remove the solvent. The residual solid was then purified by direct application to a silica gel column. Elution with CH_2Cl_2/MeOH (10/1) gave the product **14** as a fluffy yellow foam (0.810 g, 82%). ^{1}H NMR (CD_3OD, 250 MHz) δ, ppm: 1.905 (3H, s, CH_3COO–), 1.960 (3H, s, CH_3COO–), 2.032 (2H, m, 1-C–C–C**H$_2$**–C–N), 2.388 (H, m,H'_2), 2.726 (H, m, H''_2), 3.161 (3H, s, NCH_3), 3.235 (3H, s, NCH_3), 3.737 (2H, m, H'_5), 3.993 (1H, m, H'_4), 4.065 (1H, m, O–C–C(O)**H**–C–C–N), 4.278 (1H, m, O–C–C–C–C**H**–N), 4.360 (2H, m, O–C**H$_2$**–C–C–C–N), 4.546 (1H, m, $H_{3'}$), 5.115 (1H, m, O–C–C–C–C**H**–N), 6.346 (1H, t, $J = 6.8$ Hz, $H_{1'}$), 8.044 (1H, s, H_8), 8.600 (1H, s, 2-N=C**H**N); FAB-MS: m/z 495.2 ($M + H^+$).

3.5. 5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-1-(3,4-diacetoxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**15**)

1-(3,4-Diacetoxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**14**) (494 mg, 1 mmol) was co-evaporated with dry toluene (3×4 mL) and pyridine (3×4 mL). The anhydrous residue was then redissolved in pyridine (15 mL) and 4,4′-dimethoxytritylchloride (DMT-Cl, 407 mg, 1.2 mmol) was added. The mixture was stirred at room temperature for 1 h under nitrogen and then quenched with 0.75 mL MeOH, and the volatiles were removed under reduced pressure. Methylene chloride (50 mL) was then added to the residue and the organic extraction was washed with saturated $NaHCO_3$ solution then water, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/TEA: 25/1/1%) to obtain the desired product **15** (600 mg, 75%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 250 MHz) δ, ppm: 1.961 (2H, m, 1-C–C–C**H$_2$**–C–N), 1.965 (3H, s, CH_3COO–), 1.985 (3H, s, CH_3COO–), 2.535 (2H, m, H'_2), 3.037 (3H, s, NCH_3), 3.069 (3H, s, NCH_3), 3.294 (2H, m, H'_5), 3.543 (1H, m, H'_4),

3.572 (6H, s, CH_3OAr), 4.023 (1H, m, O–C–C(O)**H**–C–C–N), 4.197 (2H, m, O–**CH_2**–C–C–C–N), 4.322 (1H, m, O–C–C–C–C**H**–N), 4.601 (1H, m, $H_{3'}$), 5.600 (1H, m, O–C–C–C–C**H**–N), 6.364 (1H, t, $J = 6.8$ Hz, $H_{1'}$), 6.728 (4H, d, $J = 8.7$ Hz, ArH), 7.183 (8H, m, ArH), 7.348 (1 H, d, $J = 7.3$ Hz, ArH), 7.664 (1H, s, H_8), 8.550 (1H, s, 2-N=C**H**N); FAB-MS: m/z 797 ($M + H^+$).

*3.6. 3′-O-[N,N-Diisopropylamino-(2-cyanoethoxy)phosphinyl]-5′-O-(4,4′-dimethoxytriphenylmethyl)-1-(3,4-diacetoxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**16**)*

5′-*O*-(4,4′-Dimethoxy-triphenylmethyl)-1-(3,4-diacetoxybutyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**15**) (398 mg, 0.5 mmol) was co-evaporated with dry toluene (3×4 mL) and pyridine (3×4 mL). The residue was re-dissolved in dry CH_2Cl_2 (10 mL) and treated with triethylamine (0.56 mL) and in one portion 2-cyanoethyl diisopropylchlorophosphoramidite (208 μL). The reaction mixture was stirred at room temperature for 2 h under nitrogen and then diluted with CH_2Cl_2 (40 mL). The resulting solution was washed with saturated $NaHCO_3$ solution then water, dried (Na_2SO_4), and concentrated in vacuo to give the desired product **16** (423 mg, 85%). ^{1}H NMR ($CDC1_3$, 250 MHz) δ ^{1}H NMR ($CDC1_3$, 250 MHz) δ, ppm: 1.205 (12H, m, N(C(C**H_3**$)_2)_2$), 1.981 (2H, m, 1-C–C–C**H_2**–C–N), 2.004 (3H, s, C**H_3**COO–), 2.017 (3H, s, C**H_3**COO–), 2.478 (2H, m, H'_2), 2.592 (2H, m, C**H_2**CN), 3.105 (3H, s, NC**H_3**), 3.141 (3H, s, NC**H_3**), 3.303 (2H, m, H'_5), 3.659 (5H, m, N(C**H** $(CH_3)_2)_2$, **H'_4**), P–O–C**H_2**CCN), 3.752 (6H, s, C**H_3**OAr), 4.067 (1H, m, O–C–C(O)**H**–C–C–N), 4.252 (2H, m, O–C**H_2**–C–C–C–N), 4.379 (1H, m, O–C–C–C–C**H**–N), 4.669 (1H, m, **$H_{3'}$**), 5.267 (1H, m, O–C–C–C–C**H**–N), 6.336 (1H, t, $J = 6.8$ Hz,$H_{1'}$), 6.771 (4H, m, Ar**H**), 7.197 (8H, m, Ar**H**), 7.361 (1H, d, $J = 7.3$ Hz, Ar**H**), 7.691 (1H, s, **H_8**), 8.534 (1H, s, 2-N=C**H**N); ^{31}P NMR ($CDC1_3$, 250 MHz) δ, ppm: 149.952, 150.070; FAB-MS: m/z 997.5 ($M + H^+$).

*3.7. 3′,5′-O-Bis(tert-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-2′-deoxyguanosine (**12**)*

A mixture of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-1- (3,4-diacetoxybutyl)-N^2-dimethylaminomethylene-2′- deoxyguanosine (**11**) (73 mg, 100 μmol) and 2 mL 20% methanolic ammonia was sealed in a flask and heated at 50 °C overnight. The reaction mixture was evaporated under reduced pressure, and the residue was purified by chromatography to give the desired product **12** as a yellow solid (54 mg, 93%). ^{1}H NMR (CD_3OD, 300 MHz) δ, ppm: 0.077 (6H, s, Si–$(CH_3)_2$), 0.095 (6H, s, Si–$(CH_3)_2$), 0.905 (9H, s, C–$(CH_3)_3$), 0.913(9H, s, C–$(CH_3)_3$), 1.686 (1H, m, 1-C–C–C**H**–C–N), 1.924 (1H, m, 1-C–C–C**H**–C–N), 2.389 (2H, m, H'_2), 3.491 (3H, m, O–C**H_2**–C–C–C–N, O–C–C(O)**H**–C–C–N), 3.761 (2H, d, $J = 3.6$ Hz, H'_5), 3.983 (1H, dd, $J = 3.4$, 6.6 Hz, H'_4), 4.373 (H, m, C–C–C–C**H**–N), 4.559 (2H, m, H'_3, C–C–C–C**H**–N), 6.321 (1H, t, $J = 6.6$ Hz, $H_{1'}$), 7.876 (1H, s, H_8), 8.581 (1H, s, 2-N=C**H**N); FAB-MS: m/z 606.2 ($M + Na^+$), 584.2 ($M + H^+$).

3.8. 3-(3′,5′-O-Bis(tert-butyldimethylsilyl)-2′-deoxy-β-D-erythro-pentofuranosyl)-6-hydroxy-5,6,7,8-tetrahydropyrimido[1,2- a]purine-10(3H)-one (**4**) and 3-(3′,5′-O-bis(tert-butyldimethylsilyl)-2′-deoxy-β-D-erythro-pentofuranosyl)-6-methoxy-5,6,7,8-tetrahydropyrimido-[1,2-a]purine-10(3H)-one (**13**)

To a solution of 3′, 5′-*O*-bis(*tert*-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-2′-deoxyguanosine (**12**) (20 mg, 0.034 mmol) in MeOH (1.2 mL) was added a solution of $NaIO_4$ (500 μL, 0.1 M) in water. The reaction mixture was shaken at room temperature for 1 h and then diluted with CH_2Cl_2 (10 mL). The organic layer was separated, washed with water (5 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH: 25/1) to afford the products **4** (5 mg, 26.7%) and **13** (10 mg, 52%). Compound (**4**): ^{1}H NMR (DMSO-d_6, 300 MHz) δ, ppm: 0.02 (6H, s, Si–$(CH_3)_2$), 0.08 (6H, s, C–$(CH_3)_3$), 0.92 (18H, s, Si–$(CH_3)_2$), 1.733 (H, m, H_{7a}), 1.950 (H, m, H_{7b}), 2.215 (1H, m, $H_{2'}$a), 2.633 (1H, m,$H_{2'b}$), 3.455 (H, m, H_{8a}), 3.651 (2H, m, $H_{5'}$) 3.793 (H, m, $H_{4'}$), 4.408 (H, m, H_{8b}), 4.470 (H, m,$H_{3'}$), 4.951 (H, m, H_6), 5.927 (H, dd, $J = 1.5$, 4.8 Hz, disappears after D_2O is added, 6-OH), 6.096 (H, m, $H_{1'}$), 7.888 (H, S, H_2), 8.410 (H, m, H_5). FAB-MS: m/z 574 ($M + Na^+$), 552 ($M + H^+$); compound (**13**): ^{1}H NMR (DMSO-d_6, 300 MHz) δ, ppm: 0.024 (6H, s, Si–$(CH_3)_2$), 0.089 (6H, s, C–$(CH_3)_3$), 0.852 (18H, s, Si–$(CH_3)_2$), 1.743 (H, m, H_{7a}), 2.116 (H, m, H_{7b}), 2.236 (1H, m, $H_{2'a}$), 2.635 (1H, m, $H_{2'b}$), 3.291 (3H, m, OCH_3), 3.306 (1H, m, H_{8a}), 3.653 (2H, m, $H_{5'}$), 3.801 (H, m, $H_{4'}$), 4.470 (H, m, H_{8b}), 4.486 (H, m, $H_{3'}$, 4.496 (H, m, H_6), 6.111 (H, m, $H_{1'}$), 7.923 (H, S, H_2), 8.824 (H, d, $J = 3.9$ Hz, NH_5). FAB-MS: m/z 566 ($M + H^+$). The compounds **4** and **13** were identical in spectroscopic data with the previously prepared sample [16].

3.9. 3-(3′,5′-O-Bis(tert-butyldimethylsilyl)-2′-deoxy-β-D-erythro-pentofuranosyl)-6-hydroxy-5,6,7,8-tetrahydropyrimido-[1,2-a]purine-10(3H)-one (**4**)

To a solution of 3′, 5′-*O*-bis(*tert*-butyldimethylsilyl)-1-(3,4-dihydroxybutyl)-2′-deoxyguanosine (**12**) (20 mg, 0.034 mmol) in CH_3CN (1.2 mL) was added a solution of $NaIO_4$ (500 μL, 0.1 M) in water. The reaction mixture was shaken at room temperature for 1 h and then CH_2Cl_2 (10 mL) was added. The organic layer was separated, washed with water (5 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH: 25/1) to afford product **4** (15.3 mg, 82%).

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