Synthesis of Oligonucleotides Containing 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)guanine and 8-Amino-2'-deoxyguanosine

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The synthesis of oligonucleotides containing 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine and 8-amino-2'-deoxyguanosine was accomplished. The viable intermediate N^2-isobutyryl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (**6**) was prepared *via* a four step deoxygenation procedure from 7-β-D-ribofuranosylguanine (**1**). The 5'-hydroxyl group of **6** was protected as 4,4'-dimethoxytrityl ether and then converted to the target phosphoramidite (**8**) *via* conventional phosphitylation procedure. The amino groups of 8-amino-2'-deoxyguanosine (**9**) were protected in the form of *N*-(dimethylamino)methylene functions to give the protected nucleoside **10**, which was subsequently converted to the target phosphoramidite **12** *via* dimethoxytritylation followed by phosphitylation. The phosphoramidites **8** and **12** were incorporated into a 26-mer and a 31-mer G-rich oligonucleotide using solid-support, phosphoramidite methodology. Analysis of antiparallel triplex formation by the oligonucleotides containing 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine in place of 2'-deoxyguanosine showed no enhancement in triple helix formation.

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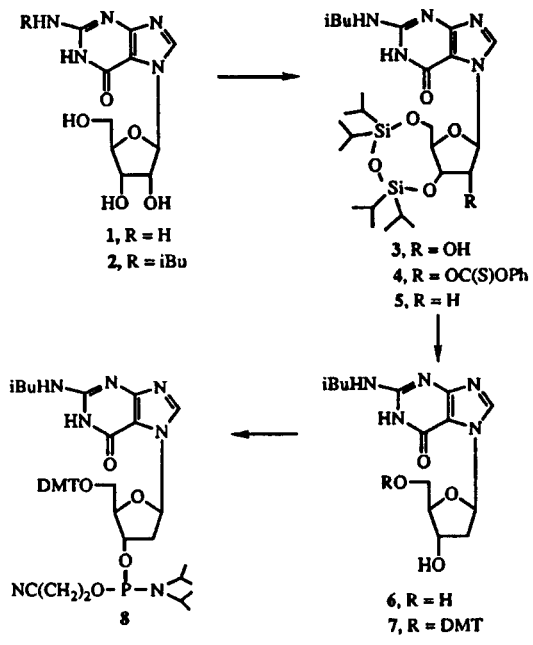
Although the existence of triple helical structures is well documented [1-3], triple helix formation is a relatively new concept as the basis for a program of pharmaceutical intervention. Recently it has been demonstrated that in the presence of divalent cations, certain short guanine-rich oligonucleotides can bind to specific sites in duplex DNA to form triple helices [4-6] at physiological *p*H. It has also been shown that the formation of such sequence specific triple helices can inhibit DNA replication [7,8], and block transcription initiation [4], thus resulting in the specific inhibition of the synthesis of target proteins. Therefore, the potential therapeutic significance of these triple helix forming oligonucleotides (TFOs) is obvious.

The major goal of TFO design is to develop molecules which can bind to any duplex DNA sequence, without regard for purine content or other symmetry considerations. Hydrogen-bonding of the Hoogsteen or reverse Hoogsteen type normally occurs with purine bases in the major groove of an underlying duplex. Consequently, a polypurine/polypyrimidine duplex target presents an orderly array of bond formers positioned on one side of the major helix groove. However, at sites of CG or TA inversion, the corresponding purine target base is placed on the opposing half of the major groove and can be reached for the purposes of hydrogen-bonding only a) upon extension of the TFO backbone, b) by distension of the duplex, or c) both. If triplex formation could be made stable at sites of CG or TA inversion, TFOs could be designed against any duplex site, rather than just at purine rich targets. Another potential problem associated with the guanine-rich TFOs is self-association leading to stable G-tetrads [9-12] by the formation of eight *H*-bonds and by coordination of the four O^6 atoms of guanine with alkali metal ions bound to the center of the quadruplex. Chemical modification of the guanine moiety that disrupts the multiple hydrogen bonding of the G-tetrad can inhibit or eliminate its formation [13]. Therefore, when considering the potential biological utility of TFOs, it is rather important to examine nucleoside modifications or other nucleoside congeners which would allow for sequence specific triplex formation at physiological *p*H. Currently we are directing our attention to solving the problems associated with G-tetrad formation and also to improving binding at CG or TA inversion sites either by modifying the base moiety of the nucleoside [13-16] or by backbone [17,18] modification. In continuation of these efforts we now report the synthesis of properly protected 7-(2-deoxy-β-D-ribosyl)guanine and 8-amino-2'-deoxyguanosine phosphoramidites as synthons suitable for incorporation into oligonucleotides employing solid-support, phosphoramidite chemistry.

Direct glycosylation [19] of the trimethylsilyl derivative of N^2-acetylguanine in anhydrous 1,2-dichloroethane in the presence of tin(IV) chloride afforded mainly the *N*7-glycosylated product, which on treatment with methanolic ammonia gave 7-β-D-ribofuranosylguanine (**1**). Compound **1** was converted to the corresponding 2'-deoxy derivative *via* reductive deoxygenation procedure [20] (Scheme 1). Since it is necessary to protect the exocyclic amino functionality of **1** during the preparation of the target building block, in order to avoid any side reactions during the synthesis of oligonucleotides, we decided to carry out this step prior to 2'-deoxygenation. Thus, 7-β-D-ribofuranosylguanine (**1**) was converted to the corresponding N^2-isobutyryl derivative *via* transient protection methodology [21]. Trimethylsilylation of **1** with chlorotrimethylsilane in anhydrous pyridine, followed by the treatment with 1.2 molar equivalents of isobutyryl chloride and removal of the silyl protecting groups with methanol gave N^2-isobutyryl-7-β-D-ribofuranosylguanine (**2**) in

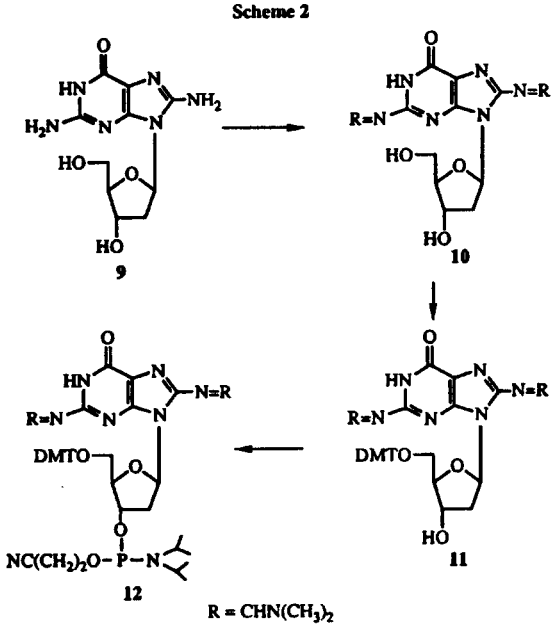
87% yield. The protection of the 3',5'-hydroxyl functions of **2** was accomplished by the treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (Markiewicz's reagent) [22] in anhydrous pyridine for 2.5 hours at room temperature. Work up of the reaction mixture, followed

Scheme 1



by purification of the product by silica gel column chromatography using 0-2% methanol in dichloromethane gave a 72% yield of **3**. Acylation of the 2'-hydroxyl group of **3** with 1.2 molar equivalents of phenyl chlorothionoformate in anhydrous acetonitrile in the presence of 4-dimethylaminopyridine gave N^2-isobutyryl-7-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-phenoxythiocarbonyl-β-D-ribofuranosyl]guanine (**4**) in a 69% yield. Reductive cleavage of the phenoxythiocarbonyl group of **4** with tributyltin hydride in the presence of 2,2'-azobis(2-methylpropionitrile) afforded the 2'-deoxy derivative **5** in a 67% yield. Removal of the protecting cyclic siloxy group was accomplished by the treatment with tetrabutylammonium fluoride in tetrahydrofuran and the desired product N^2-isobutyryl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (**6**) was isolated in a 76% yield after silica gel column chromatography. The 5'-hydroxyl group of **6** was protected as the 4,4'-dimethoxytrityl ether by treatment with 1.3 molar equivalents of 4,4'-dimethoxytrityl chloride in anhydrous pyridine. Work up of the reaction mixture and purification of the product by silica gel column chromatography afforded the dimethoxytrityl derivative **7** in a 72% yield. The target phosphoramidite synthon **8** was prepared by the treatment of **7** with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite in anhydrous dichloromethane in the presence of *N,N*-diisopropylethylamine. The pure product was isolated in a 76% yield after silica gel column chromatography.

Scheme 2



The synthesis of the target phosphoramidite building block of 8-amino-2'-deoxyguanosine (**9**) was achieved by a three step procedure (Scheme 2). Our initial attempts to protect the exocyclic amino functions using transient protection methodology was not very promising. The isolated yield of *N,N*-diisobutyryl derivative using transient protection methodology was discouragingly low, partly due to depurination during the course of the reaction. Since the above methodology was not found to be the method of choice, we decided to protect the exocyclic amino functions of **9** with an *N*-(dimethylamino)methylene group [23]. Thus, 8-amino-2'-deoxyguanosine [24] was treated with dimethylformamide diethylacetal in anhydrous dimethylformamide at room temperature for 36 hours. The solid product that crystallized after the addition of methanol to the reaction mixture was filtered to give a 75% yield of 2,8-[bis-*N*-(dimethylamino)methylene]-8-amino-2'-deoxyguanosine (**10**). Selective protection of the 5'-hydroxyl function of **10** was achieved by the treatment with 4,4'-dimethoxytrityl chloride in pyridine. Work up of the reaction mixture and silica gel column chromatography of the reaction product afforded 5'-*O*-(4,4'-dimethoxytrityl)-2,8-[bis-*N*-(dimethylamino)methylene]-8-amino-2'-deoxyguanosine (**11**) in a 74% yield. Finally the 5'-*O*-(4,4'-dimethoxytrityl) derivative (**11**) was converted to the corresponding phosphoramidite (**12**) by the treatment with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite in anhydrous dichloromethane in the pres-

ence of N,N-diisopropylethylamine. Work up of the reaction mixture followed by silica gel column chromatography afforded the pure product **12** in a 79% yield.

Synthesis, Purification and Characterization of Modified TFOs.

The TFOs containing the modified nucleosides were prepared by the phosphoramidite method using standard solid-support methodology on a 0.2 μmole scale on an ABI 380B automated DNA synthesizer. However, the modified building blocks **8** and **12**, were used in ten fold excess concentration compared to natural phosphoramidites, and with a four fold increase in coupling time. A series of TFOs was prepared with a stepwise coupling efficiency ranging from 96% to 99%. All base labile protecting groups on the oligodeoxyribonucleotides were removed by treatment with concentrated ammonium hydroxide at ambient temperature and the TFO was purified by hplc using an ion exchange Q-sepharose (Pharmacia) column. The purified product was desalted by passage through a C_{18} Sep-Pack (Waters) cartridge and analyzed on a 20% denaturing polyacrylamide gel after labeling with [γ-^{32}P]ATP using polynucleotide kinase [25]. Unmodified oligonucleotide was used as the standard for comparison of mobility and purity. The modified TFOs were found to be ≥ 95% pure and with the expected length.

Assay for Triplex Formation.

Triplex formation was assessed using the gel shift assay, essentially as described previously [4,5]. In general, trace concentrations (~ 10^{-11} *M*) of end labeled duplex were incubated with increasing concentrations of third strand in a buffer consisting of 20 m*M* Tris-HCl, *p*H 7.6, 10 m*M* magnesium chloride, and 10% sucrose. Incubations were at 37° for 20-24 hours. Samples were then separated on 12% polyacrylamide gels buffered in 89 m*M* Tris, 89 m*M* boric acid, and 10 m*M* magnesium chloride (TBM_{10}). Electrophoresis was at 80V for 2-3 hours at room temperature. Gels were dried and autoradiographed.

Triplex formation was detected by the appearance of a discrete band migrating more slowly than the duplex in samples containing added third strand. Apparent dissociation constants for triplex formation were estimated as follows. Triplex formation and dissociation were assumed to be simple bimolecular reactions:

$$\text{triplex} \leftrightarrow \text{duplex} + \text{third strand}$$

It was assumed that each sample was at equilibrium when loaded on the gel, and that gel electrophoresis did not substantially alter the relative amounts of duplex and triplex in the sample. Under these conditions, the apparent dissociation constant, K_d, is expressed by:

$$K_d = SD/T$$

where S, D, and T represent the concentrations of single strand, duplex, and triplex, respectively. Assuming that the amount of duplex is insignificant relative to the amount of added third strand, the K_d is approximately equal to the third strand concentration where 50% of the duplex has been converted to triplex. Although this approximation depends on a number of assumptions, we have found that it is generally useful for comparing the relative affinities of different triplexes, and for estimating the approximate strength of the interaction.

Table 1 shows the results of triplex binding analysis for a series of oligonucleotides. Binding of the TFO Z100-50 to the synthetic duplex ZR100-0·ZY100-0 results in the formation of a high affinity triplex (K_d = ~2x10-10 *M*). This triplex consists of a series of reverse Hoogsteen G·GC and T·AT base triplets, with the third strand antiparallel to the purine strand of the duplex, as previously described [5]. Substitution of three 2'-deoxyguanosine residues with 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-guanine (x), as in the TFO Z102-109, drastically reduced

Table 1

Summary of Binding Studies

oligo	oligo sequence	K_d(M)
ZY100-0	5'-ccccttccccccttcccccttcccc-3'	
ZR100-0	3'-ggggaaggggggaaggggaaggggg-5'	
Z100-50	5'-ggggttgggggttgggggttggggg-3'	2x10^{-10}
Z102-109	5'-ggggttgggxggttggxggttgxggg-3'	0.5-1x10^{-6}
ZY102-0	5'-ccccttcccgccttccgccttcgccc-3'	
ZR102-0	3'-ggggaagggcggaaggcggaagcggg-5'	
Z100-50	5'-ggggttgggggttgggggttggggg-3'	»1x10^{-6}*
Z102-109	5'-ggggttgggxggttggxggttgxggg-3'	»1x10^{-7}*
ZY102-7	5'-ccccttcccctccttcctcctttctccc-3'	
ZR102-7	3'-ggggaagggaggaaggaggaagagggg-5'	
Z102-56	5'-ggggttgggtggttggtggttgtgggg-3'	4x10^{-10}
Z102-109	5'-ggggttgggxggttggxggttgxggg-3'	»1x10^{-7}*
ZY102-8	5'-ccccttcccaccttccaccttcaccc-3'	
ZR102-8	3'-ggggaagggtggaaggtggaagtggg-5'	
Z100-50	5'-ggggttgggggttgggggttggggg-3'	»1x10^{-6}*
Z102-56	5'-ggggttgggtggttggtggttgtgggg-3'	»1x10^{-6}*
Z102-109	5'-ggggttgggxggttggxggttgxggg-3'	»1x10^{-7}*

x = 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)guanine. * no triplex formation detected up to the given concentration of TFO.

the apparent binding affinity for the homopurine·homopyrimidine target. In order to evaluate potential binding of x to CG base pairs in an otherwise homopurine target, we tested the binding of Z100-50 and Z102-109 to the duplex ZR102-0·ZY102-0. Neither TFO showed any detectable binding to that duplex, indicating that neither G nor x is

capable of binding with appreciable affinity to GC base pairs. Similarly, binding of x to AT and TA base pairs was tested using two additional duplex targets, ZR102-7·ZY102-7 and ZR 102-8·ZY102-8. As shown in the Table 1, x substantially reduced binding affinity for AT base pairs relative to T (compare K_d of Z102-56 and Z102-109 binding to ZR102-7·ZY102-7), and exhibited no apparent binding to TA base pairs, as well.

These results indicate that 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine is not well suited to binding within the context of a standard, antiparallel triplex consisting of reverse Hoogsteen G·GC and T·AT triplets. However, we have not ruled out potential triplex interactions with this nucleoside in other contexts. It is possible that complete substitution of all G residues with 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine may permit formation of a triplex that differs distinctly from one consisting of natural nucleosides. Furthermore, the potential value of this nucleoside in parallel triplexes consisting of Hoogsteen type T·AT and C+·GC triplets remains to be determined. The results pertinent to oligonucleotides containing 8-amino-2'-deoxyguanosine will be published elsewhere.

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. The presence of solvent as indicated by elemental analysis was verified by ^{1}H nmr spectroscopy. Thin layer chromatography (tlc) was performed on aluminum plates coated (0.2 mm) with silica gel $60F_{254}$ (EM Science). Silica gel (E. M. Science; 230-400 mesh) was used for flash column chromatography. All solvents and chemicals used were reagent grade, and were not further dried/purified unless otherwise noted. Detection of nucleoside components on tlc was by uv light, and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30°. Nuclear magnetic resonance (^{1}H nmr and ^{31}P nmr) spectra were recorded at 400 MHz with a Brüker AM400 wide bore nmr spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane (internal) for ^{1}H spectra or polyphosphoric acid (external) for ^{31}P spectra (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad).

N^2-Isobutyryl-7-β-D-ribofuranosylguanine (**2**).

7-β-D-Ribofuranosylguanine [19] (1.0 g, 3.53 mmoles) was dried by co-evaporation with anhydrous pyridine (2 x 10 ml). The dried substrate was suspended in anhydrous pyridine (10 ml), cooled in a dry ice-acetone bath (-45°) and chlorotrimethylsilane (2.25 ml) was added with stirring. After an hour, during which time the flask was allowed to warm to 15°, the mixture was again cooled to -40° (bath temperature) and isobutyryl chloride (0.45 ml, 4.3 mmoles) was added. The contents of the flask were allowed to warm to 20°. After a total of 2 hours stirring, the reaction flask was again cooled to -40° (bath temperature), the reaction mixture was diluted with dichloromethane (150 ml) and water (25 ml). The organic layer was separated and evaporated under reduced pressure. The residue was co-evaporated with methanol (3 x 25 ml) to remove the silyl protecting groups. Finally it was again co-evaporated with toluene to remove traces of pyridine. Trituration of the residue with dichloromethane (20 ml) gave a solid which was collected by filtration and dried under vacuum over phosphorous pentoxide to give 1.08 g (87%) of the title compound, mp 170-172°; ^{1}H nmr (DMSO-d_6): δ 1.13 (d, 6 H, $COCH(CH_3)_2$), 2.75 (m, 1 H, $COCH(CH_3)_2$), 3.58 and 3.69 (2 m, 2 H, $C_5'H_2$), 3.93 (m, 1 H, $C_4'H$), 4.10 (m, 1 H, $C_3'H$), 4.38 (m, 1 H, $C_2'H$), 4.92 (t, 1 H, $C_5'OH$), 5.02 (d, 1 H, $C_3'OH$), 5.31 (d, 1 H, $C_2'OH$), 6.10 (d, 1 H, J = 4.8 Hz, $C_1'H$), 8.51 (s, 1 H, C_8H), 11.46 (br s, 1 H, NH), 12.14 (br s, 1 H, NH).

Anal. Calcd. for $C_{14}H_{19}N_5O_6 \cdot 0.75\ H_2O$: C, 45.84; H, 5.63; N, 19.09. Found: C, 45.80; H, 5.43; N, 18.95.

N^2-Isobutyryl-7-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]guanine (**3**).

To a cooled (0-5°) solution of **2** (3.08 g, 8.72 mmoles) in anhydrous pyridine (45 ml) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.34 ml, 10.46 mmoles) and the mixture was allowed to warm to room temperature. The mixture was stirred at room temperature for 2.5 hours before being diluted with dichloromethane (150 ml). The organic solution was washed with saturated aqueous sodium hydrogen carbonate solution (50 ml). The organic layer was dried (sodium sulfate) and evaporated to dryness. The residue was purified by silica gel column (2.5 x 25 cm) chromatography using a gradient of 0-2% methanol in dichloromethane as the eluent. The appropriate fractions were collected and evaporated to give 3.74 g (72%) of the title compound as a colorless foam, mp 120-122°; ^{1}H nmr (DMSO-d_6): δ 1.10 (m, 28 H, 4 $CH(CH_3)_2$), 1.25 (d, 6 H, $COCH(CH_3)_2$), 2.76 (m, 1 H, $COCH(CH_3)_2$), 4.01 (m, 3 H, $C_4'H$, $C_5'H_2$), 4.40 (m, 2 H, $C_2'H$, $C_3'H$), 5.50 (d, 1 H, $C_2'OH$), 8.25 (s, 1 H, C_8H), 11.60 (br s, 1 H, NH), 12.15 (br s, 1 H, NH). ms: (FAB) m/z 597 (M+H).

Anal. Calcd. for $C_{26}H_{45}N_5O_7Si_2 \cdot 1.5\ H_2O$: C, 50.13; H, 7.76; N, 11.24. Found: C, 50.12; H, 7.52; N, 10.92.

N^2-Isobutyryl-7-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-phenoxythiocarbonyl-β-D-ribofuranosyl]guanine (**4**).

To a solution of **3** (5.0 g, 8.39 mmoles) and 4-dimethylaminopyridine (3.06 g, 25.18 mmoles) in anhydrous acetonitrile (60 ml) was added phenyl chlorothionoformate (1.38 ml, 10.07 mmoles). The mixture was stirred at room temperature for 4 hours, diluted with dichloromethane (200 ml) and the organic phase was washed sequentially with 0.1 *N* hydrochloric acid (2 x 150 ml), water (100 ml) and saturated sodium hydrogen carbonate solution (100 ml). The organic layer was dried (sodium sulfate) and then evaporated to dryness. The residue was purified by silica gel column (3 x 25 cm) chromatography using a gradient of 0-2% methanol in dichloromethane as the eluent to afford 4.2 g (69%) of the pure title compound, mp 115-117°; ^{1}H nmr (DMSO-d_6): δ1.05 (m, 34 H, $COCH(CH_3)_2$, 4 $CH(CH_3)_2$), 2.80 (m, 1 H, $COCH(CH_3)_2$), 4.10 (m, 3 H, $C_4'H$, $C_5'H_2$), 5.10 (m, 1 H, $C_3'H$), 6.20 (m, 1 H, $C_2'H$), 6.40 (d, 1 H, J = 2.84 Hz, $C_1'H$), 7.12-7.50 (m, 5 H, Ph), 8.44 (s, 1 H, C_8H), 11.60 (s, 1 H, NH), 12.10 (s, 1 H, NH).

Anal. Calcd. for $C_{33}H_{50}N_5O_8SSi_2 \cdot 2H_2O$: C, 51.53; H, 7.08; N, 9.11; S, 4.17. Found: C, 51.52; H, 6.77; N, 8.84; S, 4.02.

N^2-Isobutyryl-7-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]guanine (**5**).

To a solution of **4** (6.0 g, 8.21 mmoles) and 2,2'-azobis(2-methylpropionitrile) (AIBN, 0.25 g) in anhydrous toluene (100 ml) was added tributyltin hydride (13.5 ml, 50 mmoles) under argon atmosphere. The mixture was heated at 75° for 3.5 hours and then allowed to cool to room temperature. The solvent was removed and the residue was purified by chromatography on a silica gel column (3.5 x 35 cm) using a gradient of 0 to 0.75% methanol in dichloromethane as the eluent to give 3.2 g (67%) of the title compound, mp 118-120°; ^{1}H nmr (DMSO-d_6): δ1.10 (m, 34 H, $COCH(CH_3)_2$, 4 $CH(CH_3)_2$), 2.55 (m, 1 H, C_2'*H*), 2.73 [m, 2 H, C_2"*H* and $COCH(CH_3)_2$), 3.95 (m, 3 H, C_4'*H* and C_5'H_2), 4.73 (m, 1 H, C_3'*H*), 6.44 (dd, 1 H, J = 3.9 Hz, C_1'*H*), 8.31 (s, 1H, C_8*H*), 11.60 (br s, 1H, N*H*), 12.15 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{26}H_{45}N_5O_6Si_2$•0.5 H_2O: C, 53.03; H, 7.87; N, 11.89. Found: C, 52.91; H, 7.87; N, 11.70.

N^2-Isobutyryl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (**6**).

To a solution of **5** (2.8 g, 4.81 mmoles) in dry tetrahydrofuran (40 ml) was added tetrabutylammonium fluoride (20 ml, 20 mmoles, 1 *M* solution in tetrahydrofuran). The mixture was stirred at room temperature for 2 hours, the solvent was evaporated and the residue was purified by chromatography on a silica gel column (2.5 x 25 cm) using a gradient of 0 to 5% methanol in dichloromethane as the eluent to yield 1.23 g (76%) of **6**, mp 160-162°; ^{1}H nmr (DMSO-d_6): δ 1.14 (d, 6 H, $COCH(CH_3)_2$), 2.33 (m, 1 H, C_2'*H*), 2.47 (m, 1 H, C_2"*H*), 2.75 (m, 1 H, $COCH(CH_3)_2$), 3.55 (m, 2 H, C_5'H_2), 3.86 (m, 1 H, C_4'*H*), 4.33 (m, 1 H, C_3'*H*), 4.92 (br s, 1 H, O*H*), 5.26 (br s, 1 H, O*H*), 6.53 (t, 1 H, J = 6.48 Hz, C_1'*H*), 8.48 (s, 1 H, C_8*H*), 9.40 (br, 1 H, N*H*), 9.80 (br, 1 H, N*H*).

Anal. Calcd. for $C_{14}H_{19}N_5O_5$•H_2O: C, 47.32; H, 5.95; N, 19.71. Found: C, 47.58; H, 5.75; N, 19.57.

N^2-Isobutyryl-7-[5-*O*-(4,4'-dimethoxytrityl)-2-deoxy-β-D-*erythro*-pentofuranosyl]guanine (**7**).

To a solution of **6** (1.0 g, 2.96 mmoles) in anhydrous pyridine (20 ml) was added 4,4'-dimethoxytrityl chloride (1.3 g, 3.85 mmoles). The mixture was stirred at room temperature for 2.5 hours, diluted with dichloromethane (200 ml) and the organic phase was washed with water (50 ml). The organic layer was dried (sodium sulfate) and evaporated. The residue was co-evaporated with toluene (2 x 10 ml) to remove traces of pyridine. Finally the product was purified by silica gel column (2.5 x 25 cm) chromatography using a gradient of 0-1.5% methanol in dichloromethane as the eluent to yield 1.36 g (72%) of the title compound, mp 141-144°; ^{1}H nmr (DMSO-d_6): δ 1.10 (d, 6 H, $COCH(CH_3)_2$), 2.35 (m, 1 H, C_2'*H*), 2.63 (m, 1 H, C_2"*H*), 2.76 (m, 1 H, $COCH(CH_3)_2$), 3.20 (m, 2 H, C_5'H_2), 3.72 (s, 6 H, 2 OCH_3) 3.98 (m, 1 H, C_4'*H*), 4.32 (m, 1 H, C_3'*H*), 5.33 (d, 1 H, C_3'O*H*), 6.54 (t, 1 H, J = 8.8 Hz, C_1'*H*), 6.62 (t, 4 H, *Aromatics*), 7.23 (m, 9 H, *Aromatics*), 8.30 (s, 1 H, C_8*H*), 11.51 (br s, 1 H, N*H*), 12.10 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{35}H_{37}N_5O_7$•0.5 H_2O: C, 65.02; H, 5.99; N, 10.68. Found: C, 64.77; H, 5.98; N, 10.35.

N^2-Isobutyryl-7-[3-*O*-(P-β-cyanoethoxy-*N,N*-diisopropylaminophosphinyl)-5-*O*-(4,4'-dimethoxytrityl)-2-deoxy-β-D-*erythro*-pentofuranosyl]guanine (**8**).

To a solution of **7** (0.5 g, 0.78 mmole) and *N,N*-diisopropylethylamine (0.55 ml, 3.12 mmoles) in anhydrous dichloromethane (8 ml) was added, with stirring, 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.22 ml, 1 mmole) under an argon atmosphere. After stirring the mixture at room temperature for 20 minutes, an additional 0.15 ml of phosphitylating agent was added. The reaction was continued for a further period of 20 minutes. The reaction mixture was diluted with ethyl acetate (100 ml) and the organic phase was washed with aqueous saturated sodium hydrogen carbonate solution (25 ml). The organic layer was dried (sodium sulfate) and evaporated to dryness. The residue was purified by chromatography over a silica gel column (1 x 20 cm) using a mixture of dichloromethane:ethyl acetate:triethylamine (70:30:3, v/v) as the eluent. The appropriate fractions were pooled and evaporated. The residue was dissolved in a small amount of dichloromethane (3 ml) and the solution was added slowly to rapidly stirred pentane (150 ml, cooled in a dry ice-acetone bath). The supernatant was decanted from the precipitated solid, and the solid was collected by filtration and dried under vacuum to give 0.5 g (76%) of **8** as a colorless powder; ^{31}P nmr (acetonitrile-d_3): δ 149.74.; ^{1}H nmr (acetonitrile-d_3): δ 1.10 (m, 18 H, $COCH(CH_3)_2$, 2 $CH(CH_3)_2$), 2.70 (m, 5 H, C_2'*H*, C_2"*H*, OCH_2CH_2CN, $COCH(CH_3)_2$), 3.30-3.70 (m, 4 H, OCH_2CH_2CN, $N(CH(CH_3)_2)_2$), 3.71 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3), 4.10 (m, 3 H, C_4'*H*, C_5'H_2), 4.60 (m, 1 H, C_3'*H*), 6.56 (m, 1 H, C_1'*H*), 6.79-6.83 (m, 4 H, *Aromatics*), 7.19-7.30 (m, 7 H, *Aromatics*), 7.38-7.42 (m, 2 H, *Aromatics*), 8.06, 8.07 (2 s, 1 H, C_8*H* of the isomers).

2,8-[Bis-*N*-(dimethylamino)methylene]-8-amino-2'-deoxyguanosine (**10**).

To a suspension of dry (by co-evaporation with pyridine, 2 x 20 ml) 8-amino-2'-deoxyguanosine [24] (**9**, 1.3 g, 4 mmoles) in anhydrous *N,N*-dimethylformamide (10 ml) was added dimethylformamide diethylacetal (10 ml). After stirring the reaction mixture at room temperature for 36 hours, methanol (5 ml) was added and the mixture was left at room temperature for a day. The crystalline product that deposited was collected by filtration and washed with dichloromethane (3 x 25 ml) to give 1.17 g (75%) of the pure product, mp 226-228°; ^{1}H nmr (DMSO-d_6): δ 1.99 (m, 2 H, C_2'*H* and C_2"*H*), 3.03 and 3.12 (2 s, 12 H, $2N(CH_3)_2$), 3.50 (m, 1 H, C_5'*H*), 3.62 (m, 1 H, C_5'*H*), 3.79 (m, 1 H, C_4'*H*), 4.43 (m, 1 H, C_3'*H*), 4.87 (t, 1 H, C_5'O*H*), 5.21 (br s, 1 H, C_3'O*H*), 6.45 (t, 1 H, J = 7.12 Hz, C_1'H), 8.43, 8.46 (2 s, 2 H, $2CHN(CH_3)_2$), 11.20 (br s, 1 H, N*H*); ms:(FAB) m/z 393 (M+H).

Anal. Calcd. for $C_{16}H_{24}N_8O_4$•2 H_2O: C, 44.85; H, 6.58; N, 26.15. Found: C, 45.01; H, 6.31; N, 26.03.

5'-*O*-(4,4'-Dimethoxytrityl)-2,8-[bis-*N*-(dimethylamino)methylene]-8-amino-2'-deoxyguanosine (**11**).

To a suspension of dry (by co-evaporation with anhydrous pyridine, 2 x 20 ml) **10** (1.0 g, 2.55 mmoles) in anhydrous pyridine (30 ml) was added 4,4'-dimethoxytrityl chloride (1.12 g, 3.32 mmoles). After 2 hours of stirring at ambient temperature, an additional 0.8 g of dimethoxytrityl chloride was added and the stirring was continued for a further period of 1.5 hours. The reaction mixture was diluted with dichloromethane (150 ml) and the organic phase was washed with water (50 ml). The organic layer was dried (sodium sulfate) and evaporated. The product was purified by passing through a short silica gel column eluting with a gradient of 0-8% methanol in dichloromethane to yield

1.3 g (74%) of the title compound, mp 176-178°; ^{1}H nmr (DMSO-d_6): δ 2.10 (m, 2 H, $C_{2'}H$ and $C_{2''}H$), 2.86 (s, 3 H, NCH_3), 2.98 (s, 6 H, $N(CH_3)_2$), 3.06 (s, 3 H, NCH_3), 3.17 and 3.26 (2 m, 2 H, $C_{5'}H_2$), 3.71, 3.72 (2 s, 6 H, $2OCH_3$), 3.87 (m, 1 H, $C_{4'}H$), 4.50 (m, 1 H, $C_{3'}H$), 5.23 (d, 1 H, $C_{3'}H$), 6.47 (t, 1 H, J = 6.2 Hz, $C_{1'}H$), 6.75 (m, 5 H, *Aromatics*), 7.17, 7.30 (2 m, 8 H, *Aromatics*), 8.29, 8.38 (2 s, 2 H, $2CHN(CH_3)_2$), 11.10 (s, 1 H, *NH*).

Anal. Calcd. for $C_{37}H_{42}N_8O_6 \cdot 2\ H_2O$: C, 60.81; H, 6.34; N, 15.33. Found: C, 60.91; H, 6.15; N, 15.06.

3'-*O*-(P-β-Cyanoethoxy-*N,N*-diisopropylaminophosphinyl)-5'-*O*-(4,4'-dimethoxytrityl)-2,8-[bis-*N*-dimethylamino)methylene]-8-amino-2'-deoxyguanosine (**12**).

To a solution of **11** (0.7 g, 1 mmole) in a mixture of anhydrous dichloromethane (10 ml) and *N,N*-diisopropylethylamine (0.7 ml, 4 mmoles) was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.28 ml, 1.3 mmoles) under an argon atmosphere. The mixture was stirred at ambient temperature for 20 minutes and an additional 0.2 ml of phosphitylating agent was added. After a further period of 15 minutes, the reaction mixture was diluted with ethyl acetate (100 ml) containing 5% triethylamine and the organic phase was washed with saturated sodium hydrogen carbonate solution (50 ml). The organic layer was dried (sodium sulfate) and evaporated to dryness. The residue was purified by chromatography on a silica gel column (1 x 20 cm) using a mixture of triethylamine (3%) and methanol (1-3%) in dichloromethane. The appropriate fractions were pooled and evaporated to dryness. The residue was dissolved in a small amount of dichloromethane (4 ml) and the solution was added slowly to rapidly stirred, cold (dry ice-acetone bath) pentane (100 ml). The precipitated product was collected by filtration and dried under vacuum to give 0.71 g (79%) of **12** as a colorless powder; ^{31}P nmr (acetonitrile-d_3): δ 149.90 and 150.06; ^{1}H nmr (acetonitrile-d_3): δ 1.00 (m, 12 H, $N(CH(CH_3)_2)_2$), 2.25-2.65 (m, 4 H, $C_{2'}H$, $C_{2''}H$, OCH_2CH_2CN), 2.97, 2.98, 2.99, 3.00 (4 s, 12 H, 2 $N(CH_3)_2$), 3.15-3.70 (m, 6 H, $C_{5'}H_2$, 2 $CH(CH_3)_2$, OCH_2), 3.75, 3.76 (2 s, 6 H, 2 OCH_3), 4.10 (m, 1 H, $C_{4'}H$), 4.95 (m, 1 H, $C_{3'}H$), 6.60 (m, 1 H, $C_{1'}H$), 6.80, 7.30 (2 m, 13 H, *Aromatics*), 8.33, 8.34 (2 s, 1 H, $CHN(CH_3)_2$), 8.50 (s, 1 H, $CHN(CH_3)_2$), 9.45 (br s, 1 H, *NH*).

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