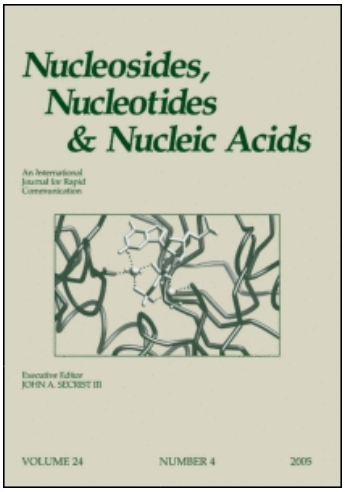

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Incorporation of 2'-Deoxy-9-deazaguanosine and 2'-Deoxy-7-deaza-6-thioguanosine into G-Rich Oligodeoxyribonucleotides

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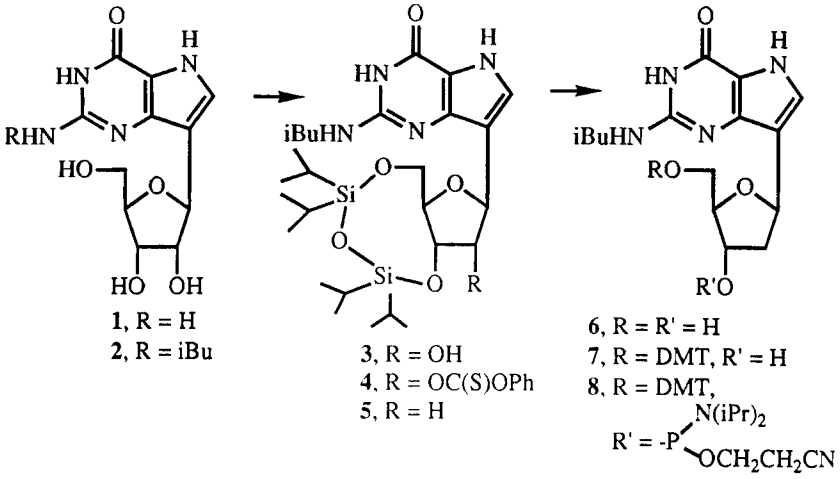
Abstract: The preparation of suitably protected monomeric phosphoramidite building blocks of 2'-deoxy-9-deazaguanosine (**8**) and 2'-deoxy-7-deaza-6-thioguanosine (**12**) and their incorporation into G-rich oligodeoxyribonucleotides are described.

The existence of intermolecular triple helical structures for nucleic acids has been recognized for over three decades.[1-3] Only recently has triple helix formation attracted considerable attention as the basis for a program of pharmaceutical development. Two major modes of triple helix formation have been identified, parallel and antiparallel. In the parallel pyr.pur.pyr motif, the protonated form of C binds to GC base pairs and T binds to AT base pairs through Hoogsteen hydrogen bonding.[4] In this pH dependent triple helix formation the protonation of C is necessary.[5] In the antiparallel pur.pur.pyr motif, G binds to GC base pairs and T binds to AT base pairs through reverse Hoogsteen hydrogen bonding.[6-10] The main advantage of the antiparallel motif is that it is pH independent and the triple helix is formed at physiological conditions. It has recently been shown[6] that under appropriate conditions, triple helix forming oligonucleotides (TFOs) utilizing a GT motif can bind with high affinity (apparent $K_d \leq 1$ nM) with high sequence selectivity and essentially no pH dependence. Such TFOs generally consist entirely of G and T residues, and are often biased in favor of G. Although these G-rich oligonucleotides hold considerable promise as first generation TFO therapeutics, it is apparent that the high G content is problematic. The major problem associated with G-rich TFOs is

self-association. It has been shown that in the presence of monovalent cations, G-rich oligonucleotides of the general structure GG-n^1-GG-n^2-GG-n^3-GG-n^4 can fold to form very stable intramolecular tetrads, so long as the regions n^1-n^4 are longer than two bases.[11-14] Since we have been studying TFOs which display sequences similar to the above tetrad mode, it is anticipated that triple helix formation will be strongly inhibited at physiological ion concentration, where a significant portion of the available TFO would be consumed by intramolecular (or intermolecular) tetrad formation. Since O^6 and N^7 of G is crucial for both H-bonding and ion coordination in a tetrad, nucleoside modifications of these sites which allow for increased triple helix formation while providing for destabilization of G-tetrads is beneficial. In this communication , we describe the synthesis of suitably protected monomeric building blocks of 2'-deoxy-9-deazaguanosine[15] and 2'-deoxy-7-deaza-6-thioguanosine, and their incorporation into oligodeoxynucleotides using the solid-support, phosphoramidite chemistry.

The synthesis of the phosphoramidite (**8**) of 2'-deoxy-9-deazaguanosine was performed as follows (*Scheme* 1). For the preparation of **8**, 9-deazaguanosine (**1**) was found to be a viable intermediate which was prepared as reported.[16] Direct glycosylation of 9-deazaguanine with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-**D**-ribofuranose in the presence of tin(IV) chloride, followed by removal of blocking benzoyl groups by treatment with methanolic ammonia gave **1**. The ribonucleoside **1** was converted to the corresponding 2'-deoxynucleoside *via* a four step deoxygenation procedure.[17] As it is necessary to protect the exocyclic amino function of the nucleoside during the preparation of the target monomeric phosphoramidite building block in order to avoid any side reactions during oligonucleotide synthesis, we decided to carry out this step prior to 2'-deoxygenation. Thus, 9-deazaguanosine (**1**) on treatment with chlorotrimethylsilane in anhydrous pyridine, followed by the reaction with isobutyryl chloride[18] and subsequent removal of the protecting silyl groups afforded N^2-isobutyryl-9-deazaguanosine (**2**) in a 76% yield. The 3'- and 5'-hydroxyl functions of **2** were protected by the treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[19] in anhydrous pyridine for 2.5 h at room temperature. The cyclic siloxy derivative **3** was isolated in a 68% yield after silica gel column chromatography. Acylation of the 2'-hydroxyl function of **3** was achieved by treatment with phenyl chlorothionoformate in anhydrous acetonitrile in the presence of 4-dimethylaminopyridine. Work up of the reaction mixture afforded a product containing a mixture of two components. The product was chromatographed on



Scheme 1

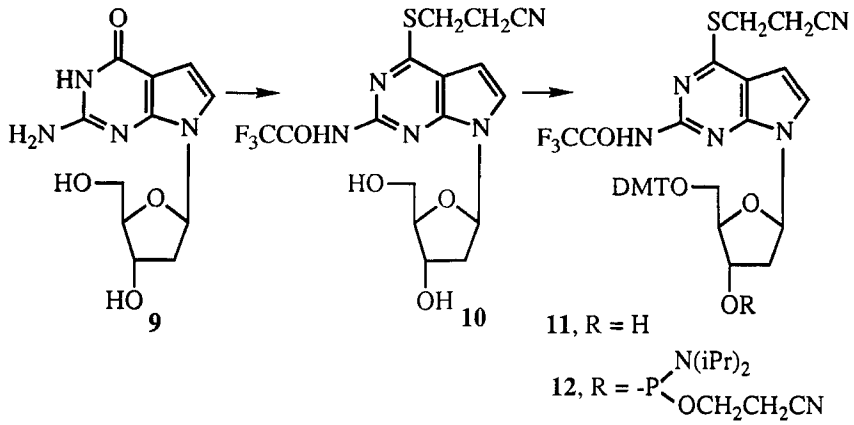
a silica gel column and the fast moving component was eluted with 15% hexanes in dichloromethane. This material was identified as N^2-isobutyryl-3',5'-*O*-[1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl]-2'-*O*-phenoxythiocarbonyl-9-deazaguanosine (**4**) and was isolated in a 59% yield. Reductive cleavage of phenoxythiocarbonyl group of **4** with tributyltin hydride in anhydrous toluene in the presence of 2,2'-azobis(2-methylpropionitrile) afforded N^2-isobutyryl-3',5'-*O*-[1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl]-2'-deoxy-9-deazaguanosine (**5**). Compound **5** was isolated in 66% yield after silica gel column chromatography. The minor component (~15 %) isolated from the phenoxythiocarbonylation reaction , appeared similar in spectral properties to that of **4** but did not give any deoxy nucleoside upon reduction. Removal of the cyclic silyloxy group of **5** was accomplished by the treatment with tetrabutylammonium fluoride and the reaction product N^2-isobutyryl-2'-deoxy-9-deazaguanosine (**6**) was isolated in a 80% yield. The 5'-hydroxyl function of **6** was protected in the form of a 4,4'-dimethoxytrityl ether by the treatment with 4,4'-dimethoxytrityl chloride in anhydrous pyridine. Work up of the reaction mixture, followed by silica gel column chromatography afforded N^2-isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-9-deazaguanosine (**7**) in a 84% yield. The target phosphoramidite (**8**) was prepared by the treatment of **7** with 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite in anhydrous tetrahydrofuran in the presence of *N*,*N*-

diisopropylethylamine. The reaction product (**8**) was purified by silica gel column chromatography and was isolated in a 84% yield.

The synthesis of 2'-deoxy-7-deaza-6-thioguanosine phosphoramidite building block (**12**) was accomplished starting from the corresponding 2'-deoxyribonucleoside (**9**) itself. Thus, 2'-deoxy-7-deazaguanosine[20] (**9**) upon reaction with trifluoroacetic anhydride in anhydrous pyridine, followed by the treatment with mercaptopropionitrile[21] (*Scheme* 2) afforded S^6-cyanoethyl-N^2-trifluoroacetyl-2'-deoxy-7-deaza-6-thioguanosine (**10**). The 5'-hydroxyl function of **10** was protected with 4,4'-dimethoxytrityl group by the treatment with 4,4'-dimethoxytrityl chloride in anhydrous pyridine and the reaction product **11** was isolated in a 66.7% yield after silica gel column chromatography. The 5'-dimethoxytrityl derivative (**11**) on treatment with 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite in anhydrous dichloromethane in the presence of diisopropylamino tetrazolide, followed by work up of the reaction mixture furnished the target phosphoramidite **12** in a 84% yield.

The synthesis of oligonucleotides containing 2'-deoxy-9-deazaguanosine or 2'-deoxy-7-deaza-6-thioguanosine residues was performed using standard phosphoramidite methodology with a stepwise coupling efficiency of 98% by coupling **8** or **12** to growing oligo and increasing the reaction time for an additional 90 seconds. Synthesis was carried out on a 0.2 μmole scale on a ABI 380B automated DNA synthesizer. All base labile protecting groups on the oligonucleotides were removed by treatment with concentrated ammonium hydroxide at 55 °C for 15 h and the oligonucleotide was purified by ion-exchange HPLC using a Q-sepharose column. The purified product was desalted by passage through a C_{18} sep-pack cartridge and analyzed on a 20% denaturing polyacrylamide gel after labeling with ^{32}P-ATP using polynucleotide kinase[22]. Unmodified oligonucleotide was used as the standard for comparison of mobility and purity. The modified oligonucleotide was found to be 96% pure and with the expected length. The compositional integrity of purified oligonucleotides containing 2'-deoxy-9-deazaguanosine and 2'-deoxy-7-deaza-6-thioguanosine were determined by enzymatic (nuclease P1/alkaline phosphatase) degradation to the corresponding nucleosides and subsequent analysis by reversed-phase HPLC using a photodiode array detector. The observed relative ratios of the nucleosides (T, dG, 2'-deoxy-9-deazaguanosine and 2'-deoxy-7-deaza-6-thioguanosine) were in excellent agreement with the calculated ratios.

The ability of these modified oligonucleotides to form triple helices was assessed by band shift gel electrophoretic analysis, essentially as described earlier [6] and the results are summarized in *Table* 1.



Scheme 2

Table 1

```
          5'- gtcactggcccttccccccttcccggtctcag-3'
          3'- cagtgaccgggaaggggggaagggccagagtc-5'  KD
                   5'-gggttgggggttggg-3'          3x10-9M
                   5'-gxgttgxgxgttgxg-3'          1x10-6M
                   5'-xxxttxxxxxttxxx-3'          1x10-6M
     x = 9-deaza-dG
5'-gatctgtacactctgttctccccccacccccctgtacactctgttctgcctag-3'
     3'-acatgtgagacaagaggggggtgggggacatgtgagacaaga-5'
           5'-gtgtgttgtgggggggtgggggtgttgtgtgtgttgt-3'  3x10-8
           5'-gtgtgttgtgggzggtggzggtgttgtgtgtgttgt-3'  3x10-7
           5'-gtgtgttgtzggzggtggzggtgttgtgtgtgttgt-3'  2x10-7
           5'-gtgtgttgtgggzgggtgzgggtgttgtgtgtgttgt-3'  1x10-7
     z = 7-deaza-6-thio-dG
```

It can be clearly seen from the above data that neither of these modifications improved the binding affinity to form triple helices when compared to the unmodified oligomers.

In summary, the synthesis of 2'-deoxy-9-deazaguanosine, was accomplished by a four step deoxygenation procedure from the corresponding ribonucleoside (**1**), which was subsequently converted to the corresponding phosphoramidite monomer (**8**). A simple synthesis of suitably protected 2'-

deoxy-7-deaza-6-thioguanosine phosphoramidite monomer (**12**) was also developed. Both of these phosphoramidite monomers were successfully incorporated into G-rich oligodeoxyribonucleotides. However, these modified oligonucleotides didn't show any enhancement in triple helix formation.

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 °C. Nuclear magnetic resonance (^{1}H NMR and ^{31}P NMR) spectra were recorded at 400 MHz with a Bruker AM400 wide bore nmr spectrometer. Mass spectra were obtained from Baylor College of Medicine Mass Spectrometry Core Facility, Houston, TX. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane (internal) for ^{1}H spectra or polyphosphoric acid (85%, external) for ^{31}P spectra (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad)

N^2-Isobutyryl-9-deazaguanosine (2).

To a suspension of 9-deazaguanosine[16] (2 g, 7.09 mmoles) in anhydrous pyridine (20 mL) was added chlorotrimethylsilane (8.99 mL, 70.91 mmoles) at 0 °C. The mixture was allowed to warm slowly to room temperature. After 2 h, isobutyryl chloride (0.90 mL, 8.51 mmoles) was added at 0 °C and the reaction mixture was stirred at room temperature for a further period of 4 h. The reaction flask was again cooled in an ice bath, water (20 mL) was added followed by dichloromethane (150 mL). The organic layer was separated and evaporated at 40 °C. The residue was co-evaporated with methanol (3 x 100 mL) followed by toluene (2 x 25 mL). The solid was triturated with dichloromethane (20 mL), the product was filtered and dried in vacuum. The filtrate which contained a small amount of the product was chromatographed on a silica gel column using 0 -

10% methanol in dichloromethane as the eluent. Total yield: 1.90 g (76%); mp 222-224 °C; ^{1}H NMR (DMSO-d_6): δ 1.10 [d, 6 H, COCH(CH_3)$_2$], 2.75 [m, 1 H, COC*H*(CH$_3$)$_2$], 3.30-3.80 (m, 3 H, C$_4$'*H*, C$_5$'*H*$_2$), 3.90 (t, 1 H, C$_3$'*H*), 4.09 (t, 1 H, C$_2$'*H*), 4.81 (d, J = 6.0 Hz, 1 H, C$_1$'*H*), 7.42 (d, 1 H, C$_8$*H*), and 11.98 (br s, 2 H, N$_2$*H*, N$_3$*H*); *Anal.* Calcd. for $C_{15}H_{20}N_4O_6$. 0.5 CH_3OH: C, 50.54; H, 6.02; N, 15.21. Found: C, 50.91; H, 5.70; N, 14.83.

N^2-Isobutyryl-3',5'-*O*-[1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl]-9-deaza-guanosine (3).

To a solution of N^2-isobutyryl-9-deazaguanosine (**2**, 2.68 g, 7.61 mmoles) in anhydrous pyridine (30 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.91 mL, 9.1 mmoles) under an argon atmosphere. The reaction mixture was stirred at room temperature for 2.5 h, diluted with dichloromethane (150 mL) and the organic solution was washed with water (50 mL). The organic layer was dried (sodium sulfate), evaporated and the residue was chromatographed on a silica gel column using 0 - 2% methanol in dichloromethane as the eluent. Yield: 3.07 g (68%); mp 148-150 °C; ^{1}H NMR (DMSO-d_6): δ 0.9-1.20 [m, 34 H, 4 C*H*(CH$_3$)$_2$, COCH(CH$_3$)$_2$], 2.75 [m, 1 H, COC*H*(CH$_3$)$_2$], 3.75-4.30 (m, 6 H, C$_2$'*H*, C$_2$''*H*, C$_3$'*H*, C$_4$'*H*, C$_5$'*H*$_2$), 4.92 (d, J = 2.8 Hz, 1 H, C$_1$'*H*), 7.28 (s, 1 H, C$_8$*H*), 11.40 (s, 1 H, N$_7$*H*), and 12.00 (br s, 2 H, N$_2$*H*, N$_3$*H*); *Anal.* Calcd. for $C_{27}H_{46}N_4O_7Si_2$. 1/3 CH_3OH: C, 54.22; H, 7.88; N, 9.25. Found: C, 54.62; H, 7.93; N, 8.83.

N^2-Isobutyryl-3',5'-*O*-[1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl]-2'-O-phenoxythiocarbonyl-9-deazaguanosine (4).

To a suspension of **3** (4.7 g, 7.89 mmoles) and 4-dimethylaminopyridine (5.78 g, 47.33 mmoles) in anhydrous acetonitrile (50 mL) was added phenyl chlorothionoformate (1.36 mL, 9.86 mmoles) under an argon atmosphere. After stirring the reaction mixture for 2 h at room temperature, it was diluted with dichloromethane (200 mL). The organic phase was washed successively with cold 1 N hydrochloric acid solution (2 x 200 mL), water (200 mL) and saturated aqueous sodium hydrogen carbonate solution (200 mL). The organic layer was dried (sodium sulfate) and the solvent evaporated. The residue, which was a mixture of two major compounds, was chromatographed on a silica gel column. The fast moving major product was eluted using 15% hexanes in dichloromethane and was characterized as compound **4**. Yield: 3.4 g (59%); mp 70-72 °C; ^{1}H NMR (DMSO-d_6): δ 0.9-1.20 [m, 34 H, 4 C*H*(CH$_3$)$_2$, COCH(CH$_3$)$_2$],

2.80 [m, 1 H, COC*H*$(CH_3)_2$], 4.00 (m, 3 H, $C_4'H$, $C_5'H_2$), 4.70 (m, 1 H, $C_3'H$), 5.31 (d, J = 3.6 Hz, 1 H, $C_1'H$), 6.05 (m, 1 H, $C_2'H$), 7.13-7.57 (m, 5 H, Ph), 8.21 (s, 1 H, C_8H), 11.62 (s, 1 H, N_7H), and 12.25 (br s, 2 H, N_2H, N_3H); MS: m/z 731 (M +H)·

N^2-Isobutyryl-3',5'-*O*-[1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl]-2'-deoxy-9-deazaguanosine (5).

To a solution of **4** (2.8 g, 3.82 mmoles) in anhydrous toluene (120 mL) was added 2,2'-azobis(2-methylpropionitrile) (AIBN, 150 mg) and tri-*n*-butyltin hydride (6.2 mL, 23 mmoles) under an argon atmosphere. The reaction flask was placed in a preheated oil bath (70 °C). After 2 h, the mixture was allowed to cool to room temperature and the solvent was evaporated in vacuum. The residual oil was chromatographed over silica gel using 0-1% methanol in dichloromethane as the eluent to yield 1.46 g (66%) of **5** as analytically pure product; mp 104-106 °C; ^{1}H NMR (DMSO-d_6): δ 1.03 [m, 28 H, 4 C*H*$(CH_3)_2$], 1.15 [d, 6 H, COCH$(CH_3)_2$], 2.21 (m, 1 H, $C_2'H$), 2.30 (m, 1 H, $C_2''H$), 2.76 [m, 1 H, COC*H*$(CH_3)_2$], 3.75 (m, 2 H, $C_5'H_2$), 3.95 (m, 1 H, $C_4'H$), 4.48 (m, 1 H, $C_3'H$), 5.15 (t, J = 6.44 Hz, 1 H, $C_1'H$), 7.30 (s, 1 H, C_8H), 11.26 (br s, 1 H, N_7H), and 11.89 (br s, 2 H, N_2H, N_3H); *Anal.* Calcd. for $C_{27}H_{46}N_4O_6Si_2$: C, 56.02; H, 8.01; N, 9.68. Found: C, 56.35; H, 8.23; N, 9.21.

N^2-Isobutyryl-2'-deoxy-9-deazaguanosine (6).

To a solution of **5** (2 g, 3.36 mmoles) in tetrahydrofuran (20 mL) was added a 1 N solution of tetrabutylammonium fluoride (13.45 mL, 13.45 mmoles) in tetrahydrofuran. The mixture was stirred at room temperature for 2.5 h and the solvent was evaporated. The reaction product was purified by silica gel column chromatography using 0-2.5 % methanol in dichloromethane as the eluent to yield 0.9 g (80%) of the title compound; mp 102 °C; ^{1}H NMR (DMSO-d_6): δ 1.12 [d, 6 H, COCH$(CH_3)_2$], 2.01 (m, 2 H, C_2'H, $C_2''H$), 2.75 [m, 1 H, COC*H*$(CH_3)_2$], 3.69 (br s, 1 H, $C_4'H$), 3.93 (br s, 2 H, $C_5'H_2$), 4.18 (br s , 1 H, $C_3'H$), 4.60 (br s, 1 H, $C_3'OH$), 4.90 (br s, 1 H, $C_5'OH$), 5.18 (t, J = 6.44 Hz, 1 H, $C_1'H$), 7.33 (s, 1 H, C_8H), 9.80 (br s, 1 H, N_7H), and 10.40 (br s, 2 H, N_2H, N_3H); *Anal.* Calcd. for $C_{15}H_{20}N_4O_5$. 1/4 H_2O: C, 52.85; H, 6.06; N, 16.46. Found: C, 52.61; H, 6.30; N, 16.20.

N^2-Isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-9-deazaguanosine (7).

To a solution of **6** in anhydrous pyridine (15 mL) was added 4,4'-dimethoxytrityl chloride (0.63 g, 1.86 mmoles) in 4 portions at 1 h intervals. After

the addition was complete the reaction was continued for an additional 2 h. Then the reaction mixture was 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 20 mL) and the product was chromatographed on a silica gel column using 0-1.5% methanol in dichloromethane as the eluent. Yield: 0.8 g (84%); mp 134-136 °C; ^{1}H NMR (DMSO-d_6): δ 1.10 [d, 6 H, COCH(CH_3)$_2$], 2.07 (m, 2 H, C_2'H, C_2''H), 2.73 [m, 1 H, COCH(CH_3)$_2$], 3.05 (m, 2 H, C_5'H_2), 3.73 (s, 6 H, 2 OCH_3), 3.85 (m, 1 H, C_4'H), 4.16 (br s , 1 H, C_3'H), 4.60 (br s, 1 H, C_3'OH), 5.22 (t, J = 7.60 Hz, 1 H, C_1'H), 6.85 (m, 4 H, 4 ArH), and 7.20-7.39 (m, 10 H, 9 ArH, C_8H); *Anal.* Calcd. for $C_{36}H_{38}N_4O_{7.}1/4$ H_2O: C, 67.22; H, 6.03; N, 8.71. Found: C, 67.19; H, 6.22; N, 8.31.

N^2-Isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-9-deazaguanosine-3'-*O*-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (8).

To a solution of **7** (0.5 g, 0.78 mmoles) and *N,N*-diisopropylethylamine (0.55 mL, 3.13 mmoles) in anhydrous tetrahydrofuran was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.21 mL, 0.94 mmoles) under an argon atmosphere. The mixture was stirred at room temperature for 25 min, diluted with ethyl acetate (100 mL) and the organic solution was washed with saturated sodium hydrogen carbonate solution (30 mL). The organic layer was dried (sodium sulfate) and evaporated. The residue was chromatographed on a silica gel column using a mixture of dichloromethane , ethyl acetate and triethyl amine (70:27:3). The appropriate fractions containing the product were pooled and evaporated. The product was dissolved in a minimum amount of dichloromethane (3 mL) and the solution was added to a rapidly stirred pentane (150 mL) at - 40 °C. The supernatant was decanted and the product was dried on a high vacuum pump to give 0.55 g (84%) of the phosphoramidite **8** as colorless foam. ^{31}P NMR (CD_3CN): δ 148.57, 148.86; ^{1}H NMR (CD_3CN): δ 1.11 [m, 20 H, 2 CH(CH_3)$_2$, COCH(CH_3)$_2$], 2.15 (m, 2 H, C_2'H, C_2''H), 2.20-2.65 [m, 3 H, OCH_2CH_2CN , COCH(CH_3)$_2$], 3.15 (m, 2 H, O$CH_2$$CH_2$CN), 3.45 (m, 2 H, C_5'H_2), 3.74 (s, 6 H, 2 OCH_3), 4.05 (m, 1 H, C_4'H), 4.50 (m , 1 H, C_3'H), 5.20 (m, 1 H, C_1'H), 6.80 (m, 4 H, 4 ArH), and 7.11-7.48 (m, 10 H, 9 ArH, C_8H); *Anal.* Calcd. for $C_{45}H_{55}N_6O_8P$: C, 64.42; H, 6.61; N, 10.02; P, 3.69. Found: C, 64.09; H, 6.76; N, 9.69; P, 3.75.

S^6-Cyanoethyl-N^2-trifluoroacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-7-deaza-6-thioguanosine (11).

2'-Deoxy-7-deazaguanosine (0.9 g, 3.38 mmoles) was dried by co-evaporation with anhydrous pyridine (2 x 10 mL). The dried nucleoside was dissolved in anhydrous pyridine (15 mL), the reaction flask was cooled in an ice bath and trifluoroacetic anhydride (2.75 mL) was added. The mixture was stirred at 0-5 °C for 30 min and 3-mercaptopropionitrile (3.2 mL) was added. The ice bath was removed and the mixture was stirred at ambient temperature for 15 h. The reaction mixture was diluted with dichloromethane (100 mL) and the organic phase was washed with water (50 mL). The aqueous layer was reextracted with dichloromethane and the combined organic layers was dried (sodium sulfate) and evaporated. The residue was co-evaporated with toluene and the product was chromatographed on a silica gel column using 0-3% methanol-dichloromethane as the eluent to yield 1.15 g (79%) of the product. ^{1}H NMR (DMSO-d_6): δ 2.22 (m, 1 H, $C_{2'}H$), 2.52 (m, 1 H, $C_{2''}H$), 3.15 (t, 2 H, CH_2CN), 3.53 (m, 4 H, $C_{5'}H_2$, SCH_2), 3.84 (br s, 1 H, $C_{4'}H$), 4.37 (t, 1 H, $C_{3'}H$), 6.58 (t, 1 H, J = 6.4 Hz, $C_{1'}H$), 6.62 (d, 1 H, C_5H), 7.76 (d, 1 H, C_6H), and 11.98 (s, 1 H, N*H*).

The above compound (0.67 g, 1.55 mmoles) was dried by co-evaporation with anhydrous pyridine (2 x 10 mL). Then it was dissolved in anhydrous pyridine (20 ml) and the flask was cooled in an ice bath. To this cooled solution was added 4,4'-dimethoxytrityl chloride (0.66 g, 1.94 mmoles) in one portion and the mixture was stirred at 0-5 °C for 15 h. Methanol (2 mL) was added and after a further period of 10 min the reaction mixture was diluted with dichloromethane (100 mL). The organic phase was washed with water (50 mL). The aqueous layer was extracted with dichloromethane and the combined organic layers was dried (sodium sulfate). The organic phase was evaporated and the residue was co-evaporated with toluene. Finally, the product was purified by silica gel column chromatography using 0-1% methanol-dichloromethane as the eluent to yield 0.67 g (67%) of analytically pure material, mp 95-97 °C; ^{1}H NMR (DMSO-d_6): δ 2.29 (m, 1 H, $C_{2'}H$), 2.64 (m, 1 H, $C_{2''}H$), 3.16 (m, 4 H, $C_{5'}H_2$, CH_2CN), 3.57 (t, 2 H, SCH_2), 3.72 (s, 6 H, 2 OCH_3), 3.94 (m, 1 H, $C_{4'}H$), 4.40 (t, 1 H, $C_{3'}H$), 5.34 (d, 1 H, $C_{3'}OH$), 6.57 (t, 1 H, J = 6.8 Hz, $C_{1'}H$), 6.61 (d, 1 H, C_5H), 6.80 (t, 4 H, 4 Ar*H*), 7.22 (m, 7 H, 7 Ar*H*), 7.33 (m, 2 H, 2 Ar*H*), 7.57 (d, 1 H, C_6H), and 11.98 (s, 1 H, N*H*); *Anal.* Calcd. for $C_{37}H_{34}F_3N_5O_6S$: C, 60.56; H, 4.67; N, 9.54; F, 7.77; S, 4.37. Found: C, 60.53; H, 4.76; N, 9.25; F, 7.59; S, 4.06.

S^6-Cyanoethyl-N^2-trifluoroacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-7-deaza-6-thioguanosine-3'-*O*-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (12).

To a solution of **11** (0.45 g, 0.61 mmole) and diisopropylamine (42 μL, 0.3 mmole) in anhydrous dichloromethane (10 mL) was added tetrazole (22.6 mg, 0.3 mmole) under an argon atmosphere. The mixture was stirred at room temperature for 30 min and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (0.28 mL, 0.9 mmole) was added. The reaction was continued for 45 min, the tetrazolide salt was removed by filtration and the filtrate was diluted with ethyl acetate (50 mL). The organic phase was washed with cold 5% sodium hydrogen carbonate solution (20 mL). The organic layer was dried (sodium sulfate), evaporated and the residue was chromatographed on a silica gel column which was packed in a mixture of dichloromethane and ethyl acetate (69:30) containing 1% triethyl amine. The product was eluted using the same solvent. The fractions containing the product were pooled and evaporated. The product was dissolved in a small amount of dichloromethane (3 mL) and the solution was added to a vigorously stirred pentane (100 mL) at room temperature. The product that precipitated was collected by filtration and dried under vacuum to yield 0.48 g (84%) of analytically pure phosphoramidite. ^{31}P NMR (CD_3CN): δ 149.42, 149.65; ^1H NMR (CD_3CN): δ 1.15 [m, 12 H, 2 $CH(CH_3)_2$], 2.60 (m, 4 H, $C_{2'}H$, $C_{2''}H$, CH_2CN), 2.85 [m, 2 H, $CH(CH_3)_2$], 3.05 (m, 2 H, CH_2CN), 3.30 (m, 2 H, $C_{5'}H_2$), 3.60 (m, 4 H, SCH_2, OCH_2), 3.73, 3.77 (2 s, 6 H, 2 OCH_3), 4.16 (m, 1 H, $C_{4'}H$), 4.75 (m, 1 H, $C_{3'}H$), 6.52 (m, 2 H, $C_{1'}H$, C_5H), 6.78 (m, 4 H, 4 ArH), 7.30 (m, 10 H, 9 ArH, C_6H), and 9.45 (brs, 1 H, NH); *Anal.* Calcd. For $C_{46}H_{51}F_3N_7O_7PS$: C, 59.15; H, 5.50; N, 10.50; P, 3.32. Found: C, 59.03; H, 5.54; N, 10.50; P, 3.35.

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