Synthesis of Deoxydinucleoside Phosphates containing 6-Thio-substituted Purine Nucleobases

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An efficient preparative method to obtain, by the hydrogen phosphonate approach in solution, dinucleoside phosphates containing either 2'-deoxy-6-thioinosine or 2'-deoxy-6-thioguanosine at their 3' end, as in compounds **6** and **7**, is described. For this purpose appropriate 5'-OH-free sulfur-modified deoxynucleoside intermediates required for this synthesis have been defined, as exemplified with the case of 2'-deoxy-6-thioguanosine.

During the last few years, particular attention has been addressed to the synthesis of sulfur-substituted purine deoxyribosides and more recently to their incorporation into oligodeoxynucleotides. Indeed there is tremendous interest in such modified purine oligonucleotides because 6-mercaptopurine- and 6-thioguanine-deoxynucleosides are antitumour agents.[1] Selective uptake of the latter in the DNA of proliferative cells[1a] is supposed to induce lethal modifications, studies of which *via* synthetic models might be of relevance to cancer chemotherapy. In another domain, oligodeoxynucleotides containing these modified bases have been recently used in photoaffinity labelling experiments to investigate nucleic acid–protein interactions and nucleic acid tertiary structure. Thus, 2'-deoxy-6-thioguanosine served as a useful substrate in a study of the recognition site of Eco RV endonuclease and Eco RV methyl transferase[2] whereas we have recently made use of the photocross-linking properties of 2'-deoxy-6-thioinosine in a study of the tertiary folding of hammerhead[3] and hairpin[4] ribozyme domains. In these particular cases, the photolabelling studies were prompted by our discovery of the remarkable capacity of 2'-deoxy-6-thioinosine to undergo covalent bonding with a pyrimidine residue in a dinucleotide model system upon exposure to UV light (360 nm).[3] At the outset of our photochemical studies, we needed an efficient preparative procedure to obtain such dimers incorporating sulfur-modified purines. Here, we report a very convenient synthesis of 2'-deoxy-5'-*O*-thymidylyl-6-thioinosine (Tpds^{6}I) **6** and of 2'-deoxy-5'-*O*-thymidylyl-6-thioguanosine (Tpds^{6}G) **7** in which thioguanine was subsequently shown to undergo photoaddition to the 5'-pyrimidine residue in line with the observations made in the case of compound **6**.[3] The synthetic strategy which we propose extends, to 6-mercaptopurines, sulfur protection based on *S*-pivaloyloxymethylation of the thiocarbonyl function, which was shown to give good results in the pyrimidine series.[5]

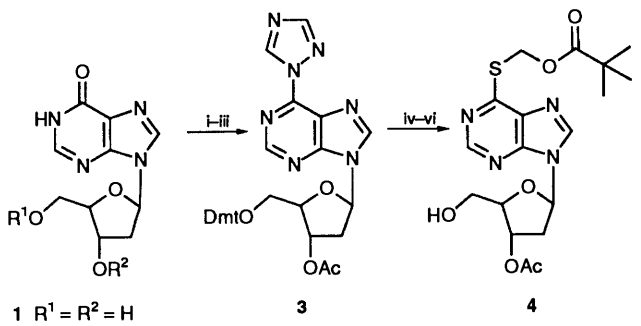
Results and Discussion

Usually, in ribonucleosides, a sulfur atom can be introduced by replacing a carbonyl oxygen atom of the heterocyclic base by treatment of an appropriate derivative with either diphosphorus pentasulfide or with Lawesson's reagent.[6] Unfortunately, in the 2'-deoxy series these methods are not suitable since, with such relatively acid- and temperature-sensitive compounds, cleavage of the glycosidic bond may occur.[1,7] For these reasons, 2'-deoxy-6-thio-inosine and -guanosine were initially obtained by condensation of a protected 2'-deoxy-D-*erythro*-pentofuranosyl chloride with various 6-chloropurine derivatives to give a mixture of α and β-isomers.[1a,8] The resulting 6-chloropurine deoxyriboside was treated with hydrogen sulfide (or sodium hydrogen sulfide) to give the desired thio compound. Enzymic procedures involving a transfer of the 2'-deoxy-D-*erythro*-pentofuranosyl group from 2'-deoxycytidine to 6-thioguanine or 6-mercaptopurine were also proposed.[9] Other routes, starting from 2'-deoxyadenosine N^1-oxide and based on a Dimroth-type rearrangement, led either to 2'-deoxy-6-thioguanosine[1b] or to 2'-deoxy-6-thioinosine[1c] in modest overall yield. Stereospecific sodium salt glycosylation procedures have also been reported.[10] Despite all these valuable efforts, it still remains attractive to prepare these compounds from their readily available oxo-analogues, 2'-deoxyinosine and 2'-deoxyguanosine. The first synthesis of 2'-deoxy-6-thioinosine which is based on this strategy appeared in 1973 and involved the chloro substitution of 2'-deoxyinosine at C-6 followed by sulfur replacement.[11] All the most recently developed methods involve the displacement of a 6-quaternary ammonium intermediate, generated by treatment of 6-trifluoroacetyl,[12] -sulfonyl[13] or -phosphorylpurine[14] derivative either with pyridine or *N*-methylpyrrolidine, by a sulfur nucleophile (sodium hydrogen sulfide or thiolacetic acid) leading to the 6-thiopurine analogue. Direct treatment of a sulfonyl derivative with lithium sulfide to give the corresponding 6-thio compound has also been reported.[15]

The ultimate goal of most of these recent syntheses was to incorporate 2'-deoxy-6-thio-guanosine or -inosine into oligonucleotides using phosphoramidite chemistry.[13b-20] With such intermediates, to avoid side-reactions which may occur during DNA synthesis, protection of the nucleophilic 6-thio (and 2-amino) group has been recommended. The sulfur can be masked either with a cyanoethyl,[15,19,20] a 2,4-dinitrophenyl,[13b,14] or a mesitylenesulfonyl group.[17] Protection of the 2-amino group of guanine is usually performed with either an isobutyryl,[13b,18,20] a benzoyl,[16,17] a phenylacetyl[13b] or a phenoxyacetyl group.[19]

The route that we have designed to synthesize compounds **6** and **7** in solution on a preparative scale relies on hydrogen-phosphonate chemistry. Our synthetic scheme was initially based upon the C-6 triazolylation of either a 2'-deoxyinosine or a 2'-deoxyguanosine derivative to give the appropriate precursor of the expected thio-modified deoxynucleoside whose 6-thiocarbonyl function would be readily protected by means of *S*-pivaloyloxymethylation.[5]

Thus, to introduce a sulfur-containing residue at the 3'-end of compound **6** (Tpds^{6}I) and **7** (Tpds^{6}G) dimers the preparation of the corresponding 5'-OH-free and 3'-*O*-acetylated deoxynucleoside unit was needed. In the case of inosine derivative **6**, the synthesis of the required 2'-deoxy-3'-*O*-acetyl-6-thio-

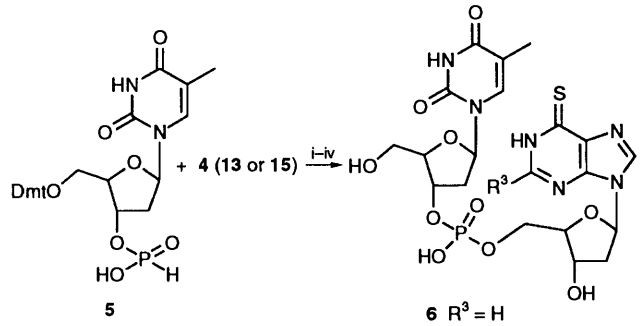


1 $R^1 = R^2 = H$
2 R^1 = Dmt, R^2 = Ac
Dmt = dimethoxytrityl

3 4

Scheme 1 *Reagents:* i, dimethoxytrityl chloride, pyridine; ii, Ac_2O, pyridine; iii, 1,2,4-triazole, triethylamine, phosphorus trichloride oxide, acetonitrile; iv, NaSH, DMF; v, pivaloyloxymethyl chloride, K_2CO_3; vi, CH_2Cl_2, 3% TFA

inosine derivative **4** starts from 2′-deoxyinosine **1**, which was dimethoxytritylated and acetylated by a one-flask, two-step procedure to give compound **2** (63% yield) (Scheme 1). Compound **2** was triazolylated by using 1,2,4-triazole and phosphorus trichloride oxide in acetonitrile in the presence of triethylamine in 63% yield. Treatment of a dimethylformamide (DMF) solution of the product **3**, with sodium hydrogen sulfide afforded the corresponding 6-thionucleoside, which was subsequently S-protected by using pivaloyloxymethyl chloride in acetone in the presence of potassium carbonate. Detritylation of the product was performed in a 3% trifluoroacetic acid (TFA)–methylene dichloride solution, leading to compound **4** in an overall yield of 48% (3 steps). Condensation of the 3′-*O*-acetyl derivative **4** with 3′-(5′-*O*-dimethoxytrityl)thymidylyl hydrogenphosphonate **5** followed by oxidation and deprotection of the resulting phosphate diester was performed according to the previously described procedure [5c] in 37.5% overall yield (4 steps) (Scheme 2).



5

Dmt = dimethoxytrityl

6 $R^3 = H$
7 $R^3 = NH_2$

Scheme 2 *Reagents:* i, bis(2-oxo-1,3-oxazolidin-3-yl)phosphinic chloride, acetonitrile–pyridine (1:1); ii, iodine, water–pyridine (1:9); iii, NH_4OH–pyridine (1:1); iv, 80% AcOH

We then tried to apply the above strategy to the preparation of 2′-deoxy-5′-*O*-thymidylyl-6-thioguanosine **7** starting from the commercially available 2′-deoxy-5′-*O*-dimethoxytrityl-2-*N*-isobutyrylguanosine which, after the same reaction sequence, gave 2′-deoxy-3′-*O*-acetyl-2-*N*-isobutyryl-6-(*S*-pivaloyloxymethyl)thioguanine. However, simultaneous deprotection of the 6-thio and 2-amino functions of this compound, by using a mixture of 30% ammonia–pyridine (1:1) at room temperature overnight, failed to regenerate the expected nucleoside. Instead, a fluorescent product was formed, which was not further characterized since it was supposed to be the corresponding 2,6-diaminopurine nucleoside in view of known observations indicating that treatment of 6-(alkylthio)guanosines with ammonia gives such a derivative.[15]

As a consequence of this failure, we had to develop another route. To circumvent the deprotection problem, we were pleased to find that it was possible to use the above proposed phosphorylation and deprotection methods without masking both the thione function and the 2-amino group or 2′-deoxy-6-thioguanine. In practice, we protected either the 6-thiocarbonyl group with a pivaloyloxymethyl group or the 2-N-amino function with an acetyl group. The 5′-hydroxy group of 2′-deoxyguanosine **8** was selectively protected as a dimethyl-(1,1,2-trimethylpropyl)silyl ether, also known as thexyldimethylsilyl ether, by treatment with thexyldimethylsilyl chloride in DMF in the presence of imidazole to give compound **9** in 53% yield (Scheme 3). Compound **9** was converted into its corresponding 6-pyridinium derivative by reaction with trifluoroacetic anhydride (TFAA) in pyridine,[8] and which, upon *in situ* treatment with sodium hydrogen sulfide, gave the 6-thio analogue **10** in 82% yield. Compound **10** was successively *S*-protected with pivaloyloxymethyl chloride as described above to give pivalate **11** in 75% yield and selectively 3′-*O*-acetylated to give compound **12** in 72% yield. Selective removal of the silyl group using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) afforded compound **13** (70% yield). To obtain the 2′-deoxy-2-*N*,3′-*O*-diacetyl-6-thioguanosine **15**, the silyl thionucleoside **10** was di-*O*,*N*-acetylated by using acetic anhydride together with 4-(dimethylamino)pyridine (DMAP) in pyridine to provide compound **14** in 63% yield which was then desilylated to give compound **15** (88%) yield).

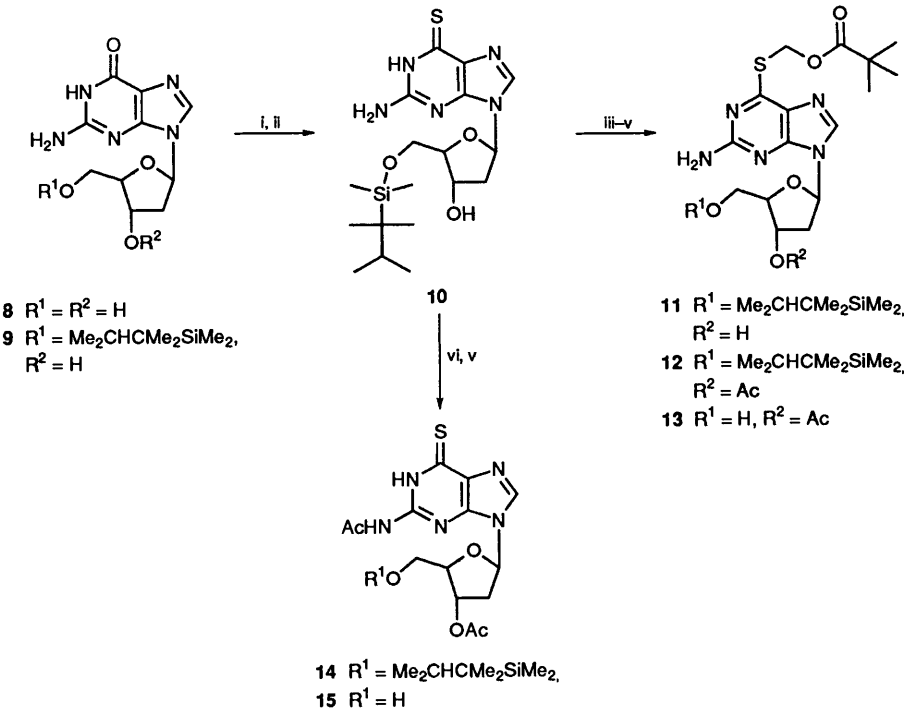
The synthesis of 2′-deoxy-5′-*O*-thymidylyl-6-thioguanosine **7** by condensation of either derivative **13** or **15** with 3′-(5′-*O*-dimethoxytrityl)thymidinyl hydrogenphosphonate **5** was performed according to the above described procedure (42%, 4 steps). Removal of the *S*-pivaloyloxymethyl group required treatment for 2 days at 60 °C with 30% ammonia–pyridine (1:1) whereas the elimination of the 2-N-acetyl group occurred smoothly in methanol–water–triethylamine (8:1:1) overnight at room temperature.

In conclusion, dinucleoside phosphates containing either 2′-deoxy-6-thioguanosine or 2′-deoxy-6-thioinosine at the 3′-end have been prepared in satisfactory yield by the hydrogenphosphonate approach in solution under mild reaction conditions and starting from readily accessible deoxynucleosides. In the 6-thioguanine case, under our initially proposed conditions, simultaneous deprotection of the 2-N and 6-S positions appeared to be problematic. This difficulty could be easily overcome since we found that efficient coupling and deprotection resulted by simple protection of only one of these two functions either by *N*-acetylation or by *S*-pivaloyloxymethylation.

Experimental

^1H, ^{13}C and ^{31}P NMR spectra were recorded on Bruker AC 250 or AM 400, Bruker AC 250, and Bruker AC 300-P spectrometers, respectively. ^1H Chemical shifts (δ_H) are reported either relative to residual $CHCl_3$ (δ 7.27) in deuteriochloroform ($CDCl_3$), to residual CHD_2SOCD_3 (δ 2.6) in [$^2H_6$]dimethyl sulfoxide ([$^2H_6$]DMSO) or to residual HOD (δ 4.8) in deuterium oxide (D_2O); *J* values are given in Hz; exchangeable protons are asterisked. ^{13}C Chemical shifts (δ_C) are reported relative to $CDCl_3$ (δ_C 77.7) in $CDCl_3$, to [$^2H_6$]DMSO (δ_C 43.5) in [$^2H_6$]DMSO, and to dioxane (δ_C 67.8) in D_2O. ^{31}P Chemical shifts (δ_P) are reported relative to an external capillary standard of 85% phosphoric acid.

Mass spectrometry was carried out using a AEI MS 50 instrument for electron-impact spectra (EI), or a Kratos MS 80 machine for fast-atom-bombardment spectra (FAB) using thioglycerol–NaCl as the matrix unless otherwise stated.



Scheme 3 *Reagents and conditions:* i, dimethylthexylsilyl chloride, imidazole, DMF, room temp.; ii, TFAA pyridine, 0 °C, NaSH, DMF, room temp.; iii, pivaloyloxymethyl chloride, K_2CO_3, acetone, room temp.; iv, Ac_2O, pyridine, room temp.; v, TBAF tetrahydrofuran (THF), room temp.; vi, Ac_2O, DMAP, pyridine

M.p.s were determined with a Reichert apparatus and are uncorrected.

Pyridine and acetonitrile (HLPC grade, water < 0.05%) were dried by heating, under reflux, with calcium hydride and diphosphorus pentaoxide (P_2O_5), respectively.

5′-*O*-Dimethoxytrityl-3′-thymidylyl hydrogenphosphonate triethylammonium salt **5** was synthesized according to a published procedure.[21] 3′-Hydrogenphosphonate and 3′-*O*-acetyl nucleoside derivatives were dried at room temperature in a desiccator over P_2O_5 *in vacuo* overnight before condensation.

All reactions were carried out at room temperature unless otherwise stated.

Chromatography was performed on Merck 7729 silica gel. TLC was run on pre-coated silica gel sheets with a luminescer (254 nm) manufactured by Schleicher and Schüll (ref. 394 732). Lichroprep RP 18 (Merck 13 900) was employed for reversed-phase chromatography.

3′-O-*Acetyl*-5′-O-*dimethoxytrityl*-2′-*deoxyinosine* **2**.—To a suspension of 2′-deoxyinosine **1** (1 g, 4 mmol) in pyridine (15 cm^3) was added portionwise dimethoxytrityl chloride (1.44 g, 1.1 mol equiv.). The reaction mixture was stirred overnight at room temperature, then acetic anhydride (4 cm^3) was added and the mixture was stirred for a further 2 h. After addition of methanol (2 cm^3), the mixture was concentrated under reduced pressure, diluted with CH_2Cl_2, washed successively with aq. sodium hydrogen carbonate and twice with brine, and dried over sodium sulfate. The solution was then concentrated under reduced pressure. The crude product was purified by chromatography using a gradient of methanol in ethyl acetate (0–10%) to give the title compound **2** (1.5 g, 63.5%); FAB *m/z* 619 ($M + Na^+$); $\delta_H(CDCl_3)$ 2.13 (3 H, s, $MeCO_2$), 2.63 (1 H, br dd, *J* 6.1 and 13.7, 2′-H), 2.92 (1 H, ddd, *J* 6.1, 8.1 and 14.1, 2′-H), 3.44 (2 H, m, 5′-H_2), 3.78 (6 H, s, MeO Dmt), 4.30 (1 H, br s, 4′-H), 5.51 (1 H, br d, *J* 6.1, 3′-H), 6.43 (1 H, dd, *J* 5.7 and 8.4, 1′-H), 6.83 (4 H, d, *J*, 7.2, CH Dmt), 7.10–7.50 (9 H, m, CH Dmt) and 7.99 and 8.17 (2 H, 2 s, 2- and 8-H); $\delta_C(CDCl_3)$ 21.6 (*Me*CO_2) 38.9 (C-2′), 55.9 (MeO Dmt), 64.3 (C-5′), 75.8 (C-3′), 85.1 (C-1′ and -4′), 87.4 (C Dmt), 113.9 (CH Dmt), 125.7 (C-5), 127.6, 128.5, 128.8 and 130.7 (CH Dmt), 136.1 (C Dmt), 138.9 (C-8), 145.0 and 145.7 (C-4 and C Dmt), 149.4 (C-2), 159.3 (C Dmt), 159.8 (C-6) and 170.9 (Me*C*O_2).

3′-O-*Acetyl*-5′-O-*dimethoxytrityl*-6-(1,2,4-*triazolyl*)-2′-*deoxyinosine* **3**.—To a mixture of dried acetate **2** (900 mg, 1.5 mmol) and 1,2,4-triazole (1.725 g, 25 mmol) in acetonitrile (12 cm^3) was added triethylamine (3.75 cm^3). The reaction mixture was chilled to 0 °C, then phosphorus trichloride oxide (560 mm^3, 6 mmol) was added and the mixture was stirred for 2 days at room temperature; then it was poured into saturated aq. sodium hydrogen carbonate and extracted with CH_2Cl_2; the extract was washed twice with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by chromatography using a gradient of ethyl acetate in heptane (0–10%) to give compound **3** (614 mg, 63%); FAB (3-nitrobenzyl alcohol–LiCl) *m/z* 654 ($M + Li^+$); $\delta_H(CDCl_3)$ 2.15 (3 H, s, $MeCO_2$), 2.73 (1 H, ddd, *J* 1.9, 5.7 and 13.7, 2′-H), 3.05 (1 H, m, 2′-H), 3.48 (2 H, m, 5′-H_2), 3.67 (6 H, s, MeO Dmt), 4.36 (1 H, br s, 4′-H), 5.58 (1 H, d, *J* 6.1, 3′-H), 6.59 (1 H, dd, *J* 6.1 and 8.0, 1′-H), 6.80 (4 H, d, *J* 9.2, CH Dmt), 7.10–7.50 (9 H, m, CH Dmt) and 8.0–9.70 (4 H, 2- and 8-H, and CH triazolyl); $\delta_C(CDCl_3)$ 21.6 (*Me*CO_2), 39.0 (C-2′), 55.8 (MeO Dmt), 64.2 (C-5′), 75.7 (C-3′), 85.4 (C-1′ and -4′), 87.5 (C Dmt), 113.9, 127.6, 128.6, 128.7 and 130.6 (C-5 and CH Dmt), 136.0 (C Dmt), 141.5 (C-8), 144.6, 144.9, 146.1, 152.8, 154.6 and 159.3 (C-2, -4, -6, CH triazolyl, and C Dmt) and 170.8 (Me*C*O_2).

3′-O-*Acetyl*-S-(*pivaloyloxymethyl*)-6-*thio*-2′-*deoxyinosine* **4**.—Sodium hydrogen sulfide (800 mg) was added to a stirred solution of compound **3** (1.25 g, 1.9 mmol) in DMF (5 cm^3). After 6 h, the reaction mixture was diluted with ethyl acetate and washed three times with brine, dried over sodium sulfate, and evaporated (1.288 g, crude product). The crude product was

dissolved in acetone (17 cm^3) and potassium carbonate (2.3 g, 8.6 mol equiv.) was added followed by pivaloyloxymethyl chloride (720 mm^3, 2.5 mol equiv.). After reaction overnight the mixture was filtered on Celite; the filtrate was concentrated, diluted with CH_2Cl_2, and washed twice with brine, dried over sodium sulfate, and concentrated to dryness.

The crude product (1.573 g) was treated with a solution of CH_2Cl_2 (40 cm^3) containing 3% TFA for 30 min at room temperature. Then methanol (4 cm^3) was added. After 5 min, the mixture was diluted with CH_2Cl_2, washed successively with saturated aq. sodium hydrogen carbonate and twice with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by chromatography using a gradient of ethyl acetate in heptane (50–100%) to give the *title compound* **4** [396 mg, 48% overall yield (3 steps)]; m.p. 107 °C (from ethyl acetate) (Found: C, 51.05; H, 5.5; N, 13.1; S, 7.5. $C_{18}H_{24}N_4O_6S$ requires C, 50.94; H, 5.70; N, 13.20; S, 7.54%); EI *m/z* 424 (M); $\delta_H(CDCl_3)$ 1.13 (9 H, s, Me_3CCO_2), 2.09 (3 H, s, $MeCO_2$), 2.47 (1 H, dd, *J* 5.3 and 14.5, 2′-H), 3.08 (1 H, ddd, *J* 6.1, 9.9 and 14.5, 2′-H), 3.91 (2 H, m, 5′-H_2), 4.24 (1 H, br s, 4′-H), 5.52 (1 H, d, *J* 5.3, 3′-H), 5.67 (1 H*, br d), 5.97 (2 H, s, $Me_3CCO_2CH_2$), 6.38 (1 H, dd, *J* 5.3 and 9.2, 1′-H) and 8.20 and 8.70 (2 H, 2 s, 2-and 8-H); $\delta_C(CDCl_3)$ 21.5 ($MeCO_2$), 27.4 ($Me_3CCO_2CH_2$), 38.4 (C-2′), 39.2 ($Me_3CCO_2CH_2$), 60.9 ($Me_3CCO_2CH_2$), 63.5 (C-5′), 76.5 (C-3′), 87.6 (C-1′ and -4′), 133.2 (C-5), 143.3 (C-8), 148.4 (C-4), 151.6 (C-2), 160.0 (C-6), 170.8 ($MeCO_2$) and 178.5 ($Me_3CCO_2CH_2$).

5′-O-*Thymidylyl-6-thio-2′-deoxyinosine* **6**.—*Condensation.* To a mixture of dried hydrogenphosphonate **5** (101 mg, 0.14 mmol) and acetate **4** (67 mg, 0.16 mmol, 1.1 mol equiv.) in anhydrous pyridine–acetonitrile (1:1) (1 cm^3) was added bis(2-oxo-1,3-oxazolidin-3-yl)phosphinic chloride (75 mg, 0.294 mmol, 2.1 mol equiv.). The reaction mixture was stirred for 1 h at room temperature, then was diluted with CH_2Cl_2 washed twice with brine, and concentrated under reduced pressure.

Oxidation. Water (30 mm^3), then iodine (32 mg), were added to the above residue diluted in pyridine (500 mm^3). After 1 h, CH_2Cl_2 was added and the organic phase was washed successively with a saturated aq. sodium thiosulfate, aq. sodium hydrogen carbonate, and twice with brine. Then the organic phase was dried over sodium sulfate and concentrated under reduced pressure.

Deprotection: Removal of the pivaloyloxymethyl and acetyl protecting groups. The above residue (160 mg) was dissolved in a mixture of NH_4OH–pyridine (1:1) (1.5 cm^3) and left overnight at room temperature. Then the solution was concentrated under reduced pressure and the residue was chromatographed on silica gel using a gradient of methanol in CH_2Cl_2 (0–20%) containing 30% NH_4OH (1%). The fractions containing the tritylated phosphate dimer were pooled and evaporated.

Detritylation. The above residue was taken up in 80% acetic acid (2 cm^3). After 1 h at room temperature, the solution was concentrated under reduced pressure after addition of methanol, then was diluted with water, washed three times with CH_2Cl_2, and concentrated. The crude product was purified on a short column (4 × 1 cm i.d.) of reversed-phase silica gel RP 18 with water as eluent (18 cm^3), then with water–acetonitrile 2% (18 cm^3), 5% (18 cm^3) and 10% (18 cm^3). Fractions (3 cm^3) were collected. Those containing title compound **6** were concentrated, then lyophilized to give a foam (30 mg, 37.5%, 4 steps); FAB *m/z* 573 ($M + H^+$); $\delta_H(D_2O)$* 1.86 (1 H, m, 2′-H) Tp), 1.88 (3 H, s, Me Tp), 2.24 (1 H, br dd, *J* 6.0 and 14.2, 2′-H Tp), 2.65 (1 H, dt, *J* 13.9 and 5.6, 2′-H I), 2.93 (1 H, dt, *J* 14.0 and 6.6, 2′-H I), 3.67 (2 H, br s, 5′-H_2 Tp), 4.05 (1 H, br s, 4′-H Tp), 4.10 (2 H, br s, 5′-H_2 I), 4.26 (1 H, br s, 4′-H I), 4.66 (1 H, br s, 3′-H Tp), 4.80 (1 H, 3′-H I), 6.02 (1 H, t, *J* 6.4, 1′-H Tp) and 6.48 (1 H, t, *J* 6.0, 1′-HI); $\delta_C(D_2O)$* 12.9 (Me Tp), 38.3 (C-2′ Tp), 39.9 (C-2′ I), 62.4 (C-5′ Tp), 66.2 (C-5′ I), 71.8 (C-3′ I), 76.8 (C-3′ Tp), 85.2 (C-1′ I), 86.1 (C-1′ Tp), 86.9 (C-4′ Tp), 87.1 (C-4′ I), 112.8 (C-5 Tp), 136.1 (C-5 I), 138.2 (C-6 Tp), 143.2 (C-8 I), 145.1 (C-4 I), 146.9 (C-2 I), 152.4 (C-2 Tp), 167.4 (C-4 Tp) and 176.5 (C-6 I).

5′-O-*Dimethylthexylsilyl-2′-deoxyguanosine* **9**.—2′-Deoxyguanosine **8** (5 g, 18.72 mmol) was dried by three coevaporations with anhydrous pyridine. To the dried foam were added dry DMF (40 cm^3), imidazole (2.7 g, 2 mol equiv.) and dimethylthexylsilyl chloride (5 cm^3, 1.35 mol equiv.). After being stirred for 30 min at room temp., the reaction medium was concentrated under reduced pressure, diluted in ethyl acetate (400 cm^3), and washed with brine. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude product was chromatographed on silica gel using a gradient of methanol in CH_2Cl_2 (0–30%) to give the title compound **9** (4.06 g, 53.1%); FAB *m/z* 432 ($M + Na^+$) and 454 ($M - H^+ + 2\ Na^+$); $\delta_H([^2H_6]DMSO)$ 0.15 (6 H, s, Me_2Si), 0.91 (12 H, $Me_2CHMe_2CMe_2Si$), 1.64 (1 H, m, $Me_2CHMe_2CMe_2Si$), 2.32 (1 H, m, 2′-H), 2.60 (1 H, m, 2′-H), 3.77 (2 H, m, 5′-H_2), 3.91 (1 H, m, 4′-H), 4.40 (1 H, m, 3′-H), 5.41 (1 H*, m), 6.20 (1 H, t, *J* 6.4 1′-H), 6.58 (2 H*, br s), 7.93 (1 H, s, 8-H) and 10.76 (1 H*, s); $\delta_C([^2H_6]DMSO)$ 0.5 (Me_2Si), 22.3 and 24.2 ($Me_2CHMe_2CMe_2Si$), 28.8 ($Me_2CHMe_2CMe_2Si$), 37.7 ($Me_2CHMe_2CMe_2Si$), 43.6 (C-2′), 67.2 (C-5′), 74.5 (C-3′), 86.5 and 91.0 (C-1′ and -4′), 120.6 (C-5), 138.9 (C-8), 154.9 (C-4), 157.7 (C-2) and 160.9 (C-6).

5′-O-*Dimethylthexylsilyl-6-thio-2′-deoxyguanosine* **10**.—After being dried by coevaporation with anhydrous pyridine, compound **9** (1.6 g, 3.91 mmol) was diluted in pyridine (80 cm^3). The solution was chilled to 0 °C and TFAA (4.6 cm^3) was added slowly under nitrogen. After being stirred for 40 min at room temperature the mixture was treated with a mixture of sodium hydrogen sulfide (6.8 g, 39 mmol) in dry DMF (120 cm^3) and kept overnight at room temperature. The mixture was then poured into vigorously stirred 0.16 mol dm^{-3} aq. ammonium hydrogen carbonate (200 cm^3). The mixture was then concentrated to dryness, diluted with CH_2Cl_2, and washed successively with 0.1 mol dm^{-3} triethylammonium hydrogen carbonate and brine. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified on silica gel using a gradient of methanol in CH_2Cl_2 (0–40%) to give the title compound **10** (1.37 g, 82.2%); FAB *m/z* 426 ($M + H^+$) and 448 ($M + Na^+$); $\delta_H([^2H_6]$-DMSO) 0.17 (6 H, s, Me_2Si), 0.91 (6 H, s, $Me_2CHMe_2CMe_2Si$), 0.94 (6 H, d, *J* 7.5, $Me_2CHMe_2CMe_2Si$), 1.67 (1 H, m, $Me_2CHMe_2CMe_2Si$), 2.34 (1 H, ddd, *J* 4.3, 6.8 and 13.7, 2′-H), 2.60 (1 H, m, 2′-H), 3.79 (2 H, m, 5′-H_2), 3.93 (1 H, m, 4′-H), 4.41 (1 H, m, 3′-H), 5.43 (1 H*, d, *J* 4.3), 6.20 (1 H, t, *J* 6.8, 1′-H), 6.93 (2 H*, br s), 8.12 (1 H, s, 8-H) and 12.06 (1 H*, br s); $\delta_C([^2H_6]DMSO)$ 0.5 (Me_2Si), 22.3 and 24.2 ($Me_2CHMe_2CMe_2Si$), 28.7 ($Me_2CHMe_2CMe_2Si$), 37.6 ($Me_2CHMe_2CMe_2Si$), 43.5 (C-2′), 67.1 (C-5′), 74.3 (C-3′), 86.6 and 91.1 (C-1′ and -4′), 132.3 (C-5), 141.7 (C-8), 151.4 (C-4), 157.1 (C-2) and 179.1 (C-6).

5′-O-*Dimethylthexylsilyl*-6-(S-*pivaloyloxymethyl*)-6-*thio*-2′-*deoxyguanosine* **11**.—To a stirred solution of compound **10** (1.72 g, 4.05 mmol) in acetone (260 cm^3) were added potassium carbonate (834 mg, 1.5 mol equiv.) and pivaloyloxymethyl chloride (1.04 cm^3, 1.8 mol equiv.). After 4 days, the reaction mixture was filtered on Celite, and the filtrate was concentrated

* Tp = thymidylyl residue, I = inosine residue.

under reduced pressure, diluted with CH_2Cl_2, washed with brine, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel, and eluted with heptane, then with a gradient of ethyl acetate in heptane (50–100%), to give compound **11** (1.64 g, 75.2%); FAB *m/z* 540 ($M + H^+$) and 562 ($M + Na^+$); $\delta_H([^2H_6]DMSO)$ 0.14 (6 H, s, Me_2Si), 0.88 (6 H, s, $Me_2CHMe_2CMe_2Si$), 0.91 (6 H, d, *J* 7.5, $Me_2CHMe_2CMe_2Si$), 1.20 (9 H, s, Me_3CCO_2), 1.64 (1 H, m, $Me_2CHMe_2CMe_2Si$), 2.36 (1 H, m, 2'-H), 2.70 (1 H, m, 2'-H), 3.79 (2 H, m, 5'-H_2), 3.93 (1 H, m, 4'-H), 4.44 (1 H, m, 3'-H), 5.42 (1 H*, d, *J* 3.8), 6.05 (2 H, s, $Me_3CO_2CH_2$), 6.31 (1 H, t, *J* 6.1, 1'-H), 6.78 (2 H*, br s) and 8.22 (1 H, s, 8-H); $\delta_C([^2H_6]DMSO)$ 0.4 (Me_2Si), 22.2 and 24.1 (Me_2CHMe_2Si), 28.7 ($Me_2CHMe_2CMe_2Si$), 30.5 (Me_3CCO_2), 37.6 ($Me_2CHMe_2CMe_2Si$), 42.1 (Me_3CCO_2), 43.1 (C-2'), 64.1 ($Me_3CCO_2CH_2$), 67.1 (C-5'), 74.3 (C-3'), 86.6 and 91.0 (C-1' and -4'), 128.1 (C-5), 143.1 (C-8), 155.3, 160.1 and 163.4 (C-4, -2 and -6) and 181.3 ($Me_3CCO_2CH_2$).

3'-O-*Acetyl*-5'-O-*dimethylthexylsilyl*-6-(S-*pivaloyloxymethyl*)-6-*thio*-2'-*deoxyguanosine* **12**.—After being dried by coevaporation with anhydrous pyridine, compound **11** (1.05 g, 1.948 mmol) was diluted in pyridine (9.7 cm^3). Then acetic anhydride (1.95 cm^3) was added. After the mixture had been stirred for 90 min at room temperature, methanol (2 cm^3) was added and, after 5 min, the reaction mixture was concentrated under reduced pressure. The residue was then diluted with CH_2Cl_2 and the organic phase was washed twice with brine, dried over sodium sulfate, and evaporated under reduced pressure. The product was then purified by column chromatography on silica gel using ethyl acetate–heptane (1:3) to give the title compound (819 mg, 72.4%); FAB *m/z* 582 ($M + H^+$) and 604 ($M + Na^+$); $\delta_H(CDCl_3)$ 0.12 (6 H, s, Me_2Si), 0.87 (12 H, $Me_2CHMe_2CMe_2Si$), 1.16 (9 H, s, Me_3CCO_2), 1.60 (1 H, m, $Me_2CHMe_2CMe_2Si$), 2.10 (3 H, s, $MeCO_2$), 2.50 (1 H, dd, *J* 13.0 and 5.3, 2'-H), 2.65 (ddd, *J* 6.1, 9.2 and 13.0, 2'-H), 3.84 (2 H, m, 5'-H_2), 4.15 (1 H, m, 4'-H), 5.03 (2 H, br s), 5.38 (1 H, m, 3'-H), 5.92 (2 H, s, $Me_3CCO_2OCH_2$), 6.31 (1 H, dd, *J* 5.3 and 8.4, 1'-H) and 7.96 (1 H, s, 8-H); $\delta_C(CDCl_3)$ −3.0 and −2.8 (Me_2Si), 19.1 and 21.0 ($Me_2CHMe_2CMe_2Si$), 21.6 ($MeCO_2$), 26.0 ($Me_2CH(Me)_2CMe_2Si$), 27.6 (Me_3CCO_2), 34.7 ($Me_2CHMe_2CMe_2Si$), 39.0 (C-2), 39.3 (Me_3CCO_2), 61.3 ($Me_3CCO_2CH_2$), 64.0 (C-5'), 75.9 (C-3'), 84.1 and 86.0 (C-1' and -4'), 126.5 (C-5), 138.9 (C-8), 151.6, 159.2 and 159.5 (C-2, -4 and -6), 171.0 ($MeCO_2$) and 178.6 ($Me_3CCO_2CH_2$).

3'-O-*Acetyl*-6-(S-*pivaloyloxymethyl*)-6-*thio*-2'-*deoxyguanosine* **13**.—To a stirred solution of compound **12** (800 mg, 1.38 mmol) in anhydrous THF (40 cm^3) was added 1 mol dm^{-3} TBAF in THF (5.5 cm^3, 4 mol equiv.). After being stirred for 15 min at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed twice with brine, dried over sodium sulfate, and evaporated to dryness. The residue was purified on silica gel using ethyl acetate–heptane (1:1) to give the *title compound* **13** (424 mg, 70.2%). Crystallization of the latter material from ethyl acetate gave crystals, m.p. 154–156 °C (Found: C, 49.3; H, 5.7; N, 15.9; S, 7.2. $C_{18}H_{25}N_5O_6S$ requires C, 49.20; H, 5.73; N, 15.93; S, 7.29%); FAB *m/z* 440 ($M + H^+$) and 462 ($M + Na^+$); $\delta_H(CDCl_3)$ 1.19 (9 H, s, Me_3CCO_2), 2.14 (3 H, $MeCO_2$), 2.40 (1 H, dd, *J* 5.3 and 14.5, 2'-H), 3.14 (1 H, ddd, *J* 6.1, 9.9 and 14.5, 2'-H), 3.94 (2 H, m, 5'-H_2), 4.26 (1 H, br s, 4'-H), 5.19 (2 H*, br s), 5.54 (1 H, d, *J* 6.1, 3'-H), 5.92 (2 H, s, $Me_3CCO_2CH_2$), 6.21 (1 H, *J* 5.3 and 9.2, 1'-H) and 7.75 (1 H, s, 8-H); $\delta_C(CDCl_3)$ 21.7 ($MeCO_2$), 27.6 (Me_3CCO_2), 37.8 (C-2'), 39.4 (Me_3CCO_2), 61.1 ($Me_3CCO_2CH_2$), 63.9 (C-5'), 77.0 (C-3'), 87.8 and 88.0 (C-1' and -4'), 127.5 (C-5), 140.5 (C-8), 150.2, 158.5 and 161.0 (C-2, -4 and -6), 170.9 ($MeCO_2$) and 178.6 ($Me_3CCO_2CH_2$).

2-N,3'-O-*Diacetyl*-5'-O-*dimethylthexylsilyl*-6-*thio*-2'-*deoxyguanosine* **14**.—To a stirred solution of compound **10** (522 mg, 1.23 mmol) in pyridine (5 cm^3) were added DMAP (147 mg, 1 mol equiv.) and acetic anhydride (4.92 cm^3). After being stirred for 3 days, the mixture was treated with methanol and then concentrated. After dilution with CH_2Cl_2, the organic phase was washed successively with aq. sodium hydrogen carbonate, then twice with water, and was dried over sodium sulfate and was then concentrated under reduced pressure. The residue was purified by flash chromatography using a gradient of ethyl acetate in heptane (25–100%) to give *compound* **14** (397 mg, 63.5%), m.p. 163–166 °C (from ethyl acetate) (Found: C, 51.6; H, 6.7; N, 13.8; S, 6.4. $C_{22}H_{35}N_5O_5SSi$ requires C, 51.84; H, 6.92; N, 13.74; S, 6.29%); FAB *m/z* 510 ($M + H^+$) and 532 ($M + Na^+$); $\delta_H(CDCl_3)$ 0.08 (6 H, s, Me_2Si), 0.80 (12 H, $Me_2CHMe_2CMe_2Si$), 1.55 (1 H, m, $Me_2CHMe_2CMe_2Si$), 2.08 (3 H, s, $MeCO_2$), 2.33 (3 H, s, MeCON), 2.54 (2 H, m, 2'-H_2), 3.81 (2 H, m, 5'-H_2), 4.13 (1 H, m, 4'-H), 5.31 (1 H, br s, 3'-H), 6.15 (1 H, t, *J* 7.2, 1'-H) and 8.14 (1 H, s, 8-H); $\delta_C(CDCl_3)$ −3.0 (Me_2Si), 19.0 and 20.8 ($Me_2CHMe_2CMe_2Si$), 21.6 ($MeCO_2$), 25.1 (*Me*CON), 25.9 ($Me_2CHMe_2CMe_2Si$), 34.5 ($Me_2CHMe_2CMe_2Si$), 39.6 (C-2'), 63.8 (C-5'), 75.5 (C-3'), 84.8 and 86.3 (C-1' and -4'), 133.2 (C-5), 139.7 (C-8), 144.4 and 147.6 (C-2 and -4), 171.1 ($MeCO_2$) and 173.2 and 175.8 (MeCON and C-6).

2-N,3'-O-*Diacetyl*-6-*thio*-2'-*deoxyguanosine* **15**.—Compound **14** (374 mg, 0.734 mmol) was desilylated as described for compound **13** to give *compound* **15** (238 mg, 88.5%), m.p. 177–183 °C (Found: C, 45.55; H, 4.6; N, 19.0; S, 8.7. $C_{14}H_{17}N_5O_5S$ requires C, 45.77; H, 4.66; N, 19.06; S, 8.70%); FAB *m/z* 368 ($M + H^+$) and 390 ($M + Na^+$); $\delta_H([^2H_6]DMSO)$ 2.18 (3 H, s, $MeCO_2$, 2.32 (3 H, s, MeCON), 2.60 (1 H, m, 2'-H), 2.95 (1 H, ddd, *J* 6.1, 9.2 and 14.5, 2'-H), 3.70 (2 H, br s, 5'-H_2), 4.16 (1 H, br s, 4'-H), 5.22 (1 H*, br s), 5.42 (1 H, d, 3'-H), 6.29 (1 H, dd, *J* 6.1 and 9.0, 1'-H) and 5.82 (1 H, s, 8-H); $\delta_C([^2H_6]DMSO)$ 24.8 ($MeCO_2$), 27.8 (MeCON), 40.7 (C-2'), 65.3 (C-5'), 78.8 (C-3'), 87.2 and 89.2 (C-1' and -4'), 135.8 (C-5), 143.8 (C-8), 149.0 and 151.4 (C-2 and -4), 173.9 ($MeCO_2$), 177.8 and 178.2 (MeCON and C-6).

5'-O-*Thymidylyl*-6-*thio*-2'-*deoxyguanosine* **7**.—*Method A: Condensation.* To a solution of dried hydrogenphosphonate **5** (152 mg, 0.214 mmol) and acetate **13** (112 mg, 0.255 mmol) in a mixture of anhydrous pyridine–acetonitrile (1:1) (1.8 cm^3) was added bis(2-oxo-1,3-oxazolidin-3-yl)phosphinic chloride (125 mg, 0.49 mmol). The reaction mixture was stirred for 1.5 h at room temperature, then was diluted with CH_2Cl_2. The organic phase was washed twice with brine and then concentrated under reduced pressure.

Oxidation. To the above residue diluted with pyridine (500 mm^3) and water (30 mm^3) was added iodine (67 mg). After 1 h, CH_2Cl_2 was added and the organic phase was washed successively with saturated aq. sodium thiosulfate, aq. sodium hydrogen carbonate, and finally twice with brine. After being dried over sodium sulfate the organic phase was concentrated under reduced pressure.

Deprotection. Removal of the S-*pivaloyloxymethyl and* O-*acetyl protecting groups.* The above residue was taken up in NH_4OH–pyridine (1:1) (3 cm^3) and the resulting solution was maintained at 60 °C for 2 days. Then the solution was concentrated under reduced pressure.

Detritylation. The residue was taken up in 80% acetic acid (2 cm^3) during 1 h at room temperature. The solution was concentrated under reduced pressure after addition of methanol, then was diluted with water and washed three times with CH_2Cl_2 and concentrated. The crude product was purified on a short column (4 × 1 cm i.d.) of reversed-phase silica gel RP 18 with water as eluent (18 cm^3), then with water–acetonitrile

1% (18 cm^3), 2% (18 cm^3), 5% (18 cm^3) and 10% (18 cm^3). Fractions of 3 cm^3 were collected. Those containing title compound **7** were concentrated, and then lyophilized to give compound **7** as a foam (53 mg, 42.4%, 4 steps).

Method B. A mixture of compound **15** (109 mg, 0.297 mmol) and hydrogenphosphonate **5** (231 mg, 0.325 mmol) was treated as described in method A except that for the first deprotection step (O- and 2-N-deacetylation) was performed in a mixture of methanol–triethylamine–water (8:1:1) overnight at room temperature to give compound **7** in 41.3% overall yield (4 steps); FAB *m/z* 588 ($M + H^+$) and 610 ($M + Na^+$); $\delta_P(D_2O)$ 0.533; $\delta_H(D_2O)$* 1.74 (1 H, m, 2'-H Tp), 1.85 (3 H, s, Me Tp), 2.21 (1 H, m, 2'-H Tp), 2.57 (1 H, m, 2'-H G), 2.86 (1 H, m, 2'-H G), 3.64 (2 H, m, 5'-H_2 Tp), 4.06 (3 H, m, 4'-H Tp and 5'-H_2 G), 4.19 (1 H, m, 4'-H, G), 4.62 (1 H, m, 3'-H Tp), 4.80 (1 H, m, 3'-H G), 6.03 (1 H, t, *J* 6.9, 1'-H Tp), 6.20 (1 H, t, *J* 6.4, 1'-H G), 7.43 (1 H, s, 6-H Tp) and 8.14 (1 H, s, 8-H G); $\delta_C(D_2O)$* 13.0 (Me Tp), 38.2 (C-2' Tp), 39.4 (C-2' G), 62.5 (C-5' Tp), 66.2 (C-5' G), 71.9 (C-3' G), 77.1 (C-3' Tp), 84.5 (C-1 G), 86.2 (C-1' Tp), 86.7 (C-4' G), 86.9 (C-4' Tp), 112.7 (C-5 Tp), 129.2 (C-5 G), 138.1 (C-6 Tp), 141.1 (C-8 G), 148.7 (C-4 G), 152.4 (C-2 Tp), 154.4 (C-2 G), 167.3 (C-4 Tp) and 175.2 (C-6 G).

* Tp = thymidylyl residue, G = guanosine residue.

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