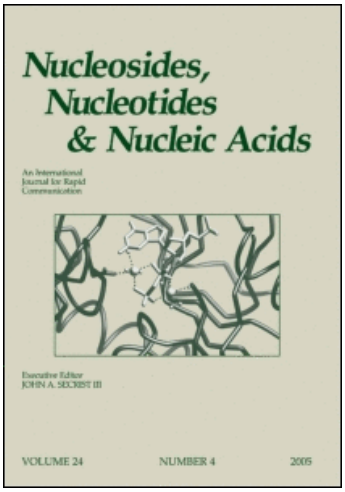

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Straightforward Synthesis of 6-Thiodeoxyguanosine and Its Incorporation into Oligodeoxynucleotides

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STRAIGHTFORWARD SYNTHESIS OF 6-THIODEOXYGUANOSINE AND ITS INCORPORATION INTO OLIGODEOXYNUCLEOTIDES

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ABSTRACT A convenient synthesis of 6-thiodeoxyguanosine from deoxyguanosine is described. Simple methods for incorporating 6-thiodeoxyguanosine into oligodeoxynucleotides are also given.

There are several reasons why it is of interest to incorporate 6-thiodeoxyguanosine into oligodeoxynucleotides. Thioguanine is an antitumour agent and it has been suggested that its mode of action involves incorporation of the drug into DNA [1-3]. Thus the properties of DNA or oligodeoxynucleotides containing 6-thiodeoxyguanosine are of potential relevance to cancer chemotherapy. The 6-keto oxygen atom of deoxyguanosine, when incorporated into B-DNA, is a potential contact point for DNA binding proteins[4]. Hydrogen bonds between DNA binding proteins and the 6-keto oxygen of deoxyguanosine often contribute to specificity of action. This has been observed for the Eco R1 restriction endonuclease[5] and several repressor proteins[6-8]. Thus replacement of the 6-keto oxygen atom with sulphur should give a probe that is of value in the study of this particular protein DNA interaction. 6-thioguanosine absorbs light at 350 nm[9] well away from the absorbance maxima of proteins and nucleic acids. Oligodeoxynucleotides containing 6-thiodeoxyguanosine should therefore be useful spectral probes. Finally 6-thioguanosine can be photooxidized to highly reactive species at 350 nm[10]. Oligodeoxynucleotides containing 6-thiodeoxyguanosine may therefore be valuable in the photoaffinity labelling of DNA binding proteins. To date there has been only one report detailing the incorporation of 6-thiodeoxyguanosine into oligodeoxynucleotides[11]. This approach used both a rather cumbersome method for the preparation of the 6-thiodeoxyguanosine base and also the seldom used phosphotriester method for oligodeoxynucleotide synthesis. This publication details a very convenient method for 6-

thiodeoxyguanosine preparation and also its incorporation into oligomers using the more common phosphoramidite method.

The usual method for preparing thionucleosides involves the reaction of an appropriately protected nucleoside with phosphorus pentasulphide or Lawesson's reagent. This approach has been successfully applied to the synthesis of 6-thioguanosine and 6-thioinosine[9] and to 4-thiothymidine and 4-thiouridine[12, 13-15]. However this method has not been used to prepare 6-thiodeoxyguanosine and there are reports in the literature (usually as unpublished observations or personal communications) that the method fails. When we treated the tribenzoyl-deoxyguanosine derivative (II) with Lawesson's reagent under conditions that successfully gave 4-thiothymidine[12], tlc indicated an extremely complex and messy reaction with several products being formed. Although one of these did correspond to the tribenzoylated 6-thiodeoxyguanosine derivative (IV) the tlc indicated both difficulties in purification and low yields. In the absence of this simple method preparations of 6-thiodeoxyguanosine have involved the reaction of protected 6-chloropurines with protected 1-chlorosugars using mercury salts as the condensing agent [16-18]. After separation of the α and β-anomers the derivatives prepared can be further converted to the desired 6-thiodeoxyguanosine. This method was used to prepare this deoxynucleoside for the only published incorporation into oligomers[11]. Although these methods could probably be improved by using the sodium salt method for glycosidic bond formation[19] it is still more attractive to prepare 6-thiodeoxyguanosine from readily available deoxyguanosine if posssible.

Our route towards this is shown in figure 1 and is based upon the sulphonylation of deoxyguanosine at the 6-keto oxygen. It is well documented that this sulphonlyation activates the 6-position to addition/elimination reactions[20-26] and this method has been used synthetically to prepare 2,6 diaminopurine- [25], 6-O-alkyl-guanine- [24] and 6-hydrazino-guanine- [26] deoxynucleoside derivatives. All these reactions involve the reaction of the sulphonylated deoxyguanosine reaction with nucleophiles. We have found that the tribenzoyl-sulphonyl deoxyguanosine derivative (III) undergoes reaction with lithium sulphide in tetrahydrofuran yielding the 6-thio derivative (IV). The reaction proceeds under very mild conditions (slight excess of the sulphide, room temperature, 2.5 h) and gives a high yield of product. This tribenzoyl-6-thio derivative (IV) can be completely debenzoylated with sodium methoxide to give 6-thiodeoxyguanosine in an extremely short and simple 4 step synthesis from deoxyguanosine. Deblocking with methoxide gave better yields than either NaOH or NH_3. As our principle aim is to incorporate 6-thiodeoxyguanosine into oligodeoxynucleotides we have also used brief NaOH treatment[27] to give the N-2-monobenzoyl-6-thiodeoxyguanosine

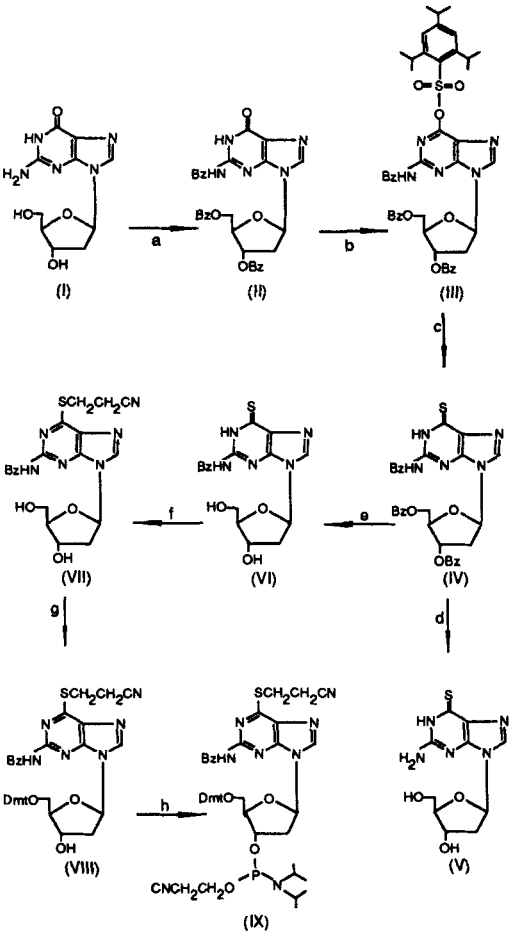

FIGURE 1 Preparation of 6-thiodeoxyguanosine (V) and a 6-thiodeoxyguanosine derivative suitable for oligodeoxynucleotide synthesis (IX) by the phosphoramidite method.
Reagents:
(a) Benzoic anhydride, (b) tri-isopropylbenzenesulphonyl-chloride, (c) lithium sulphide, (d) sodium methoxide,
(e) sodium hydroxide (brief), (f) bromopropionitrile,
(g) dimethoxytrityl chloride, (h) 2-cyanoethyl-N,N-di-isopropylchlorophosphor-amidite. Bz = benzoyl, Dmt = dimethoxytrityl.

(VI) a suitably protected base for oligonucleotide synthesis. Much of our reaction conditions for the sulphonylation and the selective debenzoylation are taken from the methods developed by McLaughlin and coworkers[26].

It is well documented that the 6-position of deoxyguanosine is active during oligodeoxynucleotide synthesis by both the phosphotriester[20,21] and phosphoramidite[28-30] method and this can lead to difficulties. With the phosphotriester approach these side reactions can be eliminated by protecting the 6-keto-oxygen[31,32] or reduced by performing an oximate deblock post synthesis[33]. With the phosphoramidite method side reactions are automatically reversed by the capping step during synthesis[28-30] and so no protection is used. 6-thioguanosine is much more reactive than guanosine[34]. For example the sulphur atom can be oxidized to disulphides and sulphonic acid derivatives with iodine. The sulphur atom is also nucleophilic and can react with reagents such as methyl iodide giving alkylated derivatives. Thus there is a possibility of side reactons with both iodine and activated deoxynucleoside phosphoramidites during DNA synthesis with 6-thiodeoxyguanosine. Therefore we felt that protection of the sulphur atom would be necessary even for synthesis by the phosphoramidite approach where deoxyguanosine itself requires no protection. We have used the cyanoethyl group for this and introduced it by reaction of N-benzoyl-6-thiodeoxyguanosine (VI) with bromopropionitrile to give the S-cyanoethyl derivative (VII). The conditions outlined in the materials and methods i.e. dropwise addition of N-benzoyl-6-thiodeoxyguanosine to a large excess of bromopropionitrile in dimethylformamide using K_2CO_3 as base are important. We have tried several variants involving different stoichiometries of the thiodeoxyguanosine derivative and bromopropionitrile, alternative bases and solvents, higher temperatures and also the use of acrylonitrile. In all cases either no reaction took place or the glycosidic bond was cleaved (presumably following alkylation at N-7). We have studied the reaction of N-benzoyl-S-cyanoethyl-6-thiodeoxyguanosine with NH_3 to check the removal of these protecting groups. Using 35% aqueous NH_3 at 55°C the cyanoethyl group was completely removed in 2h. The benzoyl group was removed to a 95% extent in 3h and completely removed in 4h. Thus the benzoyl group in 6-thiodeoxyguanosine appears to be more labile to NH_3 than it does in deoxyguanosine[27]. After 3h no 2,6-diaminopurine-deoxyriboside was formed and after 4h only about 2% of this product was observed. It is known that alkylated derivatives of 6-thioguanosine and to a very much lesser extent 6-thioguanosine itself react with NH_3 to give 2,6 diaminopurine derivatives[34]. Thus 4h appears to be optimum for NH_3 deblocking in that both the cyanoethyl and benzoyl groups

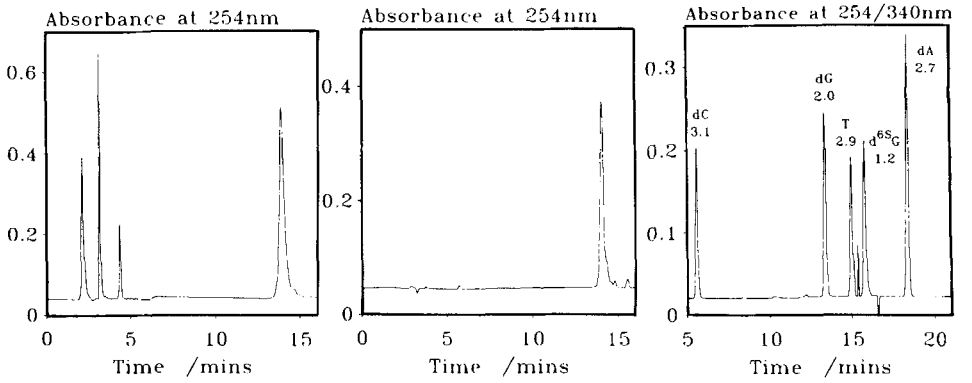

FIGURE 2 The reverse phase hplc traces of crude dmt - d(GAC[^{6S}G]ATATCGTC) (left) , purified d(GAC[^{6S}G]ATATCGTC) (middle) and the base composition analysis of d(GAC[^{6S}G]ATATCGTC) (right). The numbers by the peaks represent the mole fraction of each base. 2,6-diaminopurine-deoxyriboside elutes just after d^{6S}G. Detection was at 254 nm except for d^{6S}G which was monitored at 340 nm. The small perturbations before and after d^{6S}G are due to switching the U.V. detector between 254 and 340 nm.

are fully removed and very little of the 6-sulphur atom is displaced. This time was used to deblock the d(GAC[^{6S}G]ATATCGTC) prepared here. As it is likely that only a small number of d^{6S}G bases will need to be incorported into a single oligonucleotide this low level of conversion to 2,6 diaminopurine is probably acceptable. With the d^{6S}G synthon used the N-benzoyl group is removed more slowly than the S-cyanoethyl with NH_3 (indeed the cyanoethyl function can be removed with no 2,6 diaminopurine formation and so provides excellent protection for the thiol group). It will probably be possible to improve the method by preparing a d^{6S}G synthon in which the 2-amino group is blocked by a more ammonia labile amide protecting group and using this in conjunction with normal bases containing base labile protection[35,36].

The N-benzoyl-S-cyanoethyl-6-thiodeoxyguanosine (VII) could easily be converted to its 5′-dimethoxytrityl derivative (VIII) and then to its 3′-phosphoramidite (IX) by the standard procedures. The synthon (IX) was used to prepare d(GAC[^{6S}G]ATATCGTC) using the phosphoramidite method on a 1μmol scale. The coupling of IX proceeded in quantitative yield as monitored by trityl cation release. Thus IX behaves in the same manner as the four usual deoxynucleoside phosphoramidites giving a 100% coupling yield as measured by trityl cation release. After synthesis and deblocking with NH_3 (4h at 55°C) the crude trityl-on hplc trace is shown in figure 2. As can be seen a great deal of the oligonucleotide material runs

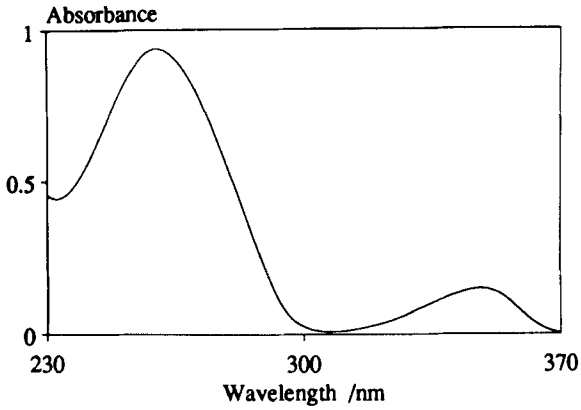

FIGURE 3 The U.V. spectrum of d(GAC[^{6S}G]ATATCGTC). The peak at 260 nm is typical for nucleic acids containing the four usual bases. That at 350 nm is due to d[^{6S}G] and does not occur in normal oligodeoxynucleotides.

as the full length trityl derivative with a smaller amount of failure sequences visible. After detritylation we have observed that normal oligodeoxynucleotides (i.e. those containing dC, dG, T and dA) of this length are pure enough to use in biochemical studies[12]. This was also observed with d(GAC[^{6S}G]ATATCGTC) which had a purity shown in figure 2 after only trityl-specific purification. We have observed that oligodeoxynucleotides that contain d^{6S}G have a tendency to form both disulphides and for the d^{6S}G to decompose to dG giving the parent oligonucleotide. These reactions do not occur during synthesis and deblocking but after these steps if the oligodeoxynucleotide is left in aqueous buffers at neutral pH values and room temperature. Thus it is important to purify oligonucleotides as soon as possible after preparation. After purification disulphide formation can be prevented by the addition of 1mM dithiothreitol. Conversion of the d^{6S}G to dG can be reduced by storing the purified products frozen at -20°C. As we have not yet found conditions that completely prevent this reaction it is important to use oligonucleotides containing d^{6S}G in experiments as soon as possible after preparation. Deoxynucleoside composition analysis of d(GAC[^{6S}G]ATATCGTC) (12) gave dC, T, dG, dA and d^{6S}G in the expected molar ratio of 3,3,2,3,1 respectively confirming the identity of the product (figure 2).

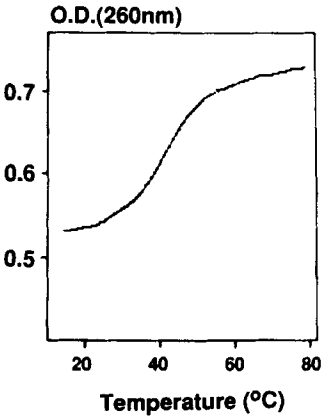

FIGURE 4 The melting temperature profile of d(GAC[^{6S}G]ATATCGTC). The melting temperature obtained from a differential plot of the above data was 44°C. d(GACGATATCGTC) has a melting temperature of 53°C[12].

The U.V. spectrum of d(GAC[^{6S}G]ATATCGTC) is shown in figure 3. As well as the usual 260 nm maximum a peak is also visible at 350 nm due to the d^{6S}G. This dodecamer is self-complementary and we have measured its Tm as shown in figure 4. The d^{6S}G containing oligodeoxynucleotide has a Tm value of 42°C, 11°C lower than the 53°C found for d(GACGATATCGTC)[12]. As the 6-position of dG forms a Watson-Crick hydrogen bond with the 4-NH_2 group of dC this destabilisation probably indicates a weaker hydrogen bond with the sulphur as compared to the oxygen. Finally the C.D. spectrum of d(GAC[^{6S}G]ATATCGTC) is shown in figure 5. This is typical of B-DNA in having a positive peak at about 280 nm and a negative at 250 nm[37,38]. However this d^{6S}G containing oligomer also shows an additional transition at about 340 nm due to the 6-thiodeoxyguanosine chromophore. Preliminary results show that d(GAC[^{6S}G]ATATCGTC) is a very poor substrate for the Eco RV endonuclease but is as good a substrate as d(GACGATATCGTC) for the Eco RV methylase (recognition site GATATC,[39,40]). Furthermore this 6-thiodeoxyguanosine containing oligodeoxynucleotide can be efficiently photocrosslinked to the Eco RV methylase by illumination with U.V. light in the 350 nm region. These studies will be reported on in more detail later.

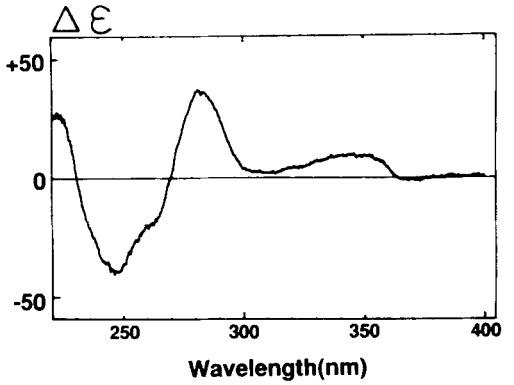

FIGURE 5 The circular dichroism spectrum of d(GAC[^{6S}G]ATATCGTC). The region below 300 nm is typical of B-DNA. The transition at about 340 nm is due to the d[^{6S}G] base and is not seen in oligodeoxynucleotides that only contain the four usual bases.

Materials and Methods Lithium sulphide, bromopropionitrile, benzoic anhydride and 2,4,6-triisopropylbenzenesulphonyl chloride were purchased from Aldrich (Gillingham, Dorset). 2-deoxyguanosine was the product of Genofit (Geneva). The source of all other reagents has been described[12]. Most of the protocols used in this paper including drying of solvents, thin layer chromatography, flash chromatography, ^{1}H NMR spectroscopy, fast atom bombardment mass spectroscopy, UV spectroscopy, melting temperature determination and CD spectroscopy have been described[12]. The reverse phase hplc methods used to purify and characterise oligodeoxynucleotides have also been documented[12]. For base compostion analysis, buffer A consisted of 0.1 M triethylammonium acetate at pH 6.5 containing 0% CH_3CN and buffer B consisted of 0.1 M trietylammonium acetate at pH 6.5 containing 65% CH_3CN. A linear gradient of 3%-23% buffer B over 20 minutes was used at room temperature with a flow rate of 1 ml min^{-1}.

3′,5′-0,N-2-tribenzoyldeoxyguanosine. 2.67g (10 mmol) of dry deoxyguanosine was suspended in 40 ml of dry pyridine. 9.05g (40 mmol) of benzoic anhydride and 0.35g of dimethylaminopyridine were added and the mixture refluxed under anhydrous conditions overnight. 5% $NaHCO_3$ solution was added and the mixture evaporated to give an oil. This

oil was dissolved in 200 ml CH_2Cl_2 and worked up (extract with 2 x 200 ml 5% $NaHCO_3$ 200 ml saturated NaCl and dry the organic layer with anhydrous Na_2SO_4. Evaporate the driec organic layer to a solid or oil). The crude product was purified by flash chromatography eluting with CH_2Cl_2 containing 4% CH_3OH. 5.29g (90%) of product was obtained which was pure by tlc. ^1H NMR ($CDCl_3$) 12.2(1Hs, N1H), 9.7(1Hs, amido H), 8.2-7.3(16Hm, aromatic, including H8), 6.35-6.28(1Ht, H1′), 5.99-5.95(1Hm, H3′), 5.10-5.03(1Hm, H5″), 4.72-4.65(2Hm, H4′+H5′), 3.33-3.24(1Hm, H2″), 2.74-2.66 (1Hm, H2′) FAB-MS 580 ($M+H^+$, 30%), 256 (heterocycle $+2H^+$, 100%).

3′,5′-0,N-2-tribenzoyl-6-(2,4,6,-triisopropylbenzenesulphonyl)deoxyguanosine. 4.63g (8mmol) of dry 3′,5′-0, N-2-tribenzoyldeoxyguanosine was dissolved in 50 ml of dry CH_2Cl_2 and 0.59g of dimethylaminopyridine followed by 5.6 ml of diisopropylethylamine was added. 4.85g (16 mmol) of 2,4,6-triisopropybenzenesulphonyl chloride was added and the mixture stirred with exclusion of moisture for 1 h. After evaporation of solvents the crude product was purified by flash chromatography using CH_2Cl_2 containing 2% CH_3OH. 4.5g (66%) of product was obtained which was pure by tlc. ^1H NMR ($CDCl_3$), 8.65 (1Hs, amido H), 8.15-7.35 (16Hm, aromatic, including H8), 7.23 (2Hs, aromatic from triisopropylbenzene), 6.59-6.55(1Ht, H1′), 5.97-5.94(1Hm, H3′), 4.87-4.83(1Hm, H5″), 4.77-4.72(1Hm, H5′), 4.70-4.66(1Hm H4′), 4.33-4.25(2Hm, 2 and 6-C<u>H</u>$(CH_3)_2$), 3.35- 3.27(1Hm, H2″), 2.96-2.86(2Hm, H2′ + 4-C<u>H</u>$(CH_3)_2$), 1.31-1.23(18Hm, CH_3). FAB-MS 846($M+H^+$, 15%), 579(M minus triisopropyl benzenesulphonyl $+H^+$, 18%), 521(M minus sugar $+H^+$, 30%) 255(M minus triisopropylbenzenesulphonyl minus sugar $+H^+$, 100%).

3′,5′-0,N-2-tribenzoyl-6-thiodeoxyguanosine. 4g (4.7 mmol) of 3′,5′-0,N-2-tribenzoyl-6-(2,4,6-triisopropylbenzenesulphonyl)deoxyguanosine was dissolved in 60 ml of dry tetrahydrofuran and 0.27g (5.9 mmol) of lithium sulphide was added. The mixture was stirred for 2.5 h, evaporated to dryness, dissolved in $CHCl_3$ and worked up as above. Purification was by flash chromatography ($CHCl_3$ containing 1% CH_3OH). 2.4g (86%) of product was obtained which was pure by tlc. ^1H NMR ($CDCl_3$) 13.4(1Hs, N1H), 9.6(1Hs, amido H), 8.23-7.34(16Hm, aromatic, including H8), 6.36-6.31(1Ht, H1′), 6.05-6.00(1Hm, H3′), 5.09-5.03(1Hm, H5″), 4.83-4.51(2Hm, H4′+H5′), 3.35-3.25(1Hm, H2″), 2.78-2.68(1Hm, H2′). FAB-MS 596($M+H^+$, 27%), 325(sugar +, 50%), 272 (heterocycle $+2H^+$, 100%).

6-thiodeoxyguanosine. 0.6g (1mmol) of 3′,5′-0, N-2-tribenzoyl-6-thiodeoxyguanosine was suspended in 23 ml of anhydrous methanol and 5 mmol of sodium methoxide added. The mixture was left at room temperature overnight. Dowex 50X-8 (pyridinium cycle) was added

in small batches until the pH was 7. The mixture was filtered and evaporated to dryness. The product was dissolved in CH_3OH, H_2O (60:40) and purified by passage over a column (10 x 2.5cm) packed with LiChroprep RP-18 (Merck 9303) and eluted with this solvent. 0.18g (65%) of product was obtained. ^1H NMR (d_6 - DMSO) 12.05 (1Hs, N1H), 8.21 (1Hs, H8), 6.9 (2Hs, NH_2), 6.21 (1Ht, H1′), 5.37 and 5.02 (2Hs, broad, 3′ and 5′-OH), 4.46-4.42 (1Hm, H3′), 3.93-3.89 (1Hm, H4′), 3.69-3.64 (1Hm, H5″), 3.63-3.57 (1Hm, H5′), 2.68-2.56 (1Hm, H2″), 2.37-2.27 (1Hm, H2′). FAB-MS 284 ($M+H^+$, 50%), 168 (heterocycle + $2H^+$, 100%). UV λmax (pH4) 341, 258; (pH6.9) 341, 255; (pH12) 319, 251. These spectra are identical to those given for 6-thioguanosine (9).

N-benzoyl-6-thiodeoxyguanosine. 2.39g (4.0 mmol) of 3′,5′-0,N-2-tribenzoyl-6-thiodeoxyguanosine was dissolved in 55 ml of pyridine containing 6.4 ml of CH_3OH and cooled to -20°C. 6.0ml of chilled 2M NaOH was added slowly over 3 min. After 30 min Dowex 50-X8 (pyridinium cycle) was added in small batches until the pH of the mixture was neutral. The mixture was filtered and evaporated to dryness. This crude product can be used directly in the next reaction. A pure sample obtained by recrystallisation from either H_2O or CH_3OH gave satisfactory spectroscopic data. ^1H NMR (d_6-DMSO) 13.75 (1Hs, N1H), 12.25 (1Hs, amido H), 8.55 (1Hs, H8), 8.22 - 7.65 (5Hm, aromatic), 6.40 (1Ht, H1′), 5.42(1Hd, 3′-OH), 5.05 (1Ht, 5′-OH), 4.53 - 4.48 (1Hm, H3′), 3.99 - 3.93 (1Hm, H4′), 3.75 - 3.60 (2Hm, H5′ + H5″), 2.77 - 2.65 (1Hm, H2″), 2.45 - 2.35 (1Hm, H2″). FAB-MS 388 ($M + H^+$, 70%), 272 (heterocycle + $2H^+$, 100%).

N-benzoyl-S-2-cyanoethyl-6-thiodeoxyguanosine. 5 ml of 3-bromopropionitrile and 6g of anhydrous K_2CO_3 were added to 100 ml of dry dimethylformamide and the mixture stirred vigorously. The crude N-benzoyl-6-thiodeoxyguanosine obtained above was dissolved in 40 ml of dry dimethylformamide and added dropwise to the stirred solution over about 10 min. The mixture was stirred overnight and then filtered to remove solid material. The oil obtained on evaporation was dissolved in 200 ml of $CHCl_3$ (80), CH_3OH (20) mixture and insoluble material removed by filtration. Evaporation of the filtrate gave a product which was >90% pure and could be used without further purification in the next reaction. A small sample of the crude product was purified for spectroscopic analysis by flash chromatography eluting with 8% CH_3OH in CH_2Cl_2. ^1H NMR (d_6-DMSO) 11.1 (1Hs, amido H), 8.7 (1Hs, H8); 8.02-8.08 (2Hm, aromatic), 7.60-7.75 (3Hm, aromatic), 6.47 (1Ht, H1′), 5.41 (1Hd, 3′-OH), 5.00 (1Ht, 5′-OH), 4.50-4.58 (1Hm, H3′), 3.94-4.00 (1Hm, H4′), 3.58-3.76 (4Hm, H5′ + H5″ + $S\underline{CH_2}CH_2CN$), 3.28 (2Ht, $SCH_2\underline{CH_2}CN$), 2.80-2.90 (1Hm, H″), 2.35-2.45 (1Hm, H2′). FAB-MS 441 ($M + H^+$, 90%), 325 (heterocycle + $2H^+$, 100%).

5′-0-dimethoxytrityl-N-benzoyl-S-2-cyanoethyl-6-thiodeoxyguanosine. The N-benzoyl-S-2-cyanoethyl-6-thiodeoxyguanosine produced above was converted to its 5′-dimethoxytrityl derivative in the standard manner using dimethoxytritylchloride[31]. After standard work up the product was purified by flash chromatography using ethylacetate containing 0.5% pyridine as eluent. 1.4g (47% yield for the 3 steps from the tribenzoyl-6-thiodeoxyguanosine) of product was obtained. ^{1}H NMR($CDCl_3$) 8.45(1Hs, amido H) 8.05 (1Hs, H8) 7.78-6.65 (18Hm, aromatic), 6.46 (1Ht, H1′), 4.85 (1Hm, H3′), 4.24 (1Hm, H4′), 3.74 (6Hs, OCH_3) 3.58 (2Hm, H5′ + H5″), 3.41 (2Hm, -S$\underline{CH_2}CH_2CN$), 3.10 (2Ht, $SCH_2\underline{CH_2}CN$), 2.94 (1Hm, H2″), 2.55 (1Hm, H2′). FAB-MS (743, M + H$^+$, 1%) 303 (dmt+, 100%).

Protected deoxynucleoside 3′-0-(N,N-diisopropylamino)-2-cyanoethylphosphoramidite derivative. The deoxynucleoside above was converted to its phosphoramidite on a 1 mmol scale as described[41]. A 70% yield was obtained and the product was pure by tlc ($CHCl_3$ 45, ethyl acetate 45, triethylamine 10) - two diastereomers with R_f values of 0.8 and 0.85.

Oligodeoxynucleotide synthesis and purification. Oligodeoxynucleotides containing 6-thiodeoxyguanosine were prepared as described[12] with 6-thiodeoxyguanosine being treated in an identical fashion to the modified bases used in this publication. Deblocking was performed using 35% aqueous NH_3 at 55°C for 4 h. Dimethoxytrityl-specific purification was performed by reverse phase hplc and the dmt group removed with aqueous acetic acid as described[12]. Sometimes the d(GAC[^{6S}G]ATATCGTC) was of sufficient purity after this step. Occasionally a further reverse phase hplc purification using a shallow CH_3CN gradient was required to remove minor contaminants[12]. Immediately prior to both hplc purification steps the pH of the oligodeoxynucleotide solution was adjusted to 8 with $NaHCO_3$ and dithiothreitol added to a final concentration of 10 mM to reduce any disulphides.

Deoxynucleoside composition analysis. This was performed as described[12] but 5mM dithiothreitol was included in the digest buffer. The product deoxynucleosides were analysed by reverse phase hplc using the gradient described above. The elution times of the bases were dC (5.6 min), dG (13.5 min), T (15.1 min), d^{6S}G (15.9 min), dA (18.6 min). Detection at 254 nm was used to quantify the four usual bases (extinction coefficients[12]). Detection was switched to 340 nm to detect and quantify d^{6S} G (extinction coefficient 20 x 10^3 M^{-1} cm^{-1} [9]).

Deblocking of N-benzoyl-S-2-cyanoethyl-6-thiodeoxyguanosine with NH_3. A small amount (≈ 10mg) of the above compound was dissolved in 5 ml of 35% aqueous NH_3 and heated at 55°C. At various times aliquots were removed, evaporated to dryness and redissolved in H_2O. The products of deblocking were examined by reverse phase hplc using

a step gradient that consisted of 100% buffer A, 0% buffer B (t = 0 to 10 min); 85% buffer A, 15% buffer B (t = 10 to 15 min); 70% buffer A, 30% buffer B (t = 15 to 30 min) (A = 0.1M triethylammonium acetate pH 6.5 containing 5% CH_3CN, B = 0.1M triethylammonium acetate pH 6.5 containing 65% CH_3CN. The relevant deoxynucleosides had the following elution times: 6-thiodeoxyguanosine (7.1 min), 2,6-diaminopurine deoxyriboside (7.7 min), N-benzoyl-6-thiodeoxyguanosine (27.8 min), N-benzoyl-S-2-cyanoethyl-6-thiodeoxyguanosine (28.5 min). Detection was at 254 nm.

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