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The synthesis of protected 5'-mercapto-2',5'-dideoxyribonucleoside-3'-O-phosphoramidites; uses of 5'-mercapto-oligodeoxyribonucleotides

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ABSTRACT

The syntheses of the four novel, base protected 5'-(S-triphenylmethyl)mercapto-2',5'-dideoxyribonucleoside-3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidites) are described. These compounds have been used to prepare 5'-(S-triphenylmethyl) mercapto-oligodeoxyribonucleotides, which are readily purified by reversed phase h.p.l.c., owing to the highly lipophilic trityl group. After cleavage of the S-trityl group by silver or mercuric ions, the free thiol moiety can be coupled to a wide variety of reagents, generating very useful probes. Fluorescent labelled 5'-mercapto-oligodeoxyribonucleotides are being used for automated DNA sequencing without radioactivity, and heavy metal labelled 5'-mercapto-oligonucleotides will be used in X-ray crystallography.

INTRODUCTION

The synthesis and use of modified oligodeoxyribonucleotides is today a very relevant subject. We were interested in preparing 5'-mercapto-oligodeoxyribonucleotides on a large scale for subsequent attachment of mercurials enabling the study of DNA-binding proteins by X-ray crystallography. Similarly, the attachment of suitable metal cluster compounds enables structural studies by electron microscopy techniques. Previously, Connolly and Rider reported the synthesis of 5'-mercaptoalkyl phospho-oligodeoxyribonucleotides (1), however the alkyl spacer has to be at least 3 C atoms long (Connolly, B. personal communication). We have used these 5'-mercaptoalkyl compounds for preparing fluorescent labelled primers for automated dideoxy DNA sequencing without radioactivity (2). In addition these compounds can be reacted with N^1-iodoacetyl-N^6-biotinyl-1,6-diaminohexane to generate biotinylated oligodeoxyribonucleotides.

5'-Thiothymidine has been previously described in the literature (3, 4), and showed no tendency to cyclise by addition of the thiol moiety across the 5, 6-double bond. No DNA synthesis has been reported incorporating this compound at the 5'-terminus. The other 5'-mercapto-2',5'-dideoxyribonucleosides have not been previously described. Relatively efficient syntheses of four appropriately protected 5'-mercapto-2',5'-dideoxyribonucleoside-3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidites) are described here. These building blocks can then be used in DNA synthesis by the phosphoramidite procedure

(5, 6) to produce 5′-mercapto-oligodeoxyribonucleotides. As examples, 5′-HS-d[GTAAAACGACGGCCAGT] has been synthesised and coupled with 5-iodoacetamido-fluorescein to generate a fluorescent M13 sequencing primer, and an organomercurial derivative of 5′-HS-d[GGGATATCCC] has been synthesised on a large scale. The advantage of having the modification at the 5′-terminus and not on any of the purine or pyrimidine rings is that there is minimal interference with the normal Watson-Crick base pairing in duplex DNA.

DISCUSSION AND RESULTS

The monomers

The triphenylmethyl (trityl) group was chosen as the protecting group for the 5′-mercapto function as it is highly lipophilic. Just like 5′-O-dimethoxytrityl protected oligodeoxy-ribonucleotides the presence of the S-trityl group enables the ready purification of 5′-(S-triphenylmethyl)mercapto-oligodeoxyribonucleotides by reversed phase h.p.l.c. (5). The lipophilic trityl group also aids in column chromatographic purification of the various intermediates. In addition, S-trityl compounds are readily visualised as yellow spots on

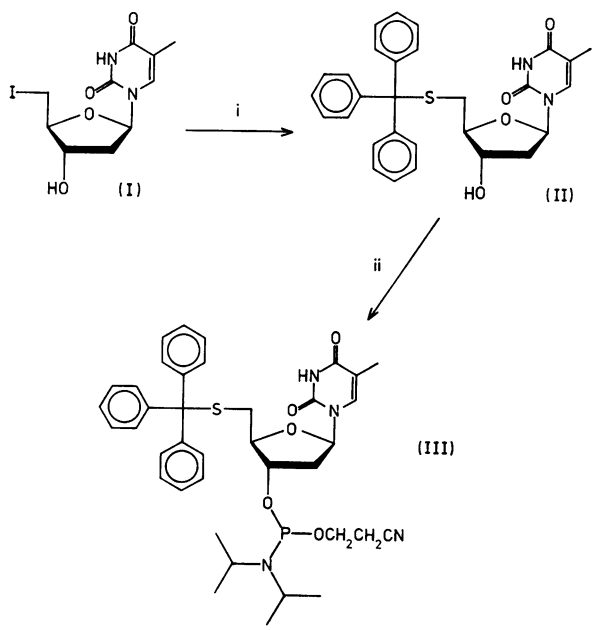

Figure 1. Scheme for the preparation of the modified thymidine building block. Reagents : i, sodium triphenylmethylmercaptide in DMF; ii, 2-cyanoethoxy bis(N,N-diisopropylamino) phosphine and diisopropylammonium tetrazolide.

t.l.c. when sprayed with perchloric acid/ethanol followed by heating. Unlike 5′-O-dimethoxytrityl the S-trityl group is relatively acid stable, however it is readily cleaved by heavy metal ions such as silver (1) or mercuric (7). S-Trityl groups are known to be labile to iodine in solution (8); the reaction involves intermolecular disulphide bond formation. This cannot occur on the relatively low functionalised controlled pore glass supports used in solid phase DNA synthesis, and has been shown not to be a problem during the final P(III) to P(V) oxidation step in the DNA synthesis (1). Standard protecting groups

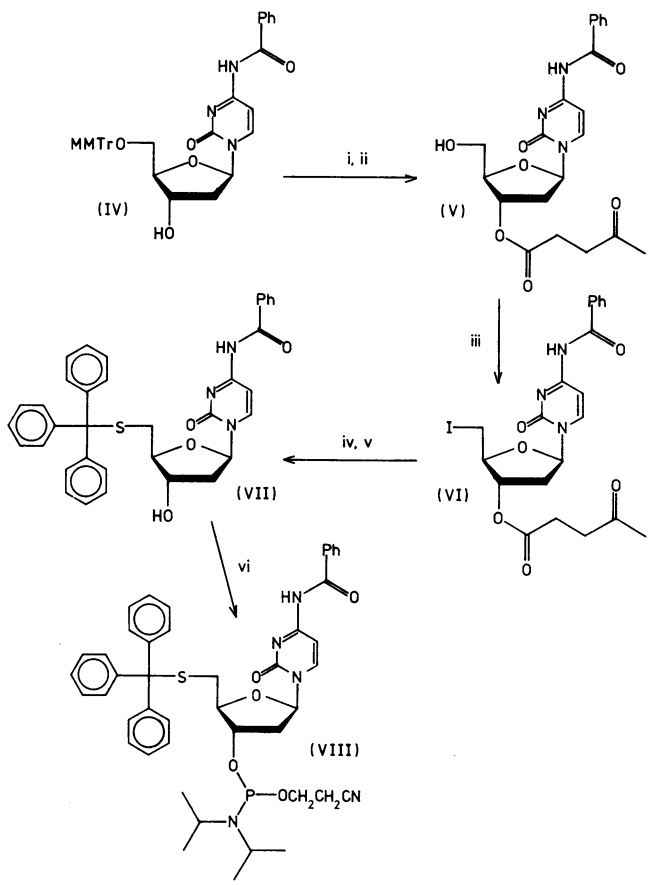

Figure 2. Scheme for the preparation of the modified 2′-deoxycytidine building block. Reagents : i, levulinic acid, N,N-dicyclohexylcarbodiimide (DCCI), and 4-dimethylaminopyridine in dioxan; ii, trichloroacetic acid in dichloromethane; iii, methyltriphenoxyphosphonium iodide in DMF; iv, sodium triphenylmethylmercaptide in DMF; v, hydrazine hydrate in pyridine/acetic acid; vi, 2-cyanoethoxy N,N-diisopropylaminochlorophosphine and N,N-diisopropylethylamine.

were used for the exocyclic amino groups of 2′-deoxycytidine and 2′-deoxyguanosine, and the 6-amino group of 2′-deoxyadenosine was protected with the trimethylacetyl (pivaloyl) group. It was expected that S_N2 displacements on suitable nucleoside 5′-derivatives with S-tritylmercaptide would proceed smoothly and in high yield, based on some previous observations in the ribonucleoside field (9).

The preparation of the modified thymidine building block (III) is illustrated in Figure 1. The known 5′-iodo-5′-deoxythymidine (I) was prepared from thymidine and methyltriphenoxyphosphonium iodide as described previously (10). The use of this phosphonium iodide for facile iodination of alcohols was first described by Landauer and Rydon (11). The iodo compound (I) was converted in 79% yield into the 5′-(S-triphenylmethyl)mercapto compound (II) with the sodium salt of triphenylmethylmercaptan in N,N-dimethylformamide (DMF). This material was then phosphitylated to give the desired 5′-(S-triphenylmethyl) mercapto-5′-deoxythymidine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (III), which was purified by short column chromatography and obtained as a white powder by precipitation into cold petroleum ether.

In the case of the modified 2′-deoxycytidine derivative, a 3′-hydroxyl protecting group was used, see Figure 2. Thus, 5′-O-monomethoxytrityl-N^4-benzoyl-2′-deoxycytidine (IV) was first protected as its 3′-O-levulinate (12), and subsequently detritylated to give the 5′-hydroxyl derivative (V) which was purified by column chromatography. This compound was smoothly iodinated to give the 5′-iodo derivative (VI) in 78% yield. A much lower yield of iodo compound was obtained when the 3′-O-protecting group was omitted, and moreover the presence of the 3′-O-levulinyl group ensured absolute specificity in the reaction. Reaction of the iodo compound (VI) with sodium triphenylmethylmercaptide followed by cleavage of the levulinyl group with hydrazine afforded the 5′-(S-triphenylmethyl)mercapto derivative (VII) in 70% isolated yield. Phosphitylation gave the desired 5′-(S-triphenylmethyl)mercapto-N^4-benzoyl-2′,5′-dideoxycytidine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (VIII) in 73% yield after short column chromatography.

In the case of the purine derivatives it was expected that attempted iodination would lead to N^3,5′-cyclonucleoside formation (10), particularly in the case of 2′-deoxyadenosine. The route for the modified 2′-deoxyguanosine building block (XIII) is illustrated in Figure 3. 5′-O-Dimethoxytrityl-N^2-isobutyryl-2′-deoxyguanosine (IX) was levulinylated and detritylated to give the 5′-hydroxy compound (X), which rather surprisingly was smoothly iodinated in high yield. Subsequent displacement of the 5′-iodo by tritylmercaptide anion gave the 5′-(S-triphenylmethyl)mercapto compound (XI) in 71% overall yield from the 5′-hydroxy compound (X). Hydrazinolyis yielded the 3′-hydroxy compound (XII), which was then phosphitylated to afford the desired 5′-(S-triphenylmethyl)mercapto-N^2-isobutyryl-2′,5′-dideoxyguanosine-3′-O-(2-cyanoethyl N,N-di-

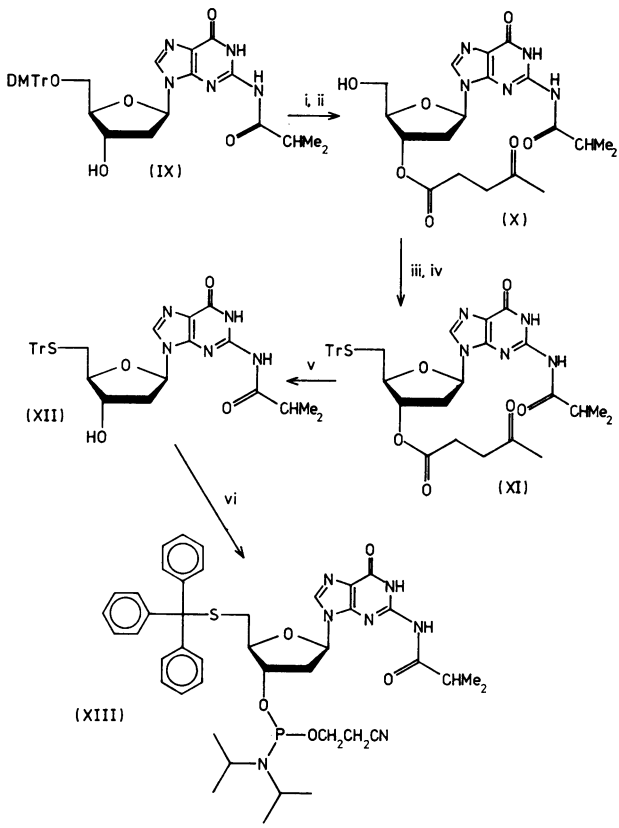

Figure 3. Scheme for the preparation of the modified 2′-deoxyguanosine building block. Reagents : i, levulinic acid, DCCI, and 4-dimethylaminopyridine in dioxan; ii, trichloroacetic acid in dichloromethane; iii, methyltriphenoxyphosphonium iodide in DMF; iv, sodium triphenylmethylmercaptide in DMF; v, hydrazine hydrate in pyridine/ acetic acid; vi, 2-cyanoethoxy N,N-diisopropylaminochlorophosphine and N,N-diisopropylethylamine.

isopropylphosphoramidite) (XIII) as a white powder in 68% yield after column purification and precipitation.

Figure 4 illustrates the route adopted for the modified 2′-deoxyadenosine building block (XVIII). The starting material 5′-O-monomethoxytrityl-N^6-pivaloyl-2′-deoxyadenosine (XIV) was prepared using the transient protection procedure of Jones (13). Levulinylation followed by detritylation afforded the 5′-hydroxy compound (XV), which was converted into the 5′-O-(4-nitrobenzenesulphonyl) compound (XVI) in good yield. It was known that N^6-acyl derivatives of adenine nucleosides resist N^3, 5′-cyclisation and that direct displacement reactions on 5′-tosylates or better still 5′-(4-nitrobenzenesulphonates) proceed smoothly (14). Thus the 4-nitrobenzenesulphonate moiety of compound

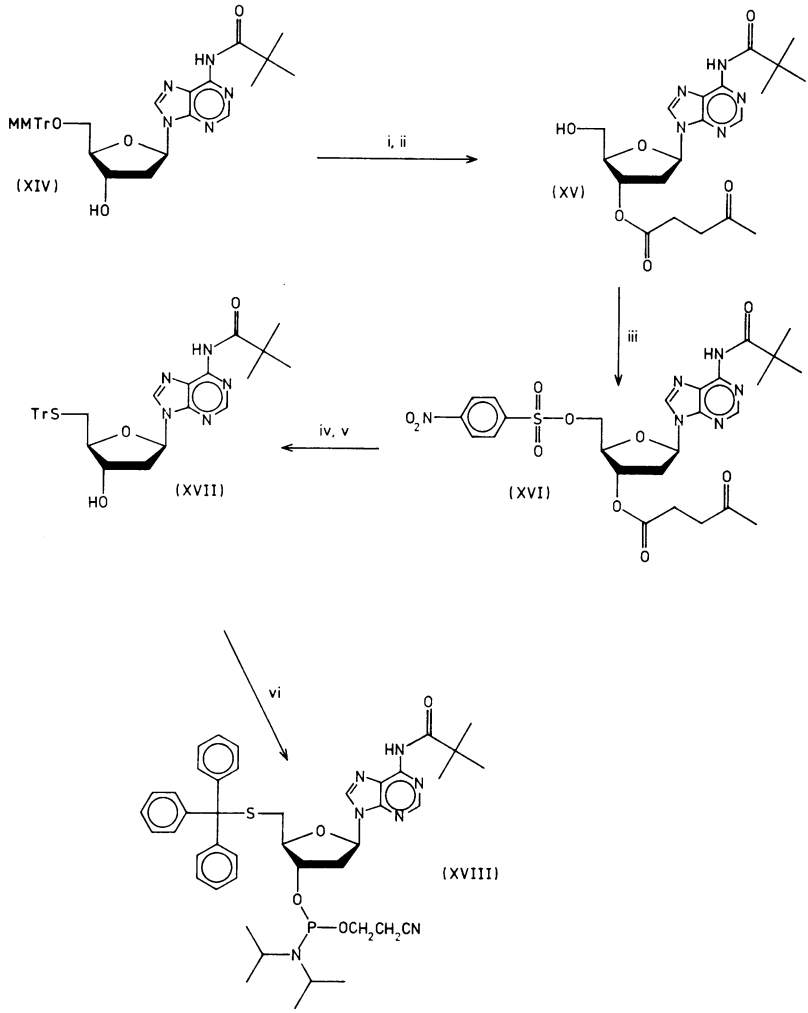

Figure 4. Reation scheme for the preparation of the modified 2′-deoxyadenosine building block. Reagents : i, levulinic acid, DCCI, and 4-dimethylaminopyridine in dioxan; ii, trichloroacetic acid in dichloromethane; iii, 4-nitrobenzenesulphonyl chloride in pyridine; iv, sodium triphenylmethylmercaptide in DMF; v, hydrazine hydrate in pyridine/acetic acid; vi, 2-cyanoethoxy N,N-diisopropylaminochlorophosphine and N,N-diisopropylethylamine.

(XVI) was readily displaced by tritylmercaptide, and subsequent hydrazine cleavage of the levulinyl protecting group afforded the 5′-(S-triphenylmethyl)mercapto compound (XVII) in excellent yield. Finally, phosphitylation gave the desired building block, 5′-(S-triphenyl-

methyl)mercapto-N^6-pivaloyl-2′,5′-dideoxyadenosine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (XVIII).

Modified oligodeoxyribonucleotides

The modified 2′-deoxyguanosine building block (XIII) was utilised under standard conditions in an Applied Biosystems DNA synthesiser as the last base addition to prepare 5′-(S-triphenylmethyl)mercapto-d[GTAAAACGACGGCCAGT], an M13 sequencing primer. This compound was isolated in 20% yield after h.p.l.c. purification. Detritylation with silver nitrate, removal of silver ions with dithiothreitol, and subsequent reaction of the 5′-mercapto-oligodeoxyribonucleotide with 5-iodoacetamidofluorescein using the described procedure (2) gave the fluorescein labelled M13 sequencing primer in 37% yield after reversed phase h.p.l.c. purification. This primer is now being used for automated DNA sequencing without radioactivity (15).

5′-(Triphenylmethyl)mercapto-d[GGGATATCCC] was prepared on a large scale using initially the manual phosphotriester method (16), and purified by preparative layer chromatography on silica gel plates (17). Obtained 4 μmol of material in this way from a 25 μmol scale synthesis. The trityl protected material was then detritylated and converted into a mercurial by treatment with 3-chloromercuri-2-methoxypropylurea. Attempts to cocrystallise the self-complementary mercuri-decamer with Eco RV will be made. Full experimental details and results will be published later.

EXPERIMENTAL

General Materials and Procedures

Dichloromethane and 1,2-dichloroethane were dried by distillation from phosphorus pentoxide. 1,4-Dioxan was passed through basic alumina to remove peroxide, and was then distilled from sodium plus benzophenone, N,N-Dimethylformamide (DMF), residue analysis grade, was obtained from Merck (Darmstadt, F.R.G.) and was used without further purification. N,N-Diisopropylethylamine was distilled initially from ninhydrin and then redistilled under nitrogen from potassium hydroxide pellets. Pyridine was first distilled from ninhydrin and then redistilled from barium oxide.

Triphenylmethylmercaptan was purchased from Aldrich (Steinheim, F.R.G.). Methyltriphenoxyphosphonium iodide was purchased from Fluka (Neu-Ulm, FRG). 5-Iodoacetamidofluorescein and 3-chloromercuri-2-methoxypropylurea were obtained from Molecular Probes, Inc. (Eugene, OR, U.S.A.) and K and K Laboratories (New York, U.S.A.) respectively. All other reagents used were of the highest available purity.

5′-Iodo-5′-deoxythymidine (I) was prepared as described previously (10). 5′-O-Monomethoxytrityl-N^4-benzoyl-2′-deoxycytidine (IV) and 5′-O-monomethoxytrityl-N^6-pivaloyl-2′-deoxyadenosine (XIV) were prepared using the Jones transient protection procedure (13). 2-Cyanoethoxydichlorophosphine was prepared by the procedure of

Tesser (18) and was converted into 2-cyanoethoxy N,N-diisopropylaminochlorophosphine as described (19). 2-Cyanoethoxy bis(N,N-diisopropylamino)phosphine was prepared according to the procedure described for the methoxy compound (20).

Phosphoramidites were obtained as powders by dropwise addition of a dichloromethane solution into cold (-40°C) vigorously stirred petroleum ether (b.p. 40 - 60°C). Precipitated material was filtered off while still cold and then dried thoroughly in high vacuum.

Column chromatography was performed on Kieselgel 60 from Fluka (Neu-Ulm, F.R.G.), and ascending mode t.l.c. was performed on aluminium foil supported silica containing a 254 nm fluor. Solvent systems used were: A, ethanol/dichloromethane (5:95 v/v) and B, ethyl acetate/dichloromethane/triethylamine (45:45:10 v/v). ^{13}C and ^{31}P n.m.r. spectra were recorded on a Bruker AM250 spectrometer using tetramethylsilane and external trimethyl phosphate as the respective references (shifts downfield from the reference are assigned as positive). ^{31}P n.m.r. spectra were recorded using broad brand proton noise decoupling. ^{13}C n.m.r.spectra and assignments of all intermediates are available upon request.

Oligodeoxyribonucleotides were synthesised on an Applied Biosystems DNA synthesiser model 380B-02 (Foster City, California, USA) using standard β–cyanoethyl phosphoramidite chemistry (6); full details will be published elsewhere.

Preparation of Monomers

5′-(S-Triphenylmethyl)mercapto-5′-deoxythymidine (II): A 60% dispersion of sodium hydride in mineral oil (400 mg, 10 mmol) was suspended in dry DMF (5ml) under argon at 0°C, and a solution of triphenylmethylmercaptan (2.76 g, 10 mmol) in dry DMF (30 ml) was added. Mixture was stirred for 10 min at room temperature, cooled in ice and a solution of 5′-iodo-5′-deoxythymidine (I; 2.9 g, 8.24 mmol) in dry DMF (20 ml) was added. After 1 h at room temperature reaction was complete by t.l.c., and solvent was evaporated *in vacuo*. The residue was dissolved in chloroform, the solution was washed with saturated brine, dried (Na_2SO_4), filtered and evaporated *in vacuo*. After evaporation of toluene the residue was chromatographed on silica gel 60 (50 g) using a gradient from 0 to 10% of ethanol in dichloromethane as eluant. Fractions containing pure product as determined by t.l.c. (R_f 0.24 solvent system A) were pooled and evaporated *in vacuo* to give a white foam. The title compound (II) was obtained as a white powder (3.26 g, 79%) by room temperature precipitation into petroleum ether (400 ml).

5′-(S-Triphenylmethyl)mercapto-5′-deoxythymidine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (III): Added diisopropylammonium tetrazolide (171 mg, 1 mmol) to a solution of 5′-(S-triphenylmethyl)mercapto-5′-deoxythymidine (1g, 2 mmol; II) in dichloromethane (10 ml) under dry argon. 2-Cyanoethoxy bis(N,N-diisopropylamino) phosphine (663 mg, 2.2 mmol) was added by gas-tight syringe and the solution was stirred

for 1 h at room temperature. The reaction mixture was diluted with dichloromethane (150 ml), and the solution was washed with saturated sodium bicarbonate solution (2 x 100 ml), dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residual almost colourless oil was purified by short column chromatography on silica gel (40 g), eluting initially with 2% triethylamine in petroleum ether/dichloromethane (40:60 v/v) and finally with 2% triethylamine in dichloromethane. Pure fractions as determined by t.l.c. (R_f 0.49 and 0.42 in solvent system B) were pooled and evaporated *in vacuo* leaving a white foam. Low temperature precipitation yielded the title compound (III) as a white powder (630 mg, 45%). ^{31}P n.m.r. spectrum (CH_2Cl_2, external D_2O lock): singlets at δ + 145.69 and 145.57 p.p.m.

Preparation of 3′-O-levulinyl-N^4-benzoyl-2′-deoxycytidine (V): Levulinic acid (2.32 g, 20 mmol) was dried by evaporation of toluene *in vacuo*. 5′-O-Monomethoxytrityl-N^4-benzoyl-2′-deoxycytidine (6.04 g, 10 mmol; IV) was dissolved in dry dioxan (150 ml), 4-dimethyl-aminopyridine (100 mg), a solution of leuvinic acid (2.32g, 20 mmol) in dioxan (50 ml), and dicyclohexylcarbodiimide (4.12 g, 20 mmol) were added. The reaction mixture was stirred overnight in the dark and judged to be complete by t.l.c. The precipitate of dicyclohexylurea was filtered off and washed with a little dichloromethane and the combined filtrates were evaporated *in vacuo*. The residue was dissolved in dichloromethane (400 ml) and the solution was washed with 5% sodium bicarbonate solution (2 x 200 ml) followed by saturated brine (250 ml), then dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel (40g) using a gradient of 0-2% ethanol in dichloromethane as eluant. Fractions containing pure product were pooled (R_f 0.7 on t.l.c. in ethanol/dichloromethane, 1:9 v/v containing 1% triethylamine) and evaporated *in vacuo* to leave a foam (6.23 g). This material was dissolved in 1,2-dichloroethane (300 ml) and cooled in ice. A solution of trichloroacetic acid (12 g) in dichloroethane (100 ml) was added and after 30 min silica gel t.l.c. showed that detritylation was complete. Methanol (40 ml) and water (10 ml) were added, and the solution was washed with 5% sodium bicarbonate solution (2 x 200 ml), then saturated brine (150 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification of the crude product on silica gel (50 g) eluting with 0-5% ethanol in dichloromethane, affored pure 3′-0-levulinyl-N^4-benzoyl-2′-deoxycytidine (V) as a white foam (2.36 g, 55%). R_f 0.61 on t.l.c. in ethanol/dichloro-methane (1:9 v/v) containing 1% triethylamine.

5′-Iodo-3′-O-levulinyl-N^4-benzoyl-2′,5′-dideoxycytidine (VI): Compound V (1.91 g, 4.45 mmol) was dissolved in dry DMF (50 ml) under argon and methyltriphenoxyphosphonium iodide (4.02 g, 8.9 mmol) was added. The mixture was stirred overnight in the dark, methanol (20 ml) was added and after 20 min the mixture was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (250 ml), and the solution was washed with sodium thiosulphate solution (100 ml, 0.1M) and then saturated brine (100 ml). After drying and

evaporation of solvent *in vacuo*, the crude product was purified by chromatography on silica gel (100 g), eluting with 0-3% ethanol in dichloromethane. Fractions containing pure product (R_f 0.57 in ethanol/dichloromethane, 1:9 v/v) were pooled and evaporated *in vacuo* to give 5′-iodo-3′-O-levulinyl-N^4-benzoyl-2′,5′-dideoxycytidine (VI) as a white foam (1.87 g, 78%).

5′-(S-Triphenylmethyl)mercapto-N^4-benzoyl-2′,5′-dideoxycytidine (VII): The 5′-iodo compound VI (1.2 g, 2.22 mmol) was treated with 3 mmol of sodium triphenylmethylmercaptide according to the procedure given for compound II above. The crude 5′-(S-triphenylmethyl)mercapto-3′-O-levulinyl derivative was purified on silica gel (40 g) using 0-4% ethanol in dichloromethane as eluant. Pure fractions were pooled and evaporated *in vacuo* to give an oil (1.5 g). This material was dissolved in pyridine/acetic acid (40 ml, 4:1 v/v) and hydrazine hydrate (1 ml) was added. After 10 min the reaction was quenched by addition of acetone (10 ml) and the solution was evaporated *in vacuo*. The residue was dissolved in dichloromethane (200 ml), washed with 5% sodium bicarbonate solution (100 ml) and then saturated brine (100 ml), dried and evaporated *in vacuo*. The residue was chromatographed on silica gel (30 g) eluting with 0-4% ethanol in dichloromethane. Pure fractions (R_f 0.22 in solvent system A) were pooled and evaporated *in vacuo*, leaving a foam, which was dissolved in dichloromethane (10 ml) and precipitated into petroleum ether (150 ml). Filtration and drying afforded 5′-(S-triphenylmethyl)mercapto-N^4-benzoyl-2′,5′-dideoxycytidine (VIII) as a white powder (920 mg, 70%).

5′-(S-Triphenylmethyl)mercapto-N^4-benzoyl-2′,5′-dideoxycytidine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite (VIII): 5′-(S-Triphenylmethyl)mercapto-N^4-benzoyl-2′,5′-dideoxycytidine (1.18 g, 2 mmol; VII) was dissolved in dry dichloromethane (10 ml) under argon, and N,N-diisopropylethylamine (1.56 ml, 9.2 mmol) was added followed by 2-cyanoethoxy N,N-diisopropylaminochlorophosphine (954 µl, 4 mmol). The reaction was monitored by t.l.c. and after 1 h at room temperature the mixture was diluted with dichloromethane (150 ml), washed with saturated brine (2 x 100 ml), dried, filtered and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (20 g) using petroleum ether/dichloro-methane (2:1 v/v) containing 2% triethylamine as eluant. Pure fractions (R_f 0.52 and 0.44 in solvent system B) were pooled and evaporated *in vacuo*. Low temperature precipitation afforded the pure title compound (VIII) as a white powder (1.16 g, 73%). ^{31}P n.m.r. spectrum (CH_2Cl_2, external D_2O lock) singlets at δ + 147.71 and 147.56 p.p.m.

3′-O-Levulinyl-N^2-isobutyryl-2′-deoxyguanosine (X): 5′-O-Dimethoxytrityl-N^2-isobutyryl-2′-deoxguanosine (6.4 g, 10 mmol; IX) was levulinylated and detritylated (15 min with trichloroacetic acid) according to the procedure for compound V above. The crude residual oil was purified on a column of silica gel (60 g) using 0-15% ethanol in dichloro-methane as eluant. Pure fractions as determined by t.l.c. were pooled and evaporated *in*

vacuo to give the title compound (X) as a practically white foam (2.87 g, 66%).

5′-(S-Triphenylmethyl)mercapto-3′-O-levulinyl-N^2-isobutyryl-2′,5-dideoxyguanosine (XI): 3′-O-Levulinyl-N^2-isobutyryl-2′-deoxyguanosine (2.87 g, 6.6 mmol) was converted to the 5′-iodo compound using the procedure described for compound VI above, and was purified on silica gel (40 g) using 0-10% ethanol in dichloromethane as eluant. 5′-Iodo-3′-O-levulinyl-N^2-isobutyryl-2′,5′-dideoxyguanosine was isolated as a foam (3 g, 5.5 mmol) and reacted with sodium triphenylmethylmercaptide (7.2 mmol) as described for compound II above. Crude product was purified by column chromatography on silica gel (30 g) eluting with a gradient of 0-5% ethanol in dichloromethane. Pure fractions were pooled and evaporated *in vacuo* to give the title compound (XI) as a white foam (3.37 g, 71%).

5′-(S-Triphenylmethyl)mercapto-N^2-isobutyryl-2′,5′-dideoxyguanosine (XII): 5′-(S-Triphenylmethyl)mercapto-3′-O-levulinyl-N^2-isobutyryl-2′,5′-dideoxyguanosine (3.27 g, 4.72 mmol; XI) was treated with hydrazine hydrate as described for compound VII above. The crude product was chromatographed on silica gel (30 g) using a gradient of 0-15% ethanol in dichloromethane as eluant. The precipitation procedure gave pure 5′-(S-triphenylmethyl)mercapto-N^2-isobutyryl-2′,5′-dideoxyguanosine (XII) as a white powder (2.0 g, 71%) of R_f 0.09 in solvent system A.

5′-(S-Triphenylmethyl)mercapto-N^2-isobutyryl-2′,5′-dideoxyguanosine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (XIII): 5′-(S-Triphenylmethyl)mercapto-N^2-isobutyryl-2′, 5′-dideoxyguanosine (1.19 g, 2 mmol; XII) was phosphitylated according to the procedure given for compound VIII above. Crude product was chromatographed on silica gel (20 g) as for compound (VIII). Low temperature precipitation afforded the title compound (XIII) as a white powder (1.08g, 68%) of R_f 0.20 in solvent system B. ^{31}P n.m.r. spectrum (CH_2Cl_2, external D_2O lock): singlets at δ + 147.73 and 147.32 p.p.m.

3′-O-Levulinyl-N^6-pivaloyl-2′-deoxyadenosine (XV): 5′-O-Monomethoxytrityl-N^6-pivaloyl-2′-deoxyadenosine (6.08 g, 10 mmol; XIV) was levulinylated and detritylated according to the procedure for compound V above. Crude product was purified by column chromatography on silica gel (100 g) eluting with 0-10% ethanol in dichloromethane. Pure fractions as determined by t.l.c. (R_f 0.45 in solvent system A) were pooled and evaporated *in vacuo* to give the title compound (XV) as a foam (2.49 g, 57%).

5′-O-(4-Nitrobenzenesulphonyl)-3′-O-levulinyl-N^6-pivaloyl-2′-deoxyadenosine (XVI): 3′-O-Levulinyl-N^6-pivaloyl-2′-deoxyadenosine (2.43 g, 5.6 mmol; XV) was dried by evaporation of pyridine *in vacuo*. Residue was dissolved in pyridine, cooled in an ice bath and 4-nitrobenzenesulphonylchloride (1.3 g, 5.87 mmol) was added with stirring and exclusion of moisture. After 3 h at 0°C the reaction was judged complete by t.l.c. (product R_f 0.55 in solvent system A). Water (2 ml) was added and the solvent was evaporated *in vacuo*. After the normal work up the crude product was purified on silica gel (70 g), eluting with 0-4% ethanol in dichloromethane. The pure title compound (XVI) was obtained as an almost colourless foam (2.1 g, 61%).

5′-(S-Triphenylmethyl)mercapto-N^6-pivaloyl-2′,5′-dideoxyadenosine (XVII): 5′-O-(4-Nitrobenzenesulphonyl)-3′-O-levulinyl-N^6-pivaloyl-2′-deoxyadenosine (2.1 g, 3.4 mmol; XVI) was treated with sodium triphenylmethylmercaptide (5 mmol) in DMF as described for compound II above. The red solution was left for 1 h at room temperature and judged complete by t.l.c. (product R_f 0.60 in solvent system A). After the usual work up, crude 5′-(S-triphenylmethyl)mercapto-3′-O-levulinyl-N^6-pivaloyl-2′,5′-dideoxyadenosine was obtained as a foam, which was dried by evaporation of pyridine and delevulinylated as described for compound VII above. After work up, the crude product was purified on silica gel (60 g) eluting with 0-5% ethanol in dichloromethane. Precipitation into petroleum ether afforded the title compound (XVII) as a white powder (1.46 g, 72.3%). R_f 0.17 on t.l.c. in solvent system A.

5′-(S-Triphenylmethyl)mercapto-N^6-pivaloyl-2′,5′-dideoxyadenosine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (XVIII): 5′-(S-Triphenylmethyl)mercapto-N^6-pivaloyl-2′,5′-dideoxyadenosine (1.19 g, 2 mmol; XVII) was phosphitylated according to the procedure given for compound VIII above. Chromatography on silica gel (30 g) eluting with dichloromethane/petroleum ether (3:2 v/v) containing 2% triethylamine followed by a low temperature precipitation afforded the title compound (XVIII) as a white powder (1.3 g, 82%). R_f 0.53 and 0.44 in solvent system B. ^{31}P n.m.r. spectrum (CH_2Cl_2 external D_2O lock): singlets at δ + 147.59 and 147.44 p.p.m.

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