Deoxyribonucleoside Phosphorodithioates. Preparation of Dinucleoside Phosphorodithioates from Nucleoside Thiophosphoramidites

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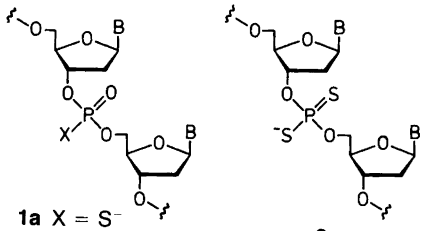
Dahl, B. H., Bjergårde, K., Sommer, V. B. and Dahl, O., 1989. Deoxyribonucleoside Phosphorodithioates. Preparation of Dinucleoside Phosphorodithioates from Nucleoside Thiophosphoramidites. – Acta Chem. Scand. 43: 896–901.

A series of protected thymidine thiophosphoramidites have been prepared and their properties evaluated. Although less reactive than phosphoramidites, thiophosphoramidites with small *N*-substituents (methyl) are useful synthons for the preparation of nucleoside phosphorodithioates, as demonstrated by the preparation of a thymidine dimer. The coupling reactions are not as clean as those of the analogous phosphoramidites since the alkylthio group is somewhat labile.

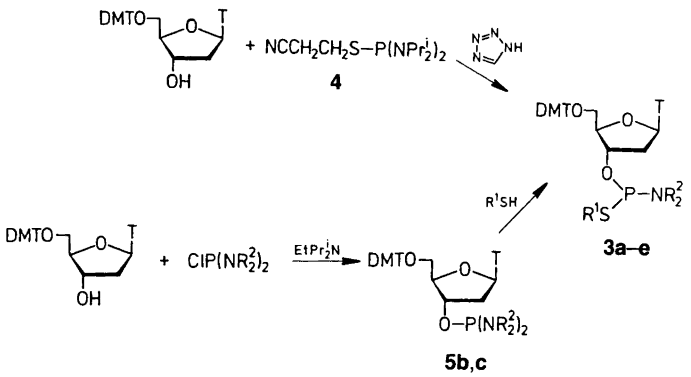
Oligodeoxyribonucleotides modified in the phosphate groups, e.g. phosphorothioates **1a**,[1] methylphosphonates **1b**,[2] phosphortriesters **1c**,[3] and phosphoramidates **1d**[4] (Scheme 1) have been used extensively for the study of enzyme recognition and stereoselectivity of nuclease cleavage. Lately, renewed interest in such compounds, particularly **1a**, has come from reports of their promising properties as nuclease-resistant antisense probes which selectively inhibit protein synthesis in living cells.[5]

The modified oligonucleotides **1a–d** all contain chiral phosphorus centers, and chemically synthesized probes with *n* modified phosphate groups are mixtures of 2^n diastereomers. This creates difficulties for the purification and characterisation of such probes. Also, since some nucleases can degrade one of the epimeric phosphorothioate configurations,[6] these probes may show variable resistance towards nuclease cleavage.



Scheme 1. Oligodeoxyribonucleotides modified in the phosphate groups.

Phosphate-modified oligonucleotides with achiral phosphorus centers would be more attractive as antisense probes, and an obvious candidate for such compounds is phosphorodithioates **2**. Here we describe our results on the preparation of deoxyribonucleoside thiophosphoramidites **3a–e** (Scheme 2) and their use in the preparation of dimeric deoxyribonucleoside phosphorodithioates.[7] Apart from two cyclic nucleoside phosphorodithioates,[8] nucleoside phosphorodithioates containing the S=P–S⁻ unit were unknown when we initiated this study. Recently, however, Caruthers *et al.* have prepared several nucleoside phosphorodithioate dimers, using different routes including one via thiophosphoramidites.[9–11] Also Gorenstein *et al.* have described a nucleoside thiophosphoramidite and briefly its potential use to prepare nucleoside phosphorodithioates.[12] Very recently Caruthers *et al.* have described a series of thiophosphoramidites derived from all four deoxyribonu-



Scheme 2. Preparation of thymidine thiophosphoramidites. R^1 = CH_2CH_2CN (**a–c**) or 2,4-dichlorobenzyl (**d**, **e**); R^2 = Pr^i (**a**), Et (**b**, **d**), Me (**c**, **e**).

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cleosides and their use in the preparation of oligodeoxyribonucleoside phosphorodithioates.[13] Although they do not use the 2-cyanoethyl protecting group on sulfur, their preparative method is similar to ours, and one of the thiophosphoramidites is identical with one of those described here (**3e**).

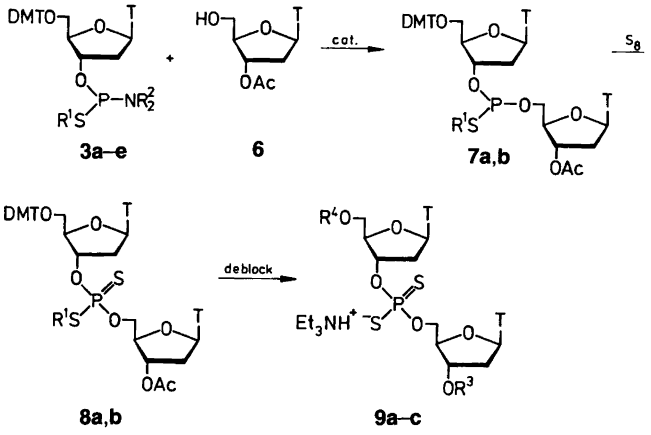
Results and discussion

Unmodified oligodeoxyribonucleotides are today usually prepared by solid-phase synthesis from protected nucleoside 2-cyanoethyl *N,N*-diisopropylphosphoramidites.[14] A similar substituted thymidine thiophosphoramidite, **3a** (Scheme 2), was therefore prepared to examine its use to obtain phosphorodithioates. The synthesis of **3a** was straightforward from 5′-*O*-(4,4′-dimethoxytrityl)thymidine, tetrazole, and the new thiophosphorodiamidite **4**. However, **3a** was surprisingly unreactive towards 3′-acetylthymidine in the presence of tetrazole; the coupling rate, estimated under the same conditions as described earlier for phosphoramidites,[15] was ca. 300 times lower than that of the analogous phosphoramidite. Attempts to increase the rate to a level suitable for solid-phase synthesis by using stronger acid catalysts were not promising. 5-(4-Nitrophenyl)tetrazole and *N*-methylimidazolium trifluoromethanesulfonate did not increase the rate sufficiently, and with even stronger acids such as 5-trifluoromethyltetrazole or *N*-methylanilinium trifluoroacetate, some loss of the DMT group occurred. However, Caruthers[10] succeeded in preparing dimers from a similar thiophosphoramidite (**3**, R^1 = 4-chlorobenzyl, R^2 = Pr^i) using the very strong acid pyridinium tetrafluoroborate.

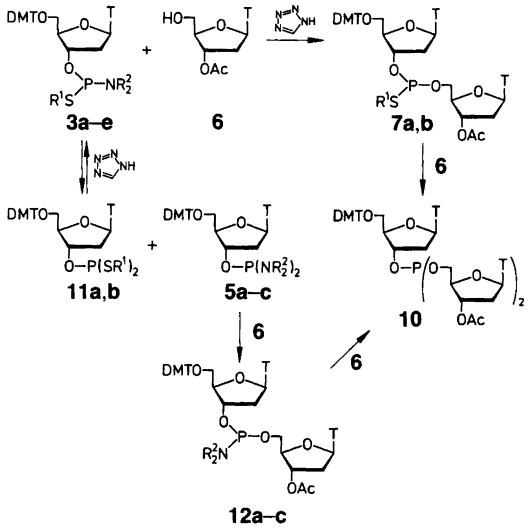
In order to increase the reactivity we turned to thiophosphoramidites with smaller *N*-substituents and prepared **3b–e** (Scheme 2). A similar route to that successful for **3a** was abandoned because of difficulties in isolating the necessary thiophosphorodiamidites analogous to **4** in a reasonably pure state. However, **3b–e** could be conveniently prepared from the nucleoside phosphorodiamidites **5b–c** and the corresponding thiol (Scheme 2). The reactions of **5b–c** with thiols were quite rapid and clean without addition of a catalyst; ethyldiisopropylammonium chloride present in the solutions presumably functioned as such. Caruthers *et al.*[13] have independently used the same method to prepare similar thiophosphoramidites.

The thiophosphoramidites were obtained fairly pure (85–95 % according to ^{31}P and ^{1}H NMR spectroscopy) after being washed with aqueous $NaHCO_3$ and precipitated into hexane, and **3b–e** were used as such for the coupling experiments. **3a** could be purified by column chromatography on silica, but **3b–e** partially decomposed on such treatment. The thiophosphoramidites with an *S*-(2,4-dichlorobenzyl) group, **3d–e**, are easily autoxidized which made degassing of solvents necessary. This has also been observed by Caruthers for *S*-(4-chlorobenzyl) thiophosphoramidites.[10] The *S*-(2-cyanoethyl) thiophosphoramidites **3a–c** were much less prone to oxidation by air and could be used in non-degassed solvents. The thermal stabilities were lower than those of the corresponding phosphoramidites; although **3c** could be kept at −20 °C for at least a month, and **3a** for at least a year without significant decomposition, **3c** was largely decomposed after 1–2 weeks at 4 °C. Solutions of **3b–e** in acetonitrile for coupling experiments should be freshly made and used within 1–2 days.

Coupling reactions. The thioamidites **3b–e** in acetonitrile all reacted with 3′-acetylthymidine **6** in the presence of tetrazole to give, after oxidation with sulfur, the protected dinucleoside phosphorodithioates **8a** or **8b** (Scheme 3). The reactions, however, were not very clean, and ^{31}P NMR spectroscopic monitoring showed that the thiophosphite **7** initially formed (in ca. 50 % yield after 3 min from equiv-



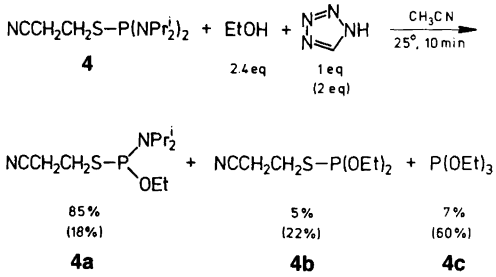
Scheme 3. Preparation of dimers. **7–9**, R^1 = CH_2CH_2CN (**a**) or 2,4-dichlorobenzyl (**b**); R^3 = acetyl (**a**) or H (**b, c**); R^4 = DMT (**a, b**) or H (**c**).



Scheme 4. Formation of by-products during the preparation of dimers **5–12**, R^1 = CH_2CH_2CN (**a**) or 2,4-dichlorobenzyl (**b**); R^2 = Pr^i (**a**), Et (**b**), or Me (**c**).

alent amounts of **3c** and **6**) slowly decomposed to the trinucleoside phosphite **10** (Scheme 4). Other major by-products were the nucleoside dithiophosphite **11** and the dinucleoside phosphoramidite **12** [all new compounds were isolated after oxidation with sulfur and characterized by ^{31}P (Table 1) and ^{1}H NMR spectroscopy as well as by comparison with authentic samples]. The slow formation of **10** from **7** shows that the alkylthio group on **7** is somewhat labile under the reaction conditions. This is in accordance with results of Pudovik *et al.*[16] and Burgada *et al.*[17] who showed that SR groups on tervalent phosphorus, under neutral or basic conditions, are leaving groups comparable to NR_2 groups. This is the case also under acidic conditions (tetrazole catalysis) since we found that **4** with 2.4 equivalents of ethanol and tetrazole in acetonitrile gave a mixture of **4a**, **4b** and **4c** (Scheme 5). The other major by-products, **11** and **12**, are probably formed from **3**, which is prone to dismutation in the presence of tetrazole. Thus **3c** with 4 equivalents of tetrazole in acetonitrile (no hydroxy compound added) gave ca. 10 % of the dithiophosphite **11a** after 15 min, and ca. 35 % after 2 h. No phosphorodiamidite **5c** was observed; however signals from its tetrazolide (δ_P 130 ppm) and its hydrolysis product (δ_P 16.3, 16.2 ppm) were seen, and **5c** (δ_P 137.5 ppm) was reformed from its tetrazolide upon addition of an excess of ethyldiisopropylamine. In contrast with the corresponding amidite the thioamidite **3c** did not form any observable amounts of tetrazolide, and the signals from **3c** remained sharp on addition of tetrazole.

These results indicate that, in order to obtain a high yield of phosphorodithioates **8**, it is necessary to use an excess of the thioamidite **3**, and to oxidize **7** with sulfur immediately after the reaction. The thioamidites **3c** and **3e** containing NMe_2 groups reacted somewhat faster than those containing NEt_2, and since the latter were neither significantly more stable nor could be purified by column chromatography we used **3c** and **3e** for the preparation of dimers. By employing 2 equivalents of **3c** or **3e** and 3 equivalents of tetrazole, and by adding an excess of sulfur after 3 min, we



Scheme 5. Model experiments showing leaving group properties of the *S*-(2-cyanoethyl) group.

obtained **8a** or **8b** in a crude yield of ca. 90 % according to ^{31}P NMR spectroscopy. The yield of purified **8** was about 60 %, but even careful chromatography failed to remove all of the impurities. A pure product **9a** or **9b**, however, was obtained after removal of the *S*-alkyl group and renewed chromatography. The dichlorobenzyl group was removed with thiophenolate,[18] and the 2-cyanoethyl group (together with the 3'-acetyl group) with concentrated aqueous ammonia in pyridine. The latter cleavage procedure gave only 1.5–2 % concomitant hydrolysis to phosphorothioate; an alternative reagent, t-butylamine in pyridine[19] was less reactive and gave slightly less phosphorothioate (0.9 %). Finally, standard removal of the DMT group (and the 3'-acetyl group of **9a**) gave the unprotected dimer phosphorodithioate **9c**. The NMR and MS data were in full agreement with the structure and correspond closely to those given by Caruthers[9] for the same compound.

Properties of dinucleoside phosphorodithioates. The ultimate goal of our studies described here is to use nucleoside thiophosphoramidites to prepare oligonucleoside phosphorodithioates **2** by solid-phase synthesis. Therefore, we evaluated the stability of phosphorodithioate groups towards the common reagents used in solid-phase syn-

Table 1. ^{31}P NMR Chemical shifts of compounds from coupling reactions between **3a–e** and **6**.

	δ_P/ppm			*P*-sulfide, δ_P/ppm	
	CH_3CN	$CDCl_3$		CH_3CN	$CDCl_3$
3a	162.3, 161.4	164.4, 162.7			88.6, 88.0
3b	168.9, 167.4	169.1, 167.3		92.8, 92.7	93.2
3c	170.3, 168.8	172.6, 170.9		95.0	95.7, 95.5
3d	167.2, 165.3	168.9, 167.4			94.3, 94.1
3e	169.8, 167.8	171.8, 170.1		95.2	96.6, 96.1
5b	132.8	134.2		77.1	
5c	137.5	138.3		82.5	76.6
7a	191.8, 191.7		**8a**	94.7, 94.5	95.7, 95.5
7b	191.7, 191.6		**8b**	94.3, 93.6	97.0, 95.6
10	139.5			66.8	67.0
11a	158.7	160.7		107.9	107.7
11b	156.9			108.0	
12c	146.6, 145.5			76.6, 76.4	77.1, 76.8

theses, *i.e.* the detritylation, capping, oxidation, and deblocking reagents.

When the fully protected dimer **8a** was treated with a detritylation reagent, 3 % $CHCl_2COOH$ in $(CH_2Cl)_2$, for 24 h at room temperature, the DMT group was quickly removed, but no other products were observed (TLC, ^{31}P NMR). By the same criterion **8a** was stable to a capping reagent, Ac_2O/2,6-lutidine/*N*-methylimidazole/THF 1:1:2:16 (v/v), for 24 h at room temperature. Attempted oxidation with 0.1 M I_2 in H_2O/2,6-lutidine/THF 1:2:7 (v/v) at room temperature gave no phosphorothioate or other products after 24 h. This latter result was somewhat surprising since partial removal of sulfur in oligonucleoside phosphorothioates, probably caused by repeated I_2 oxidation, has been observed earlier.[20] Finally, the unprotected dimer **9c** was treated with 32 % aqueous NH_3 at 55 °C for 24 h; no phosphorothioate or other hydrolysis products were observed (^{31}P NMR; detection limit ca. 1 %).

These experiments show that phosphorodithioates are stable under the conditions used to prepare oligonucleotides on solid supports. This has also been reported by Caruthers *et al.* for **8b**,[9] but not for **8a** which contains the more easily removable *S*-(2-cyanoethyl) group.

Conclusion

The results described in this paper show that thiophosphoramidites are less reactive than phosphoramidites towards hydroxy compounds, and that some by-products are formed during the coupling reaction. The nucleoside thiophosphoramidites **3c** and **3e**, however, with the nucleoside **6** and tetrazole, followed by oxidation with sulfur, do give good yields of dinucleoside phosphorodithioates rapidly, and the phosphorodithioate group is stable under normal solid-phase synthesis conditions.

Work is in progress to use **3c** and **3e**, and the corresponding derivatives of the other deoxyribonucleosides dA, dC, and dG, for solid-phase synthesis of oligodeoxyribonucleoside phosphorodithioates, and to study the properties of such modified oligonucleotides as antisense probes.

Experimental

Acetonitrile (Rathburn, HPLC grade) and carbon disulfide were dried over 4Å molecular sieves. Chloroform was washed free of ethanol with water and dried over molecular sieves, then filtered through basic alumina (ICN Biomedicals, Alumina B-Super I). Dichloromethane, tetrahydrofuran, dioxane, hexane, triethylamine, and ethyldiisopropylamine were dried by being filtered through basic alumina. Pyridine was distilled from tosyl chloride and dried over 4Å molecular sieves. All solvents had a water content of less than 20 μg ml^{-1} as determined by Karl Fischer titration (Metrohm 652 KF Coulometer). Elemental sulfur (Aldrich 99.999 %) was dried *in vacuo* over Sicapent (Merck). Tetrazole (Aldrich 98 %) was purified by sublimation at 115 °C and 0.2 mmHg. Dichloroacetic acid and thiophenol (Aldrich 99+ %) were used as received. ^{31}P NMR spectra were obtained on a JEOL FX 90 Q spectrometer at 36.4 MHz in 5 mm tubes; chemical shifts (δ_P) are positive in the low-field direction, external reference 85 % H_3PO_4; ^1H NMR spectra were obtained on the same spectrometer, internal reference $SiMe_4$.

S-*(2-Cyanoethyl)* N,N,N′,N′-*tetraisopropylthiophosphorodiamidite* (**4**). To a stirred solution of $(Pr^i_2N)_2PCl$[21] (13.3 g, 0.05 mol) and dry Et_3N (10 ml) in dry THF (50 ml), at 0 °C under N_2, was added dropwise $HSCH_2CH_2CN$[22] (4.4 g, 0.05 mol). The mixture was stirred for 3 h at room temperature, filtered under N_2 to remove $Et_3NH^+Cl^-$, and the solvent removed in a rotory evaporator. The oily residue was distilled through a small Claisen head to give the product as a yellow oil (11.0 g, 69 %), b.p. 126–127 °C at 0.5 mmHg, ca. 99 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 90.7; δ_H 3.59 (dsept, $^3J_{PH}$ 11.7, $^3J_{HH}$ 6.7 Hz, NCH), 2.75–2.64 (m, CH_2CH_2CN), 1.18 (2×d, $^3J_{HH}$ 6.7, Δ 3.8 Hz, CH_3).

O-*(5′-O-Dimethoxytritylthymidin-3′-yl)* S-*(2-cyanoethyl)* N,N-*diisopropylthiophosphoramidite* (**3a**). 5′-*O*-Dimethoxytritylthymidine[23] (545 mg, 1 mmol) was dried by coevaporation with dry $CH_3CN/CHCl_3$ (1:1 v/v, 10 ml) and redissolved in the same mixture (5 ml). To this solution was added tetrazole (2.5 ml 0.4 M in CH_3CN, 1 mmol) and **4** (510 mg, 1.6 mmol). After 18 h at room temperature the solvents were evaporated, the residue dissolved in dry CH_2Cl_2 (10 ml), and the solution extracted with saturated aqueous $NaHCO_3$ (3×10 ml), dried ($MgSO_4$) and evaporated. The residue was dissolved in a mixture of CH_2Cl_2, EtOAc, and pyridine (49:49:2 v/v; 3 ml), loaded onto a silica column (Merck Kieselgel 60, art. 9385, diam. 4 cm, height 8 cm) and eluted with the same solvent mixture. The fractions containing the product (TLC, R_f 0.60 in the elution mixture) were pooled and evaporated, the residue dissolved in dry CH_2Cl_2 (2 ml), and the product precipitated into dry hexane (30 ml) at 0 °C. Yield 550 mg (72 %) of a colourless powder, more than 98 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 164.4, 162.7; δ_H 9.6 (s, 1 H, NH), 7.6–7.5 (1 H, H-6), 7.5–7.1 and 6.9–6.7 (13 H, arom.), 6.4 (dd, *J* 6 Hz, 1 H, H-1′), 4.8–4.5 (1 H, H-3′), 4.2–4.1 (1 H, H-4′), 3.78 (s, 6 H, OCH_3), 3.8–3.2 (4 H, H-5′ and NCH), 2.8–2.3 (6 H, H-2′ and CH_2CH_2CN), 1.46 (s, 3 H, 5-CH_3), 1.3–1.0 (12 H, CH_3).

O-*(5′-O-Dimethoxytritylthymidin-3′-yl)* S-*(2-cyanoethyl)* N,N-*diethylthiophosphoramidite* (**3b**). 5′-*O*-Dimethoxytritylthymidine[23] (545 mg, 1 mmol) was dried by coevaporation with dry $CH_3CN/CHCl_3$ (1:1 v/v; 10 ml) and redissolved in dry $CHCl_3$ (2.5 ml). After the addition of dry $EtPr^i_2N$ (195 mg, 1.5 mmol) and cooling in ice, $(Et_2N)_2PCl$[24] (210 mg, 1 mmol) was added under N_2 and the mixture stirred for 10 min at room temperature. $HSCH_2CH_2CN$[22] (96 mg, 1.1 mmol) was then added and

the mixture was stirred for 0.5 h at room temperature. The clear solution was diluted with dry CH_2Cl_2 (5 ml), extracted with saturated aqueous $NaHCO_3$ (2×5 ml), the organic phase dried ($MgSO_4$) and the solvent evaporated. The residue was dissolved in dry CH_2Cl_2 (5 ml) and precipitated in dry, degassed hexane (150 ml) at 0 °C. The precipitate was finally lyophilized from dry, degassed CH_3CN (10 ml) to give 660 mg (90 %) of the product as a colourless powder, ca. 95 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 169.1, 167.3; δ_H 9.2 (s, 1 H, NH), 7.7–7.6 (1 H, H-6), 7.5–7.1 and 7.0–6.8 (13 H, arom.), 6.4 (dd, *J* 6 Hz, H-1′), 4.8–4.5 (1 H, H-3′), 4.2–4.1 (1 H, H-4′), 3.79 (s, 6 H, OCH_3), 3.6–2.3 (12 H, H-5′, H-2′, NCH_2 and SCH_2CH_2CN), 1.46 (s, 3 H, 5-CH_3), 1.06 (2×t, *J* 7 Hz, NCH_2CH_3).

O-*(5′-O-Dimethoxytritylthymidin-3′-yl)* S-*(2-cyanoethyl)* N,N-*dimethylthiophosphoramidite* (**3c**) was prepared in the same way as **3b**, using $(Me_2N)_2PCl$[25] (155 mg, 1 mmol) instead of $(Et_2N)_2PCl$. Yield 625 mg (89 %) of a pale yellow powder, ca. 95 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 172.6, 170.9; δ_H 9.4 (s, 1 H, NH), 7.7–7.5 (1 H, H-6), 7.5–7.2 and 7.0–6.7 (13 H, arom.), 6.4 (dd, *J* 6 Hz, H-1′), 4.9–4.6 (1 H, H-3′), 4.2–4.1 (1 H, H-4′), 3.78 (s, 6 H, OCH_3), 3.6–3.3 (2 H, H-5′), 3.0–2.2 (6 H, H-2′ and CH_2CH_2CN), 2.70 and 2.61 (d+d, $^3J_{PH}$ 10 Hz, 6 H, NCH_3), 1.48 (s, 3 H, 5-CH_3).

O-*(5′-O-Dimethoxytritylthymidin-3′-yl)* S-*(2,4-dichlorobenzyl)* N,N-*diethylthiophosphoramidite* (**3d**) was prepared in the same way as **3b**, using 2,4-dichlorophenylmethanethiol[26] (212 mg, 1.1 mmol) instead of $HSCH_2CH_2CN$. Yield 670 mg (90 %) of a colourless powder, ca. 95 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 168.9, 167.4; δ_H 9.3 (s, 1 H, NH), 7.6–7.5 (1 H, H-6), 7.4–7.2 and 6.9–6.8 (16 H, arom.), 6.4 (dd, *J* 6 Hz, H-1′), 4.9–4.5 (1 H, H-3′), 4.2–4.0 (1 H, H-4′), 3.9–3.7 (2 H, SCH_2 partially hidden by OCH_3), 3.78 (s, 6 H, OCH_3), 3.6–2.9 (6 H, H-5′ and NCH_2), 2.6–2.1 (2 H, H-2′), 1.44 (s, 3 H, 5-CH_3), 1.01 (2×t, *J* 7 Hz, NCH_2CH_3).

O-*(5′-O-Dimethoxytritylthymidin-3′-yl)* S-*(2,4-dichlorobenzyl)* N,N-*dimethylthiophosphoramidite* (**3e**) was prepared in the same way as **3c**, using 2,4-dichlorophenylmethanethiol[26] (212 mg, 1.1 mmol) instead of $HSCH_2CH_2CN$. Yield 730 mg (90 %) of a pale yellow powder, ca. 85 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 171.8, 170.1 (lit.[13] 172.1, 170.4); δ_H 9.1 (s, 1 H, NH), 7.6–7.5 (1 H, H-6), 7.4–7.1 and 6.9–6.8 (16 H, arom.), 6.4 (dd, *J* 6 Hz, 1 H, H-1′), 4.8–4.6 (1 H, H-3′), 4.2–4.0 (1 H, H-4′), 3.86 (d, *J* 3 Hz, 1 H, half of SCH_2), 3.78 (s, 6 H, OCH_3), 3.8–3.2 (2 H, H-5′), 2.56 and 2.53 (d+d, $^3J_{PH}$ 10 Hz, 6 H, NCH_3), 2.8–2.2 (2 H, H-2′), 1.45 (s, 3 H, 5-CH_3).

O-*(3′-O-Acetylthymidin-5′-yl)* O-*(5′-dimethoxytritylthymidin-3′-yl)* S-*(2-cyanoethyl) phosphorodithioate* (**8a**). 3′-*O*-Acetylthymidine[27] (284 mg, 1 mmol) and tetrazole (210 mg, 3 mmol) were dried by coevaporation with dry CH_3CN (10 ml). The thioamidite **3c** (1.41 g, 2 mmol) was dissolved in dry, degassed CH_3CN (3 ml) and added under N_2 to the dry mixture of 3′-*O*-acetylthymidine and tetrazole. The stirred suspension became clear after 1 min and was stirred for another 2 min when an excess of sulfur (192 mg, 6 mmol S), dissolved in CS_2/pyridine (1:1 v/v; 5 ml), was added. Analysis of the reaction mixture by ^{31}P NMR spectroscopy showed that the product (δ_P 94.7, 94.5) constituted ca. 45 % of the total phosphorus content (ca. 90 % yield).

The reaction mixture was evaporated to dryness and the residue dissolved in EtOAc (20 ml). The excess sulfur was removed by filtration, and the EtOAc phase was washed with saturated aqueous $NaHCO_3$ (3×10 ml), dried ($MgSO_4$) and evaporated. The residue was purified on a silica column (Merck Kieselgel 60, diam. 4 cm, height 20 cm), eluted with a mixture of CH_2Cl_2, EtOAc, MeOH, and pyridine (49:48:2:1 v/v). The fractions containing the product (TLC, R_f 0.11 in the same elution mixture) were collected and evaporated. Lyophilization from CH_3CN gave a pale yellow product (590 mg, 60 %), 90 % pure according to ^{31}P NMR spectroscopy. The main impurities were the sulfide of **12c** (ca. 2 %) and two unknown products (δ_P 96.1 and 94.9, 5 %). NMR ($CDCl_3$): δ_P 95.7, 95.5; δ_H 9.7 and 9.65 (2×s, 2×1 H, NH), 7.6 (s, 1 H, H-6), 7.5–7.2 and 7.0–6.8 (14 H, arom. and H-6), 6.5–6.2 (2 H, H-1′), 5.6–5.2 (2 H, H-3′), 4.5–4.1 (4 H, H-5′ and H-4′), 3.5 (2 H, H-5′), 3.3–2.2 (8 H, H-2′ and CH_2CH_2CN), 2.1 (s, 3 H, CH_3CO), 1.9 and 1.5 (2×s, 2×3 H, CH_3-5).

O-*(3′-O-Acetylthymidin-5′-yl)* O-*(5′-dimethoxytritylthymidin-3′-yl)* S-*(2,4-dichlorobenzyl) phosphorodithioate* (**8b**) was prepared in the same way as **8a**, using **3e** (1.62 g, 2 mmol) instead of **3c**. In the crude mixture **8b** (δ_P 94.3, 93.6) constituted ca. 48 % of the total phosphorus content (ca. 95 % yield). Column chromatography on silica (diam. 4 cm, height 10 cm) with a mixture of CH_2Cl_2, EtOAc, MeOH, and Et_3N (45:45:5:5 v/v) gave a pale yellow product (R_f 0.60, 650 mg, 60 %), ca. 85 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 97.0, 95.6; δ_H 7.6 and 7.5 (2×s, 2×1 H, H-6), 7.4–7.1 and 6.9–6.7 (16 H, arom), 6.5–6.2 (2 H, H-1′), 5.5–5.2 and 5.1–5.0 (2×1 H, H-3′), 4.3–3.9 (6 H, H-4′, H-5′, and SCH_2), 3.78 (s, 6 H, OCH_3), 3.5–3.3 (2 H, H-5′), 2.7–2.1 (4 H, H-2′), 2.1 (s, 3 H, $COCH_3$), 1.9 and 1.5 (2×s, 2×3 H, CH_3-5).

Triethylammonium O-*thymidin-5′-yl* O-*thymidin-3′-yl phosphorodithioate* (**9c**). To a solution of **8a** (98 mg, 0.1 mmol) in pyridine (0.3 ml) was added 32 % aqueous NH_3 (0.3 ml). Analysis by ^{31}P NMR spectroscopy (**8a** δ_P 94.8, 94.5, **9b** δ_P 115.4) showed that the 2-cyanoethyl group was removed with a $t_{1/2}$ of 7 min at 26 °C, with 1.5–2 % concomitant hydrolysis to monothioate (δ_P 55.5, 55.1). An alternative reagent, Bu^tNH_2/pyridine 1:9 (v/v),[19] removed the 2-cyanoethyl group with a $t_{1/2}$ of 12 min and gave **9a** together with 0.9 % monothioate.

The pyridine/aqueous NH_3 mixture was evaporated after 1 h at room temperature and the residue coevaporated twice with dry CH_3CN. Chromatography on a silica column (Merck Kieselgel 60, diam. 1 cm, height 5 cm), eluted with a mixture of CH_2Cl_2, MeOH, and Et_3N (90:9:1 v/v), gave triethylammonium *O*-thymidin-5′-yl *O*-(5′-dimethoxytritylthymidin-3′-yl) phosphorodithioate **9b** (R_f 0.20 in the elution mixture), 69 mg, 70 %, more than 99 % pure according to ^{31}P NMR spectroscopy. NMR ($CDCl_3$): δ_P 113.2. The dimethoxytrityl group was removed with 80 % acetic acid for 1 h at room temperature, followed by evaporation and extraction of dimethoxytrityl alcohol with ether. The product **9c** was obtained after lyophilization from water as a colourless powder in nearly quantitative yield. The purity was more than 99 % according to ^{31}P NMR spectroscopy. NMR (D_2O): δ_P 113.2 (lit.[9] δ_P 113.3); δ_H 7.8 and 7.7 (2×s, 2×1 H, H-6), 6.4–6.2 (2 H, H-1′), 5.4–4.5 (2 H, H-3′), 4.2 (4 H, H-5′ and H-4′), 3.8 (2 H, H-5′), 3.2 (q, 6 H, CH_3CH_2N), 2.6–2.2 (4 H, H-2′), 2.0 and 1.9 (2×s, 2×3 H, CH_3-5), 1.3 (t, 9 H, CH_3CH_2N). FAB^+ MS (glycerol): 579.2 ($M-Et_3NH^+ + 2H^+$. Calc. 579.16).

Acknowledgement. Financial support from the Danish Natural Science Research Council is gratefully acknowledged.

References

1. Eckstein, F. *Annu. Rev. Biochem. 54* (1985) 367.
2. Miller, P. S., Reddy, M. P., Murakami, A., Blake, K. R., Lin, S.-B. and Agris, C. H. *Biochemistry 25* (1986) 5092.
3. Gallo, K. A., Shao, K.-L., Phillips, L. R., Regan, J. B., Koziolkiewicz, M., Uznanski, B., Stec, W. J. and Zon, G. *Nucleic Acids Res. 14* (1986) 7405.
4. Froehler, B., Ng, P. and Matteucci, M. *Nucleic Acids Res. 16* (1988) 4831.
5. Marcus-Sekura, C. J., Woerner, A. M., Shinozuka, K., Zon, G. and Quinnan, G. V., Jr. *Nucleic Acids Res. 15* (1987) 5749; Matsukura, M., Shinozuka, K., Zon, G., Mitsuya, M., Reitz, M., Cohen, J. S. and Broder, S. *Proc. Natl. Acad. Sci. USA 84* (1987) 7706.
6. Stein, C. A., Subasinghe, C., Shinozuka, K. and Cohen, J. S. *Nucleic Acids Res. 16* (1988) 3209, and references therein.
7. *Preliminary report*: Dahl, B. H., Bjergårde, K., Sommer, V. B. and Dahl, O. *Nucleosides & Nucleotides 8* (1989). *In press.*
8. Eckstein, F. *J. Am. Chem. Soc. 92* (1970) 4718; Baraniak, J. and Stec, W. *J. Chem. Soc., Perkin Trans. 1* (1987) 1645.
9. Nielsen, J., Brill, W. K.-D. and Caruthers, M. H. *Tetrahedron Lett. 29* (1988) 2911.
10. Brill, W. K.-D., Nielsen, J. and Caruthers, M. H. *Tetrahedron Lett. 29* (1988) 5517.
11. Grandas, A., Marshall, W. S., Nielsen, J. and Caruthers, M. H. *Tetrahedron Lett. 30* (1989) 543.
12. Farschtschi, N. and Gorenstein, D. G. *Tetrahedron Lett. 29* (1988) 6843.
13. Brill, W. K.-D., Tang, J.-Y., Ma, Y.-X. and Caruthers, M. H. *J. Am. Chem. Soc. 111* (1989) 2321.
14. Farrance, I. K., Eadie, J. S. and Ivarie, R. *Nucleic Acids Res. 17* (1989) 1231.
15. Dahl, B. H., Nielsen, J. and Dahl, O. *Nucleic Acids Res. 15* (1987) 1729.
16. Kostin, V. P., Sinyashin, O. G., Karelov, A. A., Batyeva, E. S. and Pudovik, A. N. *J. Gen. Chem. USSR 55* (1984) 1931.
17. Willson, M., Goncalves, H., Boudjebel, H. and Burgada, R. *Bull. Soc. Chim. Fr.* (1975) 615; Goncalves, H., Burgada, R., Willson, M., Boudjebel, H. and Mathis, F. *Ibid.* 621; Burgada, R., Willson, M., Goncalves, H. and Boudjebel, H. *Ibid.* 2207.
18. Brill, W. K.-D. and Caruthers, M. H. *Tetrahedron Lett. 29* (1988) 1227.
19. Cosstick, R. and Williams, D. M. *Nucleic Acids Res. 15* (1987) 9921.
20. Stein, C. A., Subasinghe, C., Shinozuka, K. and Cohen, J. S. *Nucleic Acids Res. 16* (1988) 3209.
21. Foss, V. L., Lukashev, N. V. and Lutsenko, I. F. *J. Gen. Chem. USSR 50* (1980) 1000.
22. Bauer, L. and Welsh, T. L. *J. Org. Chem. 26* (1961) 1443.
23. Jones, R. A. In: Gait, M. J., Ed., *Oligonucleotide Synthesis, A Practical Approach,* IRL Press, Oxford 1984, Chap. 2.
24. van Wazer, J. R. and Maier, L. *J. Am. Chem. Soc. 86* (1964) 811.
25. Nöth, H. and Vetter, H.-J. *Chem. Ber. 96* (1963) 1109.
26. Kulka, M. *Can. J. Chem. 34* (1956) 1093.
27. Michelson, A. M. and Todd, A. R. *J. Chem. Soc.* (1953) 951.

Received April 19, 1989.