Synthesis of *S*-alkyl esters of protected 2′-deoxyribonucleoside 3′-phosphorothioates. Building blocks for the large-scale synthesis of phosphorothioate analogues of oligodeoxyribonucleotides by the phosphotriester approach in solution

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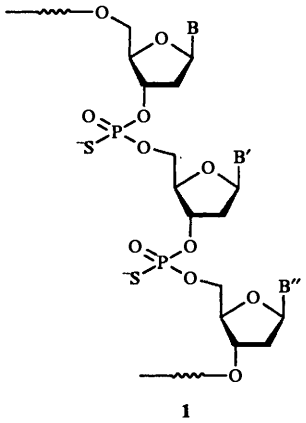
Triethylammonium salts of 5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxyribonucleoside 3′-(*H*-phosphonates) **23**, **33a**, **33b** and **33c**, derived from thymidine, 6-*N*-pivaloyl-2′-deoxyadenosine, 4-*N*-benzoyl-2′-deoxycytidine and 2-*N*-phenylacetyl-2′-deoxyguanosine, react with *N*-(2-cyanoethylsulfanyl)phthalimide **21** in the presence of chlorotrimethylsilane and 4-methylmorpholine to give the corresponding 3′-phosphorothioate *S*-(2-cyanoethyl) esters **24c**, **34a**, **34b** and **34c**, respectively, in good yield. The *S*-(2-cyanoethyl) group appears to be suitable for the protection of internucleotide linkages in the synthesis of oligonucleotide phosphorothioates by the phosphotriester approach in solution.

Introduction

The potential importance of antisense chemotherapy[1] has stimulated organic chemists to undertake the synthesis of a variety of oligonucleotide analogues and especially those analogues in which the internucleotide linkages and sugar residues are modified. Perhaps the most widely investigated analogues in this context are oligodeoxyribonucleotide phosphorothioates[1-3] (*e.g.* **1**). Indeed it is believed[4] that several such sequences are at present undergoing clinical trials. By far the most convenient method of preparing small (say, of the order of 10^{-6} mol) quantities of the latter analogues is by automated solid-phase synthesis using standard phosphoramidite building blocks,[5] and replacing the normal iodine-promoted oxidation by a sulfur-transfer step.[2,3] Although this approach does not lead to control of the stereochemistry of the chiral phosphorothioate internucleotide linkages, the synthetic analogues appear[6] to have satisfactory hybridisation properties.



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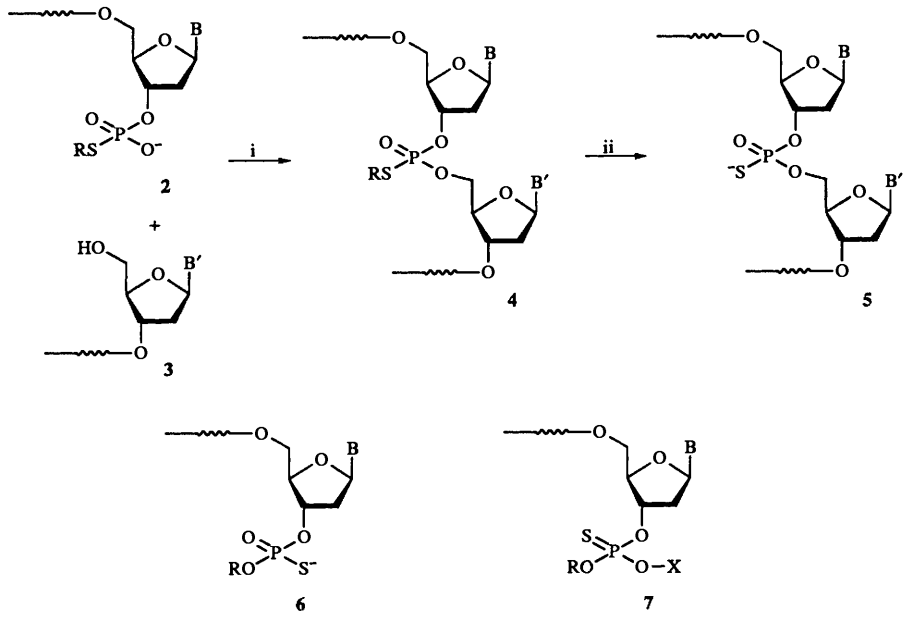
It would appear from the literature[3] that attempts to address the potential need for much larger (say, of the order of 10^{-3} mol or possibly considerably more) quantities of specific oligodeoxyribonucleotide phosphorothioates have mainly involved the scale-up of phosphoramidite-based solid-phase synthesis. The particular advantages of solid-phase synthesis, at least on the 10^{-6} molar scale, are that it can readily be automated, coupling reactions are generally very fast and efficient, and it is very flexible in that, as only one nucleotide residue is added at a time, the target sequence can very easily be changed. However, when the large-scale synthesis of a specific oligonucleotide sequence is to be undertaken, there may well be a number of drawbacks to the use of solid-phase synthesis. For example, a relatively large excess of phosphoramidite building block is likely to be required[7] in each coupling step, and coupling efficiencies may well fall as the scale increases. Furthermore, it seems likely that the addition of more than one nucleotide in each coupling step would be costly and perhaps also inconvenient. For these reasons, it is quite likely that the phosphotriester approach in solution[8] will prove to be a superior method for the synthesis of large quantities of specific oligonucleotides and their analogues. The most obvious merits of the phosphotriester approach are (i) that scale-up should not present a problem, (ii) that only a relatively small (say, 25–50%) excess of building block is likely to be required[9] in each coupling step, and (iii) that the addition of two or more nucleotide residues at a time (*i.e.*, block synthesis) would be a routine operation. We therefore propose to investigate the feasibility of the large-scale synthesis of oligonucleotide phosphorothioates by the phosphotriester approach in solution. The present report is concerned with the choice of a protecting group for the internucleotide linkages and the preparation of suitable monomeric building blocks.

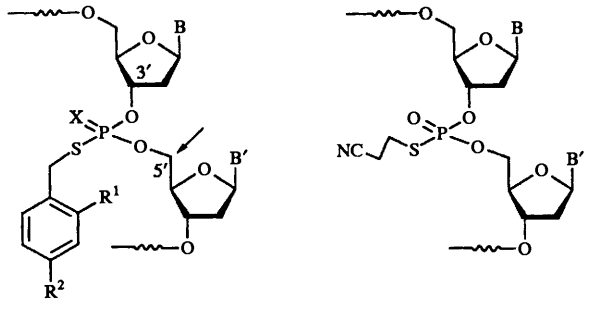
Results and discussion

A general strategy for the synthesis of oligodeoxyribonucleotide phosphorothioates by the phosphotriester approach is indicated in outline in Scheme 1. The key step (step i) involves coupling (effected by a condensing agent; see below) between a protected monomer or oligomer **2** terminating in an S-protected 3′-phosphorothioate diester and a protected monomer or oligomer **3** terminating in a 5′-hydroxy function to give the fully protected phosphorothioate triester **4**. It is essential that the 3′-phosphorothioate **2** should be protected on sulfur. If substrate **2** were to be replaced by the corresponding O-protected phosphorothioate **6** and activation by the condensing agent occurred on sulfur, loss of sulfur would occur during the coupling reaction. However, even if activation of substrate **6**



Scheme 1

occurred entirely on oxygen (to give an intermediate such as **7** containing a P=S double bond), the ensuing phosphorylation reaction might proceed relatively slowly and then be accompanied by the concomitant direct attack of the condensing agent on the 5′-hydroxy function of substrate **3**, thereby leading to a diminished yield of the required product **4**.



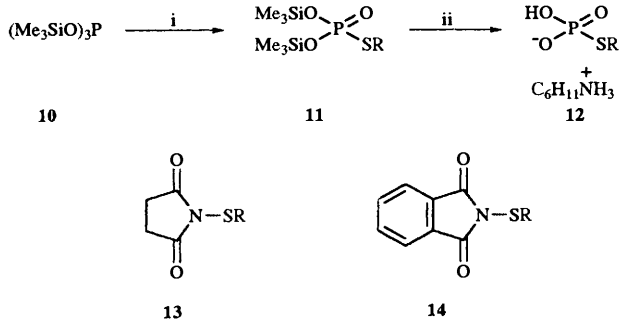
8a; X = S, $R^1 = R^2 = Cl$
b; X = S, $R^1 = R^2 = NO_2$
c; X = O, $R^1 = NO_2$, $R^2 = H$
d; X = O, $R^1 = H$, $R^2 = NO_2$

9

A crucial decision to be made at the outset is the choice of the protecting group R (Scheme 1) for the internucleotide linkages. R must fulfil at least three main criteria. First, it must be such that pure phosphodiester intermediates **2** are readily accessible in high yields. Secondly, it must remain completely intact during the assembly of the required fully protected oligonucleotide sequences **4**. Finally, it must be easily removable [Scheme 1, step ii] in such a way that only unprotected oligonucleotide analogues **5** with *exclusively* phosphorothioate internucleotide linkages are obtained. In order to meet this last criterion, the protecting group R must clearly be removed by cleavage of the R–S rather than the S–phosphoryl bond.

Substituted benzyl protecting groups, such as 2,4-dichlorobenzyl[10] (as in compound **8a**) and 2,4-dinitrobenzyl[11] (as in compound **8b**), have recently been used in the preparation of phosphorodithioate analogues of oligonucleotides. Such protecting groups may be removed by the nucleophilic attack of thiolate ions (*e.g.*, the conjugate bases of thiophenol[10] and *p*-thiocresol[11]) on the benzylic CH_2 groups. As carbon–oxygen bonds are much more readily cleaved by this process than are carbon–sulfur bonds[12] there is a real danger of concomitant nucleophilic attack occurring[13] on the C-5′ carbon atoms adjacent to the internucleotide linkages (as indicated by the arrow in structure **8**), resulting in internucleotide cleavage. In order to avoid this most undesirable side-reaction,[13] it is essential that a particularly labile benzyl group (such as 2,4-dinitrobenzyl as in compound **8b**) should be used. 2-Nitrobenzyl[14] (as in compound **8c**) and 2-cyanoethyl[15,16] (as in compound **9**) have been suggested as S-protecting groups in the synthesis of phosphorothioate analogues of oligonucleotides. The latter (*i.e.*, 2-cyanoethyl) protecting group may be removed by a base-catalysed β-elimination process. Largely with a consideration of the ease of unblocking of the internucleotide linkages (Scheme 1, step ii) in mind, we have restricted our investigation of possible protecting groups (R, Scheme 1) to 4-nitrobenzyl (as in structure **8d**) which seemed likely[17] to be more readily removable by attack of thiolate ions than would be 2-nitrobenzyl (as in structure **8c**), 2,4-dinitrobenzyl (as in compound **8**, X = O, $R^1 = R^2 = NO_2$) and 2-cyanoethyl (as in structure **9**).

Müller and Roth recently reported[18] that tris(trimethylsilyl) phosphite **10** reacted rapidly with *N*-(alkylsulfanyl)- and *N*-(arylsulfanyl)-succinimides **13** to give (Scheme 2) the corresponding bis(trimethylsilyl) *S*-alkyl and *S*-aryl phosphorothioates **11**, respectively, and that the latter products readily

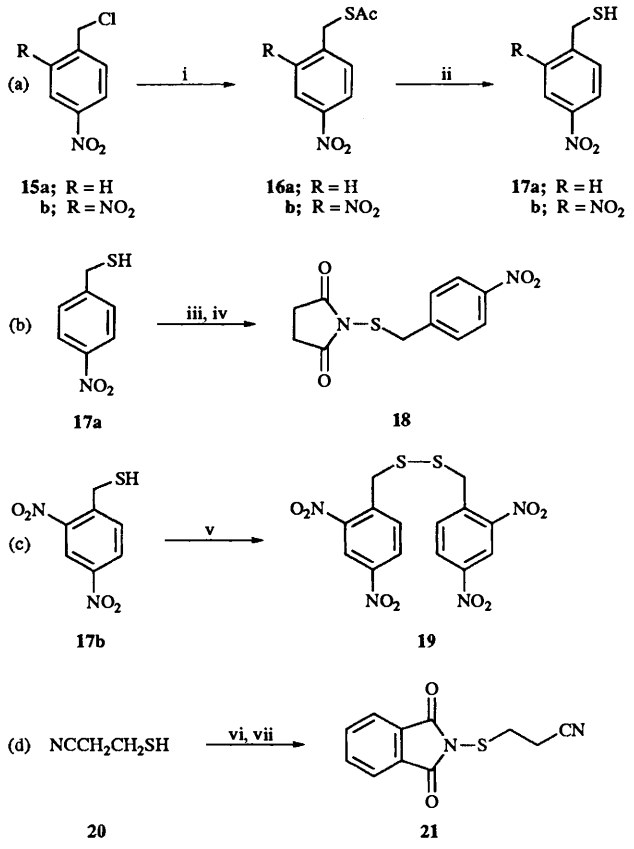


Scheme 2 *Reagents and conditions:* i, **13** (R = alkyl or aryl) or **14** (R = alkyl or aryl); ii, (a) hydrolysis; (b) cyclohexylamine

underwent hydrolysis to give *S*-alkyl and *S*-aryl phosphorothioates **12**; these workers also reported[18] that *N*-(alkylsulfanyl)- and *N*-(arylsulfanyl)-phthalimides **14** could be used instead of the corresponding succinimide derivatives **13**. van Boom and his co-workers[19] then showed that dinucleoside *H*-phosphonates can be converted into *S*-benzyl and *S*-phenyl esters of dinucleoside phosphorothioates by treatment with *N*-(benzylsulfanyl)- and *N*-(phenylsulfanyl)-succinimides (**13**, R = $PhCH_2$ and **13**, R = Ph, respectively) in the presence of di-isopropylethylamine. We decided to attempt to prepare the monomeric *S*-alkyl phosphorothioate building blocks that we required (see below) for the synthesis of phosphorothioate analogues of oligonucleotides by the phosphotriester approach in solution, by using a modification of Müller and Roth's method.[18] We therefore needed *N*-(alkylsulfanyl)-succinimide or -phthalimide derivatives (**13** or **14**) or their equivalents derived from 4-nitrobenzyl, 2,4-dinitrobenzyl and 2-cyanoethyl thiols (**17a**, **17b** and **20**, respectively).

The two-step procedure for the preparation of 4-nitrobenzyl[20] and 2,4-dinitrobenzyl[21] thiols (**17a** and **17b**, respectively) is indicated in outline in Scheme 3(a). Thus, when 4-nitrobenzyl chloride **15a** was treated with an excess both of thioacetic acid and pyridine in tetrahydrofuran (THF) solution at 50 °C, 4-nitrobenzyl thioacetate **16a** was obtained in 77% yield. When the latter compound **16a** was heated, under reflux, in sulfuric acid–water (1 : 1 v/v), 4-nitrobenzyl thiol **17a** was obtained in almost quantitative yield. 2,4-Dinitrobenzyl thiol[21] **17b** was similarly prepared from 2,4-dinitrobenzyl chloride[22] **15b** in 73% overall yield. *N*-(4-Nitrobenzylsulfanyl)succinimide **18** was prepared [Scheme 3(b) and Experimental section] in 65% yield by heating a suspension of the lead(II) salt[23] of the thiol **17a** with *N*-bromosuccinimide (NBS) in benzene. Preliminary attempts to convert 2,4-dinitrobenzyl thiol **17b** into the corresponding succinimide and phthalimide derivatives [**13** and **14**, R = 2,4-$(O_2N)_2C_6H_3$] were unsuccessful. However, bis-(2,4-dinitrobenzyl) disulfide[21] **19**, which was easily prepared in good yield by the iodine-promoted oxidation [Scheme 3(c) and Experimental section] of the thiol **17b**, proved (see below) to be an equally effective reagent for the required purpose. Finally, 3-mercaptopropanonitrile[24] **20** was converted[25] [Scheme 3(d) and Experimental section] *via* a putative intermediate sulfenyl chloride into 3-(phthalimidosulfanyl)propanonitrile **21**, which was isolated without chromatography as a crystalline solid in 58% yield.

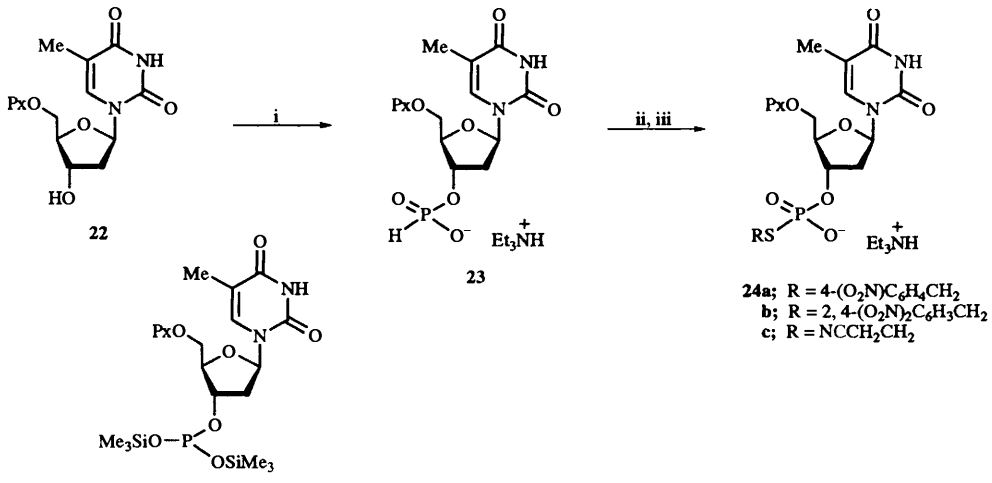
It was decided in the first instance to convert 5′-*O*-(9-phenylxanthen-9-yl)thymidine[26] **22** into the three *S*-alkyl phosphorothioate building blocks **24a**, **24b** and **24c** (Scheme 4). The nucleoside derivative **22** was treated with an approximately four-fold excess of the reagent derived from phosphorus trichloride and three molecular equivalents each of 1*H*-1,2,4-triazole and triethylamine in dry THF at −35 °C, and the intermediate was hydrolysed with 0.5 mol dm^{-3} aq. triethylammonium hydrogen carbonate to give[27] the triethylammonium salt of the 3′-(*H*-phosphonate), compound **23**. Following chromatography of the products on silica gel, the latter material **23** was isolated as a solid precipitate in almost quantitative yield (Table 1, entry no. 1). When a dry solution of the latter *H*-phosphonate **23** and a slight excess of *N*-(4-nitrobenzylsulfanyl)-succinimide **18** in dichloromethane was treated with ~4 mol equiv. of chlorotrimethylsilane and ~6 mol equiv. of triethylamine at room temp. for 2.5 h and the products were worked up with aq. triethylammonium hydrogen carbonate buffer, the *S*-(4-nitrobenzyl) phosphorothioate **24a** was obtained. Following chromatography of the products on silica gel, the latter compound **24a** was isolated as a pale yellow precipitated solid in 86% yield. It may be assumed that the reaction proceeds by the electrophilic attack of the succinimide derivative **18** on an intermediate bis(trimethylsilyl) phosphite (such as **25**). The *S*-



Scheme 3 *Reagents and conditions:* i, AcSH, C_5H_5N, THF; ii, H_2SO_4–water (1 : 1 v/v), reflux; iii, $Pb(OAc)_2$, MeOH, room temp.; iv, NBS, C_6H_6, 50 °C, 14 h; v, I_2, CH_2Cl_2, room temp., 16 h; vi, Cl_2, CH_2Cl_2, 0 °C, 20 min; vii, phthalimide, Et_3N, DMF, 0 °C to room temp.

(2,4-dinitrobenzyl) phosphorothioate **24b** was similarly prepared from the *H*-phosphonate **23**, bis-(2,4-dinitrobenzyl) disulfide **19**, chlorotrimethylsilane and 4-methylmorpholine. As the benzylic protons of 2,4-dinitrobenzyl derivatives are likely to be particularly acidic, it is advisable to avoid the use of strong bases such as triethylamine. The desired product **24b** was isolated as a precipitated solid in 67% yield. Finally, the *S*-(2-cyanoethyl) phosphorothioate **24c** was prepared in the same way from the *H*-phosphonate **23**, 3-(phthalimidosulfanyl)-propanonitrile **21**, chlorotrimethylsilane and 4-methylmorpholine; it was isolated as a pure (^{31}P NMR and HPLC, see Table 1, entry no. 2) precipitated solid in 92% yield. This represents a considerable improvement both in methodology and yield over a previously reported[16] preparation of a related 2′-deoxynucleoside 3′-*S*-(2-cyanoethyl) phosphorothioate derivative.

The comparative suitabilities of the above three phosphorothioate S-alkyl protecting groups (R in Schemes 4 and 5) were examined by undertaking the synthesis of the simple dinucleoside phosphorothioate **30** (Scheme 5). First, the triethylammonium salt of 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-phosphorothioate *S*-(4-nitrobenzyl) ester **24a** and 3′-*O*-acetylthymidine[28] **26** were coupled together in the presence of mesitylene-2-sulfonyl chloride (MSCl) and 3-nitro-1*H*-1,2,4-triazole (NT)[29] **27** in pyridine solution to give the fully protected dinucleoside phosphate **28a**. The latter material was isolated in 85% yield and was characterized on the basis of NMR spectroscopic data (particularly ^{31}P: $\delta_P[(CD_3)_2SO]$ 27.47 and 27.61). When an acetonitrile solution (~0.02 mol dm^{-3}) of compound **28a** was treated[17] at room temp. with *ca.* 10 mol equiv. of toluene-4-thiol and ~5 mol. equiv. of triethylamine, the main products (~98%) were the diastereoiso-



Scheme 4 *Reagents and conditions:* i, (a) PCl_3, 1*H*-1,2,4-triazole, Et_3N, THF, −35 °C; (b) Et_3N–water, room temp.; ii, for **24a**:**18**, Me_3SiCl, Et_3N, CH_2Cl_2, room temp.; for **24b**:**19**, Me_3SiCl, 4-methylmorpholine, CH_2Cl_2, room temp.; for **24c**:**21**, Me_3SiCl, 4-methylmorpholine, CH_2Cl_2, room temp.; iii, aq. Et_3NH^+ HCO_3^-

Table 1 Data relating to protected 2′-deoxynucleoside 3′-(*H*-phosphonates) and *S*-(2-cyanoethyl) 2′-deoxynucleoside 3′-phosphorothioate esters

Entry	Compound	Yield (%)	^{31}P NMR[a]	t_R (min)[b]
1	**23**	98	1.0 (d, $J_{P,H}$ 592)	9.42
2	**24c**	92	12.7 (s)	10.43
3	**33a**	98	0.9 (d, $J_{P,H}$ 587)	10.05
4	**34a**	88	12.7 (s)	11.01
5	**33b**	86	0.6 (d, $J_{P,H}$ 597)	11.36
6	**34b**	92	12.8 (s)	12.29
7	**33c**	72	0.4 (d, $J_{P,H}$ 593)	10.31
8	**34c**	93	12.6 (s)	11.13

[a] NMR spectra were measured at 145.8 MHz in $(CD_3)_2SO$. [b] HPLC was carried out on a Jones Apex Octyl 10μ column which was eluted with 0.1 mol dm^{-3} aq. triethylammonium acetate buffer–acetonitrile mixtures according to programme 1 (see Experimental section).

meric, partially protected dinucleoside phosphorothioates **29** [Fig. 1(*a*), t_R 10.73 and 10.98 min]. However, two other products (~0.8 and 1.1%) which corresponded to 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-phosphorothioate *S*-(4-nitrobenzyl ester) **24a** (t_R 11.87 min) and 3′-*O*-acetyl-5′-*S*-(4-methylphenyl)-5′-thiothymidine **31** (t_R 12.05 min), respectively, were detected by HPLC [Fig. 1(*a*)]. It seems reasonable to conclude that the latter products resulted[13] from the attack of toluene-4-thiolate ions at C-5′ adjacent to the internucleotide linkage (as indicated by the arrow in structure **28**). A level of ~2% cleavage per internucleotide linkage would be quite unacceptable if the synthesis of an oligonucleotide phosphorothioate even of moderate size were undertaken. It was therefore concluded that the 4-nitrobenzyl protecting group was unsuitable for the present purposes.

Unfortunately, the 2,4-dinitrobenzyl protecting group also proved to be unsuitable in that the NT **27**/MSCl-promoted coupling reaction (Scheme 5) between 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-phosphorothioate *S*-(2,4-dinitrobenzyl) ester **24b** and 3′-*O*-acetylthymidine **26** did not lead to a detectable quantity of the desired, fully protected dinucleoside phosphate **28b**. Although the *S*-(2,4-dinitrobenzyl) group is known[11] to be particularly susceptible to nucleophilic attack, this was still a surprising result. However, the NT **27**/MSCl-promoted coupling reaction between 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-phosphorothioate *S*-(2-cyanoethyl) ester **24c** and 3′-*O*-acetylthymidine **26** proceeded satisfactorily to give the fully protected dinucleoside phosphorothioate **28c**. When the latter product **28c**, which was isolated as a precipitated solid (δ_P[$(CD_3)_2SO$] 27.62 and 27.94) in 90% yield, was treated with a large excess of *tert*-butylamine[16] in pyridine solution at room temp., it was cleanly unblocked to give the expected diastereo-

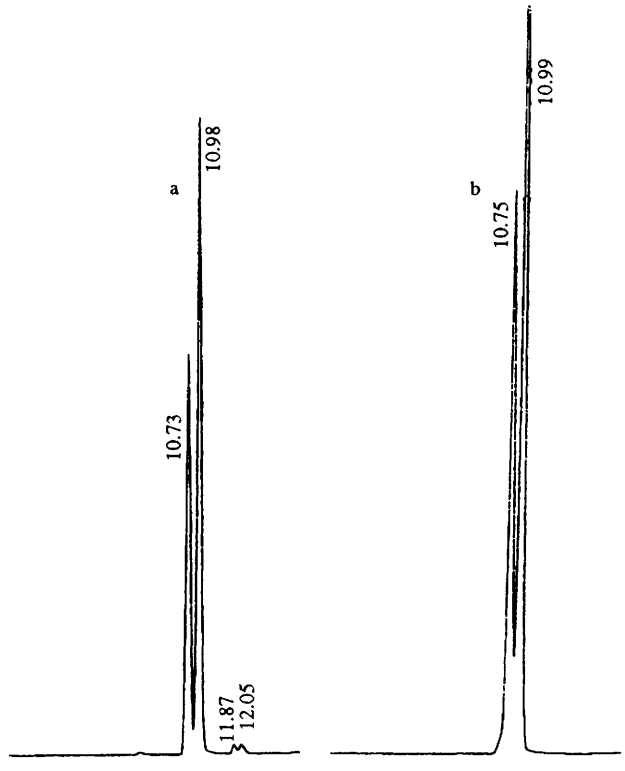
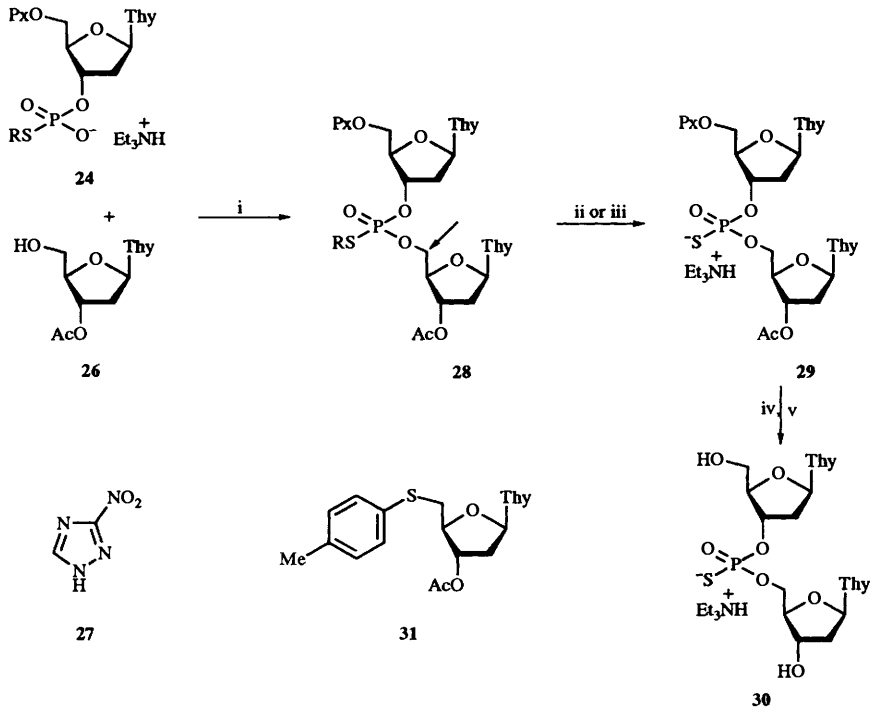


Fig. 1 HPLC profiles [Jones APEX Octyl 10μ column eluted with 0.1 mol dm^{-3} aq. triethylammonium acetate (pH 7.0)–acetonitrile according to programme 1 (see Experimental section)] of the triethylammonium salt of *O*-[3′-*O*-acetylthymidin-5′-yl] *O*-[5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl] hydrogen phosphorothioate **29** generated (a) by the action of toluene-4-thiol and triethylamine on the fully protected dinucleoside *S*-(4-nitrobenzyl) phosphorothioate ester **28a** in acetonitrile solution and (b) by the action of *tert*-butylamine on the fully protected dinucleoside *S*-(2-cyanoethyl) phosphorothioate ester **28c** in dry pyridine solution

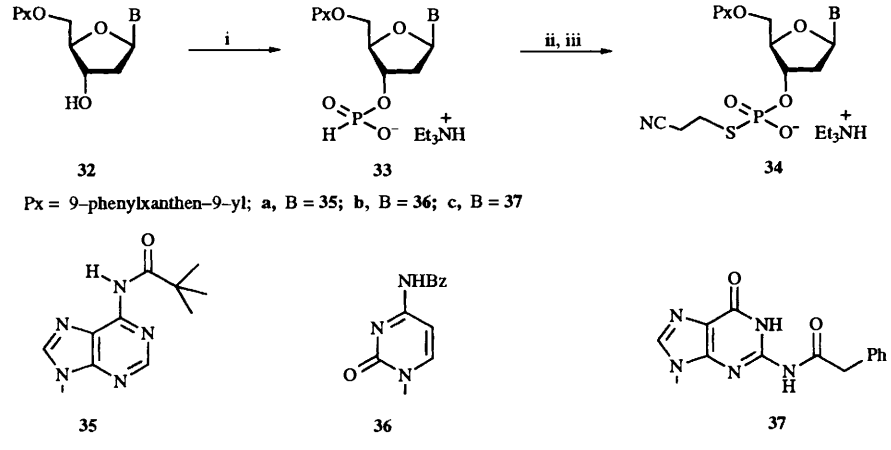


Px = 9-phenylxanthen-9-yl; Thy = thymin-1-yl; **a**, R = 4-$(O_2N)C_6H_4CH_2$; **b**, R = 2,4-$(O_2N)_2C_6H_3CH_2$; **c**, R = CH_2CH_2CN

Scheme 5 *Reagents and conditions:* i, NT **27**, MSCl, C_5H_5N, room temp., 50 min; ii, 4-MeC_6H_4SH, Et_3N, MeCN, room temp., 4.5 h; iii, Bu^tNH_2, C_5H_5N, room temp., 100 min; iv, conc. aq. NH_3 (*d* 0.88), room temp., 40 min; v, water–AcOH (96:4, v/v), room temp., 4 h

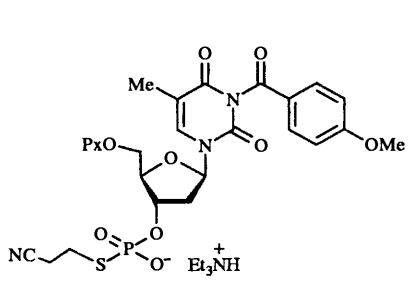
isomeric mixture of partially protected dinucleoside phosphorothioates **29** (δ_P[$(CD_3)_2SO$] 54.60 and 54.94) as the sole [see Fig 1(*b*) for HPLC profile] nucleotide products. Further unblocking of this material **29** to give the fully unprotected mixture of diastereoisomeric dinucleoside phosphorothioates **30** was readily effected (Scheme 5, steps iv and v) by treatment, at room temp., first with conc. aq. ammonia and then with 4% acetic acid. The high purity of the unprotected dinucleoside phosphorothioate **30** obtained was established[30] on the basis of NMR spectroscopic [δ_P[(D_2O)] 56.00 and 56.36] and HPLC data.

The above studies led us to conclude that the 2-cyanoethyl group was likely to be suitable for the protection of the internucleotide linkages in the large-scale synthesis of oligodeoxyribonucleotide phosphorothioates by the phosphotriester approach in solution. The preparation of the required monomeric phosphorothioate building blocks derived from 2′-deoxyadenosine, 2′-deoxycytidine and 2′-deoxyguanosine was then undertaken. 5′-*O*-(9-Phenylxanthen-9-yl)-6-*N*-pivaloyl-2′-deoxyadenosine[31] **32a** was converted (Scheme 6) *via* an intermediate *H*-phosphonate **33a** into the triethylammonium salt of its 3′-phosphorothioate *S*-(2-cyanoethyl) ester **34a** by the same procedure as was used (Scheme 4) for the conversion of 5′-*O*-(9-phenylxanthen-9-yl)thymidine **22** into the corresponding monomeric building block **24c**. It can be seen from Table 1 that good yields both of the *H*-phosphonate **33a** (entry no. 3) and the phosphorothioate **34a** (entry no. 4) were obtained. Furthermore, the high purity of both products was established by HPLC and NMR spectroscopy (Table 1). In the same way, 4-*N*-benzoyl-5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxycytidine[26] **32b** and 2-*N*-phenylacetyl-5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxyguanosine[32] **32c** were converted *via* intermediate 3′-(*H*-



Px = 9-phenylxanthen-9-yl; **a**, B = **35**; **b**, B = **36**; **c**, B = **37**

Scheme 6 *Reagents and conditions:* i, (*a*) PCl_3, 1*H*-1,2,4-triazole, Et_3N, THF, −35 °C; (*b*) aq. Et_3N, room temp.; ii, **21**, Me_3SiCl, 4-methylmorpholine, CH_2Cl_2, room temp.; iii, aq. Et_3NH^+ HCO_3^-



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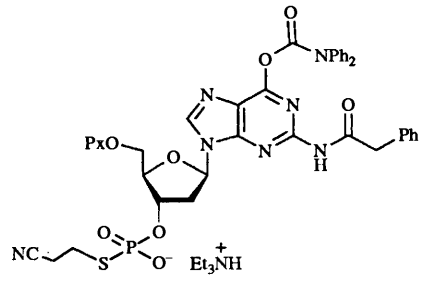

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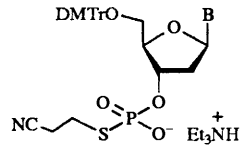

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Px = 9-phenylxanthen-9-yl; DMTr = bis-(*p*-methoxyphenyl)phenylmethyl

phosphonates) **33b** and **33c** (entries nos. 5 and 7, respectively) into the required 3′-phosphorothioate *S*-(2-cyanoethyl) esters **34b** and **34c** (entries nos. 6 and 8, respectively). All of the products were isolated as pure (HPLC, NMR) precipitated solids, and all of the yields except that of protected 2′-deoxyguanosine 3′-(*H*-phosphonate) **33c** (entry no. 7) were very satisfactory.

Earlier studies had suggested that, in the synthesis of oligonucleotides by the phosphotriester approach in solution, it is most probably desirable [33] to protect thymine (uracil) residues and to protect guanine residues both on the 2-amino and on the 1,6-lactam functions. Therefore it may be advisable to replace the monomeric phosphorothioate building blocks **24c** and **34c** by the corresponding building blocks **38** and **39**, respectively, using essentially Hata's aglycone-protecting-group strategy.[34] Preliminary studies have shown [35] that both of the latter monomers **38** and **39** are readily accessible in good yield. We believe that we have now completed the first stage of a general strategy for the synthesis of oligonucleotide phosphorothioates in solution in that a suitable protecting group for the internucleotide linkages has been identified, and methods for the preparation of the required monomeric building blocks (*i.e.*, **34**) have been developed. Alternative monomeric building blocks of general structure **40** in which the 5′-hydroxy functions are protected with bis-(*p*-methoxyphenyl)phenylmethyl [36] (DMTr) groups would be expected to be equally suitable. We ourselves prefer the 9-phenylxanthen-9-yl (Px) protecting group as its use generally leads [26] to crystalline nucleoside derivatives.

Experimental

^{1}H and ^{13}C NMR spectra were measured, unless otherwise stated, at 360.1 and 90.6 MHz, respectively, with a Bruker AM 360 spectrometer; tetramethylsilane was used as internal standard. *J* Values are given in Hz. ^{31}P NMR spectra were measured at 145.8 MHz with the same spectrometer. Merck silica gel 60 F_{254} pre-coated plates (Art 5715 and 5642), which unless otherwise stated were developed in solvent system A [chloroform–methanol (9:1 v/v)], were used for TLC. Liquid chromatography (HPLC) was carried out on a Jones Apex Octyl 10μ column which was eluted with 0.1 mol dm^{-3} triethylammonium acetate buffer/acetonitrile mixtures: programme 1 involved a linear gradient over a period of 10 min (flow rate 1.5 cm^3 min^{-1}) starting with buffer–acetonitrile (7:3 v/v) and ending with buffer–acetonitrile (3:7 v/v); programme 2 involved a linear gradient over a period of 20 min (flow rate 1.5 cm^3 min^{-1}) starting with buffer–acetonitrile (19:1 v/v) and ending with buffer–acetonitrile (4:1 v/v). Merck Kieselgel H (Art 7736) silica gel was used for short column chromatography. Acetonitrile, pyridine, THF, and triethylamine were dried by heating, under reflux, with calcium hydride for 3–5 h; dichloromethane was dried by heating, under reflux, over phosphorus pentaoxide. These solvents were then distilled at atmospheric pressure and stored over molecular sieves (no. 4 Å). Light petroleum refers to the fraction with distillation range 60–80 °C except where stated otherwise.

N-(4-Nitrobenzylsulfanyl)succinimide 18 (carried out by Dr D. C. Capaldi)

Thioacetic acid (20 cm^3, 0.28 mol) and anhydrous pyridine (15.2 cm^3, 0.188 mol) were added to a solution of 4-nitrobenzyl chloride (12.0 g, 70 mmol) in anhydrous THF (200 cm^3). The stirred reactants were heated at 50 °C for 24 h. The cooled products were filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in dichloromethane (300 cm^3) and the resulting solution was washed with saturated aq. sodium hydrogen carbonate (2 × 250 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallised from ethyl acetate–light petroleum (boiling range 60–80 °C) to give 4-nitrobenzyl *S*-thioacetate **16a** (11.43 g, 77%) as pale yellow needles, mp 55.5–57.5 °C.

Conc. sulfuric acid–water (1:1 v/v; 17 cm^3) was added to a stirred suspension of 4-nitrobenzyl *S*-thioacetate **16a** (8.0 g, 37.9 mmol) in methanol (150 cm^3) and the reactants were heated, under reflux, for 3 h. The cooled products were poured into water (1.0 dm^3) and the resulting mixture was extracted with diethyl ether (2 × 400 cm^3). The combined organic extracts were washed with water (2 × 200 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure to give 4-nitrotoluene-α-thiol **17a** as a pale yellow solid (6.31 g, 98%).

Lead(II) acetate trihydrate (0.759 g, 2.0 mmol) and 4-nitrotoluene-α-thiol (0.745 g, 4.4 mmol) were stirred together in methanol (24 cm^3) solution at room temp. After 1 h, the products were filtered off and the residue was washed with a small volume of cold methanol before being dried *in vacuo* over calcium chloride and then suspended in dry acetonitrile (20 cm^3). Following evaporation of the solvent under reduced pressure, the residue was suspended in dry benzene (20 cm^3) and the solvent was again evaporated under reduced pressure. Finally, the residue was re-suspended in dry benzene (64 cm^3), and NBS (0.783 g, 4.4 mmol) was added in three portions over a period of 45 min to the stirred suspension at room temp. The reactants were then heated at 50 °C, under argon, for 14 h. The cooled products were filtered and the filtrate was evaporated under reduced pressure to give a solid. The latter material was crystallised from ethyl acetate–hexane to give N-(*4-nitrobenzylsulfanyl*)*succinimide* **18** (0.76 g, 65%) (Found: C, 49.85; H, 3.8; N, 10.4. $C_{11}H_{10}N_2O_4S$ requires C, 49.6; H, 3.8; N, 10.5%), mp 146–148 °C; $\delta_H(CDCl_3)$ 2.73 (4 H, s), 4.18 (2 H, s), 7.50 (2 H, m) and 8.17 (2 H, m); $\delta_C(CDCl_3)$ 28.4, 40.5, 123.8, 130.3, 141.9, 147.5 and 176.2.

Bis(2,4-dinitrobenzyl) disulfide 19 (carried out according to Dr Z. Zhao's procedure[21])

Redistilled thioacetic acid (25.73 cm^3, 0.36 mol) and then dry pyridine (19.9 cm^3, 0.246 mol) were added to a stirred solution of 2,4-dinitrobenzyl chloride (13.0 g, 60.0 mmol) in dry THF (180 cm^3) at room temp. After 2 h, the products were filtered off and the filtrate was concentrated under reduced pressure. The residual solid obtained was dissolved in dichloromethane (300 cm^3) and the resulting solution was washed successively with saturated aq. sodium hydrogen carbonate (300 cm^3), 1.0 mol dm^{-3} sulfuric acid (200 cm^3) and saturated aq. sodium hydrogen carbonate (200 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure and the residue was crystallised from ethyl acetate–light petroleum to give *S-(2,4-dinitrobenzyl) thioacetate* **16b** as a pale yellow solid (12.32 g, 80%) (Found: C, 42.3; H, 3.1; N, 10.9. $C_9H_8N_2O_5S$ requires C, 42.2; H, 3.15; N, 10.9%), mp 78–79 °C; $\delta_H(CDCl_3)$ 2.35 (3 H, s), 4.49 (2 H, s), 7.91 (1 H, d, *J* 8.5), 8.41 (1 H, dd, *J* 2.4 and 8.5) and 8.88 (1 H, d, *J* 2.3); $\delta_C(CDCl_3)$ 30.2, 30.7, 120.7, 127.6, 134.2, 140.6, 147.1, 148.0 and 194.7.

A stirred suspension of *S*-(2,4-dinitrobenzyl) thioacetate **16b** (8.2 g, 32 mmol) in conc. sulfuric acid–water (1:1 v/v; 14.08 cm^3) and methanol (128 cm^3) was heated, under reflux, for 1.5 h. The cooled products were poured into water (300 cm^3) and the resulting mixture was extracted with diethyl ether (2 × 400 cm^3). The combined organic extracts were washed with water (300 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure to give *2,4-dinitrotoluene-α-thiol* **17b** as a yellow oil (6.75 g, 98%) (Found: M^+, 214.0084. $^{12}C_7{}^1H_6{}^{14}N_2{}^{16}O_4{}^{32}S$ requires *M*, 214.0048); $\delta_H(CDCl_3)$ 2.21 (1 H, t, *J* 8.7), 4.10 (2 H, d, *J* 8.7), 7.75 (1 H, d, *J* 8.7), 8.44 (1 H, dd, *J* 2.4 and 8.5) and 8.77 (1 H, d, *J* 2.4); $\delta_C(CDCl_3)$ 26.2, 121.0, 127.8, 133.0, 143.6, 146.9 and 148.0.

A solution of iodine (0.321 g, 1.26 mmol) and 2,4-dinitrotoluene-α-thiol **17b** (0.493 g, 2.3 mmol) in dichloromethane (25 cm^3) was stirred at room temp. overnight. More dichloromethane (25 cm^3) was added and the products were washed successively with 0.2 mol dm^{-3} aq. sodium hydrogen sulfite (20 cm^3), water (20 cm^3), and saturated aq. sodium hydrogen carbonate (20 cm^3). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the residue was crystallised from absolute ethanol to give *bis(2,4-dinitrobenzyl) disulfide* **19** (0.402 g, 82%) (Found: C, 39.4; H, 2.4; N, 13.0. $C_{14}H_{10}N_4O_8S_2$ requires C, 39.4; H, 2.4; N, 13.1%), mp 108–109 °C; $\delta_H(CDCl_3)$ 4.22 (4 H, s), 7.66 (2 H, d, *J* 8.5), 8.46 (2 H, dd, *J* 2.4 and 8.4) and 8.91 (2 H, d, *J* 2.4); $\delta_C(CDCl_3)$ 40.0, 121.1, 127.5, 133.9, 139.4, 147.4 and 148.0.

3-(Phthalimidosulfanyl)propanonitrile 21

A solution of chlorine in dichloromethane (1.4 mol dm^{-3}, 10.0 cm^3, 14.0 mmol) was added dropwise to a stirred solution of 3-mercaptopropanonitrile[24] **20** (1.22 g, 14.0 mmol) in dichloromethane (21 cm^3) at 0 °C (ice–water-bath). After the products had been allowed to warm up to room temp., they were added dropwise to a stirred slurry of phthalimide (1.47 g, 10.0 mmol) and triethylamine (1.94 cm^3, 14.0 mmol) in dry dimethylformamide (DMF) (12 cm^3) at 0 °C (ice–water-bath). The stirred reactants were allowed to warm up to room temp. After 2 h, the products were poured into saturated aq. sodium hydrogen carbonate (25 cm^3) and the resulting mixture was extracted with dichloromethane (2 × 50 cm^3). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallised from ethanol to give *3-(phthalimidosulfanyl)propanonitrile* **21** as needles (1.345 g, 58%) (Found: C, 57.0; H, 3.4; N, 11.8. $C_{11}H_8N_2O_2S$ requires C, 56.9; H, 3.5; N, 12.1%), mp 162–164 °C; $\delta_H(CDCl_3)$ 2.79 (2 H, t, *J* 7.2), 3.12 (2 H, t, *J* 7.2), 7.83 (2 H, m) and 7.95 (2 H, m); $\delta_C(CDCl_3)$ 18.7, 34.5, 117.5, 124.1, 131.7, 134.9 and 168.1.

Triethylammonium salt of 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen *H*-phosphonate) 23

Triethylamine (9.04 cm^3, 65.0 mmol) and phosphorus trichloride (1.75 cm^3, 20.1 mmol) were added to a stirred solution of 1*H*-1,2,4-triazole (4.14 g, 60.0 mmol; recrystallised from dry acetonitrile) in dry THF (120 cm^3) at −35 °C (methanol–solid CO_2-bath). After 15 min, a solution of 5′-*O*-(9-phenylxanthen-9-yl)thymidine[26] **22** (2.49 g, 5.0 mmol) in THF (100 cm^3) was added. After a further period of 15 min, triethylamine–water (1:1 v/v; 25 cm^3) was added and the reactants were allowed to warm up to room temp. The products were then evaporated under reduced pressure. The residue was dissolved in chloroform (300 cm^3) and the solution was washed with 0.5 mol dm^{-3} triethylammonium hydrogen carbonate buffer (pH 7.5; 2 × 150 cm^3). The organic layer was dried ($MgSO_4$), and evaporated under reduced pressure. Toluene (25 cm^3) was added, and removed by evaporation to give a glass, which was then fractionated by short column chromatography on silica gel: appropriate fractions, which were eluted with chloroform–methanol (90:10–85:15 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (30 cm^3) was added dropwise to stirred light petroleum (30–40 °C) (750 cm^3) to give the triethylammonium salt of 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen *H*-phosphonate), compound **23** (3.273 g, 98%) as a precipitated solid; t_R 9.42 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 1.42 (3 H, s), 2.38 (2 H, m), 3.13 (1 H, dd, *J* 3.5 and 10.3), 3.19 (1 H, dd, *J* 3.1 and 10.3), 4.04 (1 H, m), 4.76 (1 H, m), 6.19 (1 H, t, *J* 6.8), 6.62 (1 H, d, $J_{P,H}$ 592), 7.05–7.45 (13 H, m), 7.57 (1 H, s) and 11.41 (1 H, s); $\delta_P[(CD_3)_2SO]$ 1.0 (d, $J_{P,H}$ 592).

Triethylammonium salt of *S*-(4-nitrobenzyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen phosphorothioate) 24a

A solution of the triethylammonium salt of 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen *H*-phosphonate) **23** (0.664 g, 1.0 mmol) and *N*-(4-nitrobenzylsulfanyl)succinimide **18** (0.346 g, 1.3 mmol) in dichloromethane–acetonitrile (2:1 v/v; 15 cm^3) was evaporated to dryness under reduced pressure. The residue was redissolved in dichloromethane–acetonitrile (2:1 v/v; 15 cm^3) and the solution was again evaporated under reduced pressure. The residue was then dissolved in dry dichloromethane (20 cm^3), and triethylamine (0.835 cm^3, 6.0 mmol) and chlorotrimethylsilane (0.508 cm^3, 4.0 mmol) were added to the stirred solution at room temp. After 2.5 h, the products were poured into 0.2 mol dm^{-3} aq. triethylammonium hydrogen carbonate (200 cm^3), and the resulting mixture was extracted with dichloromethane (200 cm^3, followed by 2 × 100 cm^3). The dried ($MgSO_4$), combined organic extracts were concentrated under reduced pressure and the residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, eluted with chloroform–methanol (88:12 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (6 cm^3) was added dropwise to stirred light petroleum (30–40 °C; 150 cm^3) at room temp. to give the triethylammonium salt of *S*-(4-nitrobenzyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-phosphorothioate, **24a** as a pale yellow solid (0.72 g, 86%); t_R 11.87 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 1.39 (3 H, d, *J* 0.8), 2.15–2.4 (2 H, m), 2.91 (1 H, dd, *J* 3.5 and 10.2), 3.82 (2 H, m), 3.92 (1 H, m), 4.68 (1 H, m), 6.14 (1 H, dd, *J* 5.8 and 8.6), 7.0–7.45 (13 H, m), 7.50 (2 H, m), 7.54 (1 H, m), 8.03 (2 H, m) and 11.37 (1 H, s); $\delta_P[(CD_3)_2SO]$ 13.4.

Triethylammonium salt of *S*-(2,4-dinitrobenzyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen phosphorothioate) 24b

A solution of the triethylammonium salt of 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen *H*-phosphonate) **23** (0.199 g, 0.3 mmol) and bis(2,4-dinitrobenzyl) disulfide **19** (0.153 g, 0.36 mmol) in dichloromethane–acetonitrile (1 : 1 v/v; 6 cm^3) was evaporated to dryness under reduced pressure. The residue was dissolved in the same solvent mixture (6 cm^3), and the solution was again evaporated under reduced pressure. The residue was then dissolved in dry dichloromethane (6 cm^3), and 4-methylmorpholine (0.34 cm^3, 3.1 mmol) and chlorotrimethylsilane (0.15 cm^3, 1.2 mmol) were added to the stirred solution at room temp. After 3 h, the products were poured into 0.5 mol dm^{-3} aq. triethylammonium hydrogen carbonate (50 cm^3), and the resulting mixture was extracted with dichloromethane (2 × 50 cm^3). The dried ($MgSO_4$), combined organic extracts were concentrated under reduced pressure and the residue was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with chloroform–methanol (9 : 1 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (2 cm^3) was added dropwise to stirred light petroleum (30–40 °C; 50 cm^3) at room temp. to give the triethylammonium salt of *S*-(2,4-dinitrobenzyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen phosphorothioate) **24b** (0.177 g, 67%); t_R 10.17 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 1.40 (3 H, s), 2.25 (2 H, m), 3.08 (1 H, dd, *J* 3.2 and 10.6), 3.97 (1 H, m), 4.11 (2 H, m), 4.65 (1 H, m), 6.08 (1 H, t, *J* 7.2), 6.95–7.5 (13 H, m), 7.53 (1 H, s), 7.92 (1 H, d, *J* 8.6), 8.41 (1 H, dd, *J* 2.4 and 8.5), 8.63 (1 H, d, *J* 2.4) and 11.36 (1 H, br s); $\delta_P[(CD_3)_2SO]$ 12.4.

Triethylammonium salt of *S*-(2-cyanoethyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen phosphorothioate) 24c

A solution of the triethylammonium salt of 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen *H*-phosphonate) **23** (0.199 g, 0.3 mmol) and 3-(phthalimidosulfanyl)propanonitrile **21** (0.091 g, 0.4 mmol) in dichloromethane–acetonitrile (2 : 1 v/v; 6 cm^3) was evaporated to dryness under reduced pressure. The residue was dissolved in the same solvent mixture (6 cm^3), and the solution was again evaporated under reduced pressure. The residue was then dissolved in dry dichloromethane (6 cm^3), and 4-methylmorpholine (0.206 cm^3, 1.9 mmol) and chlorotrimethylsilane (0.15 cm^3, 1.2 mmol) were added to the stirred solution at room temp. After 3 h, the products were worked up, and purified as in the above preparation of the corresponding *S*-(2,4-dinitrobenzyl) ester. The triethylammonium salt of *S*-(2-cyanoethyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen phosphorothioate) was isolated as a precipitated solid (0.208 g, 92%); t_R 10.43 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 1.41 (3 H, d, *J* 0.7), 2.40 (2 H, m), 2.67 (2 H, m), 2.84 (2 H, m), 3.14 (1 H, dd, *J* 3.6 and 10.2), 3.19 (1 H, dd, *J* 3.0 and 10.2), 4.15 (1 H, m), 4.81 (1 H, m), 6.21 (1 H, dd, *J* 6.1 and 8.2), 7.05–7.5 (13 H, m), 7.59 (1 H, m) and 11.41 (1 H, s); $\delta_P[(CD_3)_2SO]$ 12.7.

Triethylammonium salt of *O*-[3′-*O*-acetylthymidin-5′-yl] *O*-[5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl] hydrogen phosphorothioate 29

(a) A solution of the triethylammonium salt of *S*-(4-nitrobenzyl) 5′-*O*-(9-phenylxanthen-9-yl)thymidine 3′-(hydrogen phosphorothioate) **24a** (0.299 g, 0.36 mmol), 3′-*O*-acetylthymidine[28] **26** (0.085 g, 0.3 mmol) and NT[29] (0.171 g, 1.5 mmol) in dry pyridine (5 cm^3) was evaporated under reduced pressure. The residue was redissolved in dry pyridine (5 cm^3) and the solution was re-evaporated. After this process had been repeated once more, the residue was dissolved in dry pyridine (3 cm^3) and solid MSCl (0.229 g, 1.05 mmol) was added. After the reaction solution had been stirred at room temp. for 50 min, saturated aq. sodium hydrogen carbonate (0.5 cm^3) was added and the products were partitioned between dichloromethane (20 cm^3) and 0.2 mol dm^{-3} aq. triethylammonium hydrogen carbonate. The layers were separated and the aqueous layer was extracted with dichloromethane (2 × 10 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was co-evaporated with toluene (5 cm^3) and the residue was fractionated by short-column chromatography on silica gel: appropriate fractions, which were eluted with chloroform–methanol (99 : 1 to 98 : 2 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (2 cm^3) was added dropwise to stirred light petroleum (30–40 °C; 50 cm^3) to give the fully protected dinucleoside phosphorothioate *S*-(4-nitrobenzyl) **28a** as a precipitated solid (0.254 g, 85%); $\delta_P[(CD_3)_2SO]$ 27.47 and 27.61.

Toluene-4-thiol (0.062 g, 0.5 mmol) and then triethylamine (0.035 cm^3, 0.25 mmol) were added to a stirred solution of the latter material (0.50 g, 0.05 mmol) in dry acetonitrile (2.8 cm^3) at room temp. After 4.5 h, allyl chloride[31,37] (0.5 cm^3, 6.1 mmol) was added and, after a further period of 5 min, the products were evaporated under reduced pressure. HPLC analysis revealed no starting material **28a** and four nucleotide or nucleoside products with the following t_R-values/min (programme 1): 10.73 (37.5%), 10.98 (60.55%), 11.87 (0.8%) and 12.05 (1.14%). The two lower-t_R components were believed to be the desired diastereoisomeric, partially protected dinucleoside phosphorothioates **29** and the other two components (t_Rs 11.87 and 12.05 min) corresponded to phosphorothioate ester **24a** and 3′-*O*-acetyl-5′-*S*-(4-methylphenyl)-5′-thiothymidine **31**, respectively. The products were fractionated by short-column chromatography on silica gel: appropriate fractions, which were eluted with chloroform–methanol (85 : 15 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (1 cm^3) was added to stirred light petroleum (30–40 °C; 50 cm^3) to give the triethylammonium salt of *O*-(3′-*O*-acetylthymidin-5-yl) *O*-[5′-*O*-(9-phenylxanthen-9-yl)thymidin-3-yl] hydrogen phosphorothioate **29** (0.047 g, 97%) as a precipitate; $\delta_P[(CD_3)_2SO]$ 53.99 and 54.07.

(b) A dry solution of the triethylammonium salt of phosphorothioate **24c** (0.269 g, 0.36 mmol), 3′-*O*-acetylthymidine **26** (0.085 g, 0.3 mmol) and NT (0.239 g, 2.1 mmol) in pyridine (3 cm^3) was prepared by the procedure described in section (a) above. MSCl (0.229 g, 1.05 mmol) was added to the stirred solution at room temp. and the reaction was allowed to proceed for 40 min. The products were then worked up and fractionated according to the above procedure [section (a)] to give the fully protected dinucleoside phosphorothioate *S*-(2-cyanoethyl) ester **28c**, which was isolated as a precipitated solid (0.246 g, 90%); $\delta_P[(CD_3)_2SO]$ 27.62 and 27.94. *tert*-Butylamine (0.20 cm^3, 1.9 mmol) was added to a stirred solution of the latter material (0.046 g, 0.05 mmol) in dry pyridine (0.8 cm^3) at room temp. After 100 min, the products were evaporated under reduced pressure, redissolved in chloroform (25 cm^3), and the resulting solution was washed with 0.5 mol dm^{-3} aq. triethylammonium hydrogen carbonate (2 × 15 cm^3). The dried ($MgSO_4$) organic layer was filtered, and concentrated under reduced pressure. A solution of the residue in chloroform (1 cm^3) was added to stirred light petroleum (30–40 °C; 50 cm^3) to give the triethylammonium salt of *O*-(3′-*O*-acetylthymidin-5′-yl) *O*-[5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl] hydrogen phosphorothioate, compound **29** (0.048 g, 99%) as a precipitated solid; t_R (programme 1) 10.69 and 10.93 min; $\delta_P[(CD_3)_2SO]$ 54.60 and 54.94.

3'-*O*-Acetyl-5'-*S*-(4-methylphenyl)-5'-thiothymidine 31
A solution of 3'-*O*-acetylthymidine **26** (0.511 g, 1.8 mmol) and toluene-4-sulfonyl chloride (0.514 g, 2.7 mmol) in dry pyridine (3 cm^3) was stirred at room temp. After 16 h, saturated aq. sodium hydrogen carbonate (2 cm^3) was added and, after a further 10 min, the products were partitioned between chloroform (50 cm^3) and saturated aq. sodium hydrogen carbonate (50 cm^3). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, eluted with chloroform–methanol (99:1 v/v), were combined, and evaporated under reduced pressure. The residue was dissolved in acetonitrile (2.5 cm^3) at room temp. and triethylamine (0.90 cm^3, 6.5 mmol) and toluene-4-thiol (0.805 g, 6.5 mmol) were added to the stirred solution, which was kept under argon. After 60 h, the products were concentrated under reduced pressure and the residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, eluted with chloroform–methanol (99:1 v/v), were combined, and evaporated under reduced pressure to give 3'-O-*acetyl*-5'-S-(4-*methylphenyl*)-5'-*thiothymidine* **31** as a glass (0.60 g, 85% based on 3'-*O*-acetylthymidine) (Found: M^+, 390.1249. $^{12}C_{19}{}^{1}H_{22}{}^{14}N_2{}^{16}O_5{}^{32}S$ requires M, 390.1249); t_R 12.05 min (programme 1); $\delta_H[(CD_3)_2SO]$ 1.76 (3 H, d, *J* 0.9), 2.05 (3 H, s), 2.25 (1 H, m), 2.26 (3 H, s), 2.49 (1 H, m), 3.32 (2 H, m), 4.02 (1 H, m), 5.18 (1 H, m), 6.13 (1 H, dd, *J* 6.0 and 8.6), 7.14 (2 H, d, *J* 7.9), 7.29 (2 H, d, *J* 8.2), 7.51 (1 H, m) and 11.38 (1 H, br s); $\delta_C[(CD_3)_2SO]$ 12.1, 20.5, 20.8, 35.2, 35.4, 75.7, 81.9, 83.9, 110.0, 129.1, 129.7, 131.6, 135.7, 136.0, 150.5, 163.6 and 169.9.

Triethylammonium salt of *O*-[thymidin-3'-yl] *O*-[thymidin-5'-yl] hydrogen phosphorothioate 30
The triethylammonium salt of *O*-(3'-*O*-acetylthymidin-5-yl) *O*-[5'-*O*-(9-phenylxanthen-9-yl)thymidin-3-yl] hydrogen phosphorothioate, compound **29** (0.036 g, ~0.037 mmol), prepared from its *S*-(2-cyanoethyl) ester **28c**, was dissolved in conc. aq. ammonia (*d* 0.88; 4 cm^3) at room temp. The solution was stirred for 40 min and was then evaporated under reduced pressure. The residue was dissolved in methanol (5 cm^3) and the solution was evaporated under reduced pressure. After this process had been repeated with ethanol (3 × 5 cm^3), the residue was dissolved in acetic acid–water (4:96 v/v; 4 cm^3) at room temp. After 4 h, the products were evaporated under reduced pressure and the residue was partitioned between chloroform (10 cm^3) and water (5 cm^3). The aq. layer was separated, extracted with chloroform (10 cm^3), and the extract was evaporated under reduced pressure. The residue was fractionated on a column (17 cm × 2 cm diameter) of DEAE Sephadex A-25 which was eluted with a linear gradient of aq. triethylammonium hydrogen carbonate buffer (pH 7.5; 0.001–1.0 mol dm^{-3} over 1000 cm^3): the appropriate fractions (eluted with an average buffer concentration of 0.28 mol dm^{-3}) were combined, and evaporated under reduced pressure. The residue was re-evaporated from ethanol (2 × 10 cm^3) solution to give the triethylammonium salt of *O*-(thymidin-3'-yl) *O*-(thymidin-5'-yl) hydrogen phosphorothioate **30** (554 A_{265} units) as a solid; t_R (programme 2) 14.98 and 15.85 min; $\delta_H(D_2O)$ includes the following signals: 1.82 (3 H, s), 1.87 and 1.88 (3 H, 2 s), 2.32 (3 H, m), 2.50 (1 H, m), 3.78 (2 H, m), 4.13 (4 H, m), 4.54 (1 H, m), 4.91 (1 H, m), 6.16 (1 H, m), 6.27 (1 H, t, *J* 6.9), 7.62 (1 H, s) and 7.68 (1 H, s); $\delta_P(D_2O)$ 56.00 and 56.36.

Triethylammonium salt of 5'-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyl-2'-deoxyadenosine 3'-(hydrogen *H*-phosphonate) 33a
This intermediate was prepared on the same scale and in precisely the same way as the simple thymidine derivative **23** described above. 5'-*O*-(9-Phenylxanthen-9-yl)-6-*N*-pivaloyl-2'-deoxyadenosine[31] **32a** (2.96 g, 5.0 mmol) was converted into the triethylammonium salt of 5'-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyl-2'-deoxyadenosine 3'-(hydrogen *H*-phosphonate) **33a** (3.67 g, 98%). This product was isolated as a precipitated solid; t_R 10.05 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 1.28 (9 H, s), 2.56 (1 H, m), 3.09 (1 H, dd, *J* 5.6 and 10.0), 3.28 (1 H, dd, *J* 4.4 and 10), 4.17 (1 H, m), 4.93 (1 H, m), 6.41 (1 H, t, *J* 6.7), 6.67 (1 H, d, $J_{P,H}$ 590), 6.82–7.45 (13 H, m), 8.50 (1 H, s), 8.52 (1 H, s) and 10.21 (1 H, s); $\delta_P[(CD_3)_2SO]$ 0.9 (d, $J_{P,H}$ 587).

Triethylammonium salt of *S*-(2-cyanoethyl) 5'-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyl-2'-deoxyadenosine hydrogen 3'-phosphorothioate 34a
This monomeric building block was prepared in the same way as the corresponding thymidine derivative **24c** described above. The triethylammonium salt of 5'-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyl-2'-deoxyadenosine 3'-(hydrogen *H*-phosphonate) **33a** (1.14 g, 1.5 mmol) was converted into the triethylammonium salt of *S*-(2-cyanoethyl) 5'-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyl-2'-deoxyadenosine 3'-(hydrogen phosphorothioate), compound **34a** (1.12 g, 88%). The product was isolated as a precipitated solid; t_R 11.01 min; $\delta_H[(CD_3)_2SO]$ includes the following signals: 1.29 (9 H, s), 2.63 (1 H, m), 2.76 (2 H, m), 2.90 (2 H, m), 3.33 (1 H, m), 4.27 (1 H, m), 4.98 (1 H, m), 6.42 (1 H, t, *J* 6.9), 6.85–7.45 (13 H, m), 8.51 (1 H, s), 8.53 (1 H, s) and 10.20 (1 H, s); $\delta_P[(CD_3)_2SO]$ 12.7.

Triethylammonium salt of 4-*N*-benzoyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxycytidine 3'-(hydrogen *H*-phosphonate) 33b
This intermediate was prepared on the same scale and in precisely the same way as the simple thymidine derivative **23** described above. 4-*N*-Benzoyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxycytidine[26] **32b** (2.94 g, 5.0 mmol) was converted into the triethylammonium salt of 4-*N*-benzoyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxycytidine 3'-(hydrogen *H*-phosphonate) **33b** (3.25 g, 86%). The product was isolated as a precipitated solid; t_R 11.36 min (programme 1); $\delta_H[(CD_3)_2SO + D_2O]$ includes the following signals: 2.33 (1 H, m), 2.59 (1 H, m), 3.12 (1 H, dd, *J* 3.8 and 10.6), 3.25 (1 H, dd, *J* 3.2 and 10.6), 4.14 (1 H, m), 4.75 (1 H, m), 6.13 (1 H, t, *J* 5.9), 6.60 (1 H, d, $J_{P,H}$ 597), 7.1–7.5 (14 H, m), 7.54 (2 H, m), 7.63 (1 H, m), 8.00 (2 H, m) and 8.27 (1 H, m); $\delta_P[(CD_3)_2SO]$ 0.6 (d, $J_{P,H}$ 597).

Triethylammonium salt of 4-*N*-benzoyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxycytidine *S*-(2-cyanoethyl) 3'-(hydrogen phosphorothioate) 34b
This monomeric building block was prepared in the same way as the corresponding thymidine derivative **24c** described above. The triethylammonium salt of 4-*N*-benzoyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxycytidine 3'-(hydrogen *H*-phosphonate), compound **33b** (1.13 g, 1.5 mmol) was converted into the triethylammonium salt of 4-*N*-benzoyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxycytidine *S*-(2-cyanoethyl) 3'-(hydrogen phosphorothioate) **34b** (1.16 g, 92%). The product was isolated as a precipitated solid; t_R 12.29 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 2.28 (1 H, m), 2.67 (3 H, m), 2.85 (2 H, m), 3.14 (1 H, dd, *J* 4.4 and 10.4), 3.23 (1 H, dd, *J* 3.2 and 10.4), 4.27 (1 H, m), 4.73 (1 H, m), 6.14 (1 H, t, *J* 6.4), 7.1–7.65 (17 H, m), 8.02 (2 H, d, *J* 7.4), 8.17 (1 H, d, *J* 7.5) and 11.33 (1 H, br s); $\delta_P[(CD_3)_2SO]$ 12.8.

Triethylammonium salt of 2-*N*-phenylacetyl-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxyguanosine 3'-(hydrogen *H*-phosphonate) 33c
This intermediate was prepared on the same scale and in precisely the same way as the simple thymidine derivative **23** described above. 2-*N*-Phenylacetyl-5'-*O*-(9-phenylxanthen-9-

yl)-2′-deoxyguanosine[32] **32c** (3.21 g, 5.0 mmol) was converted into the triethylammonium salt of 2-*N*-phenylacetyl-5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxyguanosine 3′-(hydrogen *H*-phosphonate) **33c** (2.92 g, 72%). The product was isolated as a precipitated solid; t_R 10.31 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 2.55 (1 H, m), 2.75 (1 H, m), 3.15 (2 H, m), 3.80 (2 H, s), 4.15 (1 H, m), 4.85 (1 H, m), 6.23 (1 H, t, *J* 6.4), 6.70 (1 H, d, $J_{P,H}$ 596), 6.85–7.45 (18 H, m) and 8.01 (1 H, s); $\delta_P[(CD_3)_2SO]$ 0.4 (d, $J_{P,H}$ 593).

Triethylammonium salt of *S*-(2-cyanoethyl) 2-*N*-phenylacetyl-5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxyguanosine 3′-(hydrogen phosphorothioate) 34c

This monomeric building block was prepared in the same way as the corresponding thymidine derivative **24c** described above. The triethylammonium salt of 2-*N*-phenylacetyl-5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxyguanosine 3′-(hydrogen *H*-phosphonate) **33c** (1.21 g, 1.5 mmol) was converted into the triethylammonium salt of *S*-(2-cyanoethyl) 2-*N*-phenylacetyl-5′-*O*-(9-phenylxanthen-9-yl)-2′-deoxyguanosine 3′-(hydrogen phosphorothioate) **34c** (1.25 g, 93%). The product was isolated as a precipitated solid; t_R 11.13 min (programme 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 2.55–2.95 (10 H, m), 3.17 (2 H, m), 3.80 (2 H, s), 4.24 (1 H, m), 4.85 (1 H, m), 6.25 (1 H, t, *J* 6.9), 6.9–7.45 (18 H, m) and 8.01 (1 H, s); $\delta_P[(CD_3)_2SO]$ 12.6.

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