

Solution phase synthesis of ISIS 2922 (Vitravene) by the modified H-phosphonate approach

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The synthesis of Vitravene, a 21-mer oligonucleotide phosphorothioate (d[Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G]) by the modified H-phosphonate approach in solution is reported. The synthetic strategy adopted involves the use of only four different trimer building blocks. The dimer and trimer blocks are prepared by a novel four component procedure in which the coupling agent (diphenyl phosphorochloridate **5b**) and the sulfur-transfer reagent (*N*-[(2-cyanoethylsulfanyl)succinimide] **19**) are added at the same time. Yields both of the building blocks and in the block coupling reactions are very satisfactory. The fully-protected 21-mer **38** is unblocked by a five-step procedure to give Vitravene, which is isolated as its sodium salt.

Introduction

The antisense^{1,2} and antigene² approaches to chemotherapy could well revolutionise the treatment of cancer, certain viral infections and a number of other diseases in years to come. One antisense oligonucleotide analogue, Vitravene has already been approved³ by U.S. Food and Drug Administration for the topical treatment of cytomegalovirus retinitis in AIDS patients and a number of other oligonucleotide sequences are presently undergoing advanced human clinical trials. For this reason, the large-scale synthesis of oligonucleotides and particularly of their phosphorothioate analogues has become a matter of considerable importance and indeed of urgency. So far, the demand for the relatively large (say, up to 1.0 kg) quantities of material required for clinical trials has been met⁴ by scaling-up phosphoramidite-based solid phase synthesis. However, should an oligonucleotide drug be approved for a systemic treatment, then most probably hundreds of kilograms or perhaps even tonnes of that specific sequence will be required. With this in mind, we have recently turned our attention to the development of a solution phase oligonucleotide synthesis that has the potential to be scaled-up to the level likely to be required.

Until fairly recently, the phosphotriester approach⁵ appeared to be the method of choice for the synthesis of oligonucleotides in solution. However, we then developed an alternative approach,^{6,7} which is based on H-phosphonate coupling followed by the *in situ* addition of a sulfur transfer reagent. We now believe that this modified H-phosphonate approach, which could also be considered to be a modified phosphotriester approach, is the preferred method of solution phase oligonucleotide synthesis and that it is particularly suitable for the preparation of phosphorothioate analogues of oligonucleotides. The protocol that we followed in our initial studies⁶ on the modified H-phosphonate approach is illustrated for the preparation of a dinucleoside phosphate and a dinucleoside phosphorothioate in Scheme 1. Coupling between a protected nucleoside 3'-H-phosphonate **1** and a partially-protected nucleoside **2** with a free 5'-hydroxy function was rapidly effected with bis(2-chlorophenyl) phosphorochloridate **5a** in pyridine-dichloromethane solution at -40 °C. No attempt was made to isolate the resulting relatively sensitive⁸ H-phosphonate diester. When a standard phosphodiester internucleotide linkage was required, 2-[(4-chlorophenyl)sulfanyl]isoindole-1,3(2*H*)-dione⁹

6a was added *in situ* and the products were then worked up to give the *S*-(4-chlorophenyl) phosphorothioate triester **3a**. Following 'detritylation' and acetylation (steps iii and iv), the products were treated with *E*-2-nitrobenzaloxime **7** and *N*¹,*N*¹,*N*³,*N*³-tetramethylguanidine (TMG) in acetonitrile solution to give the partially-protected dinucleoside phosphate **4a**. The protecting groups were then removed from the base residues and terminal hydroxy functions to give the completely unblocked dinucleoside phosphate. If a phosphorothioate diester internucleotide linkage (as in **4b**) was required, the *S*-(4-chlorophenyl) sulfur transfer reagent **6a** was replaced by 4-[(2-cyanoethyl)sulfanyl]morpholine-3,5-dione¹⁰ **8**. Following the 'detritylation' and acetylation steps, the 2-cyanoethyl group was removed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of chlorotrimethylsilane (to ensure anhydrous conditions) to give the partially-protected dinucleoside phosphorothioate **4b**. The protecting groups were then removed from the base residues and the terminal hydroxy functions in the same way.

Phosphoramidite-based solid phase synthesis, irrespective of the target sequence, almost always involves the stepwise addition of monomeric building blocks. Thus no particular synthetic strategy is possible. However, this need not be the case both in the phosphotriester and the modified H-phosphonate approaches. It can be seen from Fig. 1 that the Vitravene

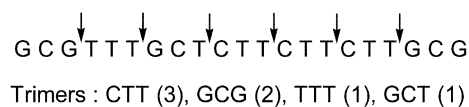
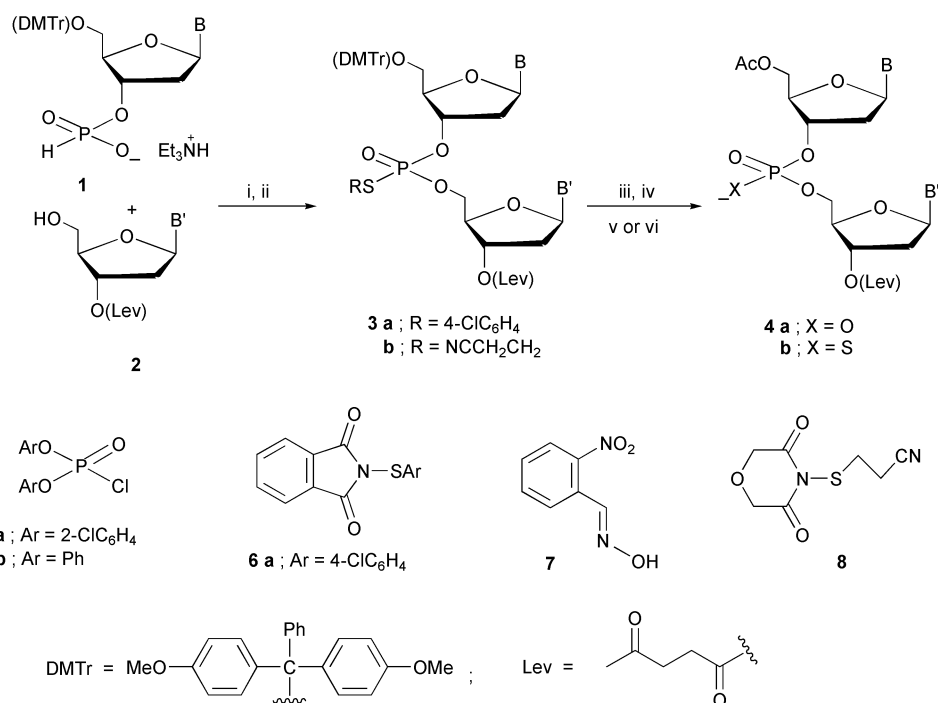


Fig. 1 Trimer components of Vitravene.

sequence (d[Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G])¹¹ can be divided into seven trimers of which one (CTT) occurs three times and another (GCG) occurs twice. Therefore it is possible to base the synthesis of Vitravene on only four trimer blocks. This is clearly advantageous and would particularly be so if a really large scale synthesis were to be undertaken. Another obvious advantage of basing a synthesis on trimer building blocks is that contamination of the product with *n* - 1 and *n* - 2 sequences is avoided. This is very unlikely



Scheme 1 Reagents and conditions: i, **5a**, C₅H₅N, CH₂Cl₂, -40 °C, 5–10 min; ii, a, **6a** or **8**, C₅H₅N, CH₂Cl₂, -40 °C, 15 min, b, C₅H₅N–water (1 : 1 v/v), -40 °C to room temp.; iii, HCl, CH₂Cl₂, -50 °C, 5 min; iv, Ac₂O, C₅H₅N, room temp., 15 h; v, **7**, TMG, MeCN, room temp., 12 h; vi, DBU, Me₃SiCl, CH₂Cl₂, room temp., 30 min.

to be the case in a synthesis based on monomeric building blocks. We now report the synthesis, by the modified H-phosphonate procedure in solution of Vitravene in which this 21-mer oligonucleotide phosphorothioate is assembled in six coupling steps, each involving a trimer H-phosphonate building block.

Results and discussion

1 Monomeric building blocks

Vitravene is derived from 2'-deoxycytidine, 2'-deoxyguanosine and thymidine, and it is necessary to prepare two monomeric building blocks from each nucleoside. These building blocks were obtained from 4-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine **9**; B = **10**, 5'-*O*-(4,4'-dimethoxytrityl)-2-*N*-isobutyryl-2'-deoxyguanosine **9**; B = **11** and 5'-*O*-(4,4'-dimethoxytrityl)thymidine **9**; B = **13**, all of which were commercially available. In order to minimize side-reactions and to increase the solubility of the protected oligonucleotide intermediates in organic solvents, it was decided to protect guanine residues also on *O*-6 with the 2,5-dichlorophenyl group¹² and thymine residues on *O*-4 with the phenyl group.¹³ The 2'-deoxyguanosine derivative **9**; B = **11** was converted in high yield into its 6-*O*-(2,5-dichlorophenyl) derivative **9**; B = **12** by a previously reported procedure¹² and the thymidine derivative **9**; B = **13** was converted (Scheme 2a and Experimental) into its 4-*O*-phenyl derivative **9**; B = **14** in 82% isolated yield. All three 5'-*O*-DMTr derivatives **9**; B = **10**, **12** and **14** were converted (Scheme 2b, step iii) into the triethylammonium salts of their 3'-H-phosphonates **1**, B = **10**, **12** and **14**, respectively, in high yields by a previously reported procedure¹⁴ and into the corresponding 3'-*O*-levulinyl derivatives **15**, B = **10**, **12** and **14** in good yields. With the exception of the 4-*O*-phenylthymidine derivatives **1** and **15**, B = **14** (see Experimental), these preparations have already been reported.⁷

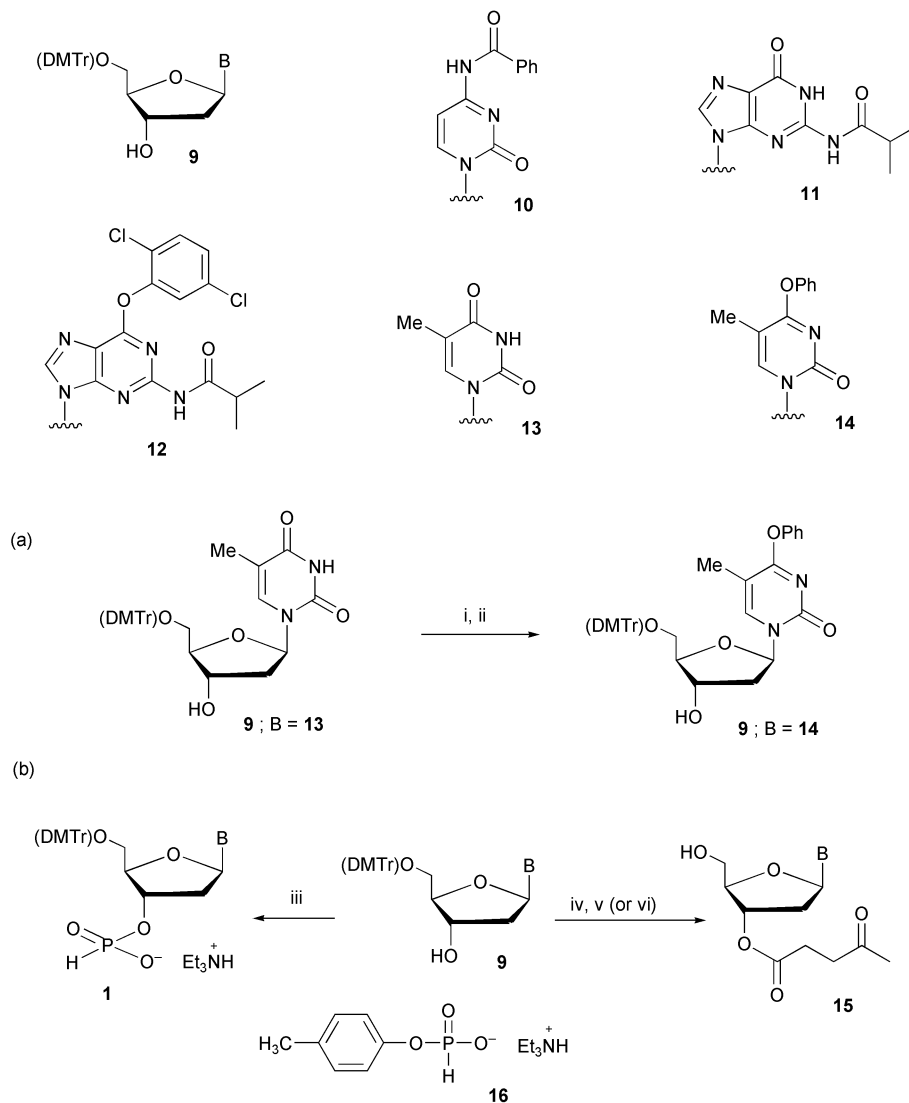
2 Four component synthesis of dimer and trimer blocks

In the initial work^{6,7} on the modified H-phosphonate approach, fully-protected blocks (e.g. **3b**) were prepared in two separate

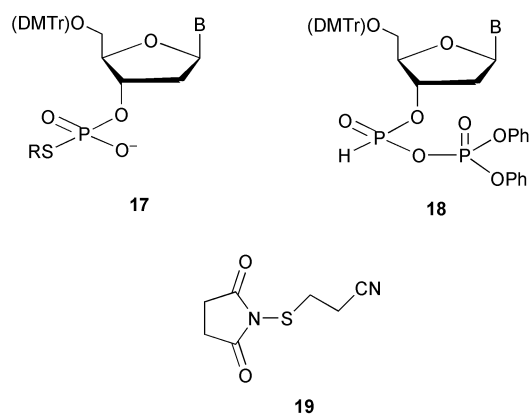
steps (Scheme 1). First, a solution containing both the 3'-H-phosphonate **1** and the component **2** with the free 5'-hydroxy function was treated with the coupling reagent **5a** at -40 °C (Scheme 1, step i) and then, after 5–10 min, the sulfur-transfer reagent (e.g. **8**) was added (step ii). This procedure is perfectly satisfactory for small scale synthesis. However, coupling reactions (step i) will most probably be exothermic and therefore, in a large scale synthesis, it may well be necessary to mix the reactants much more slowly, perhaps by the controlled addition of the coupling reagent to a solution containing the other two components. This could lead to the intermediate H-phosphonate diester being exposed to the coupling reagent for a considerable time and therefore to the possible occurrence of undesired side-reactions.¹⁵ Furthermore, it is inconvenient to carry out reactions on a very large scale at as low a temperature as -40 °C.

In order to address the last point, it was decided to look into the possibility of carrying out coupling reactions at room temperature. As both coupling and sulfur-transfer reagents are electrophilic, it seemed that it might be possible to carry out four component reactions by adding the two reagents simultaneously to a solution containing a mixture of the 3'-H-phosphonate **1** and the component **2** with the free 5'-hydroxy function. This would allow the intermediate H-phosphonate diester to react with the sulfur-transfer reagent as soon as it was formed and its exposure to the excess of coupling reagent would thereby be minimized. A possible problem that could then arise is that the H-phosphonate monoester **1** might react with the sulfur-transfer reagent (RS-X) to give the corresponding phosphorothioate diester **17** before coupling occurred. This reaction could be promoted by the coupling reagent in that the sulfur-transfer reagent might be expected to react more readily with a mixed anhydride (e.g. **18**), formed between the H-phosphonate monoester **1** and the coupling reagent (e.g. **5b**), than with the H-phosphonate monoester **1** itself.

The reaction between 5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-H-phosphonate **1**; B = **13** and the morpholine-3,5-dione reagent¹⁴ **8** in pyridine-d₅ was monitored at the ambient temperature by ³¹P NMR spectroscopy and found to be very slow indeed. It is noteworthy that the corresponding reaction with *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** was found to proceed



Scheme 2 Reagents and conditions: i, 1-methylpyrrolidine, Me_3SiCl , MeCN, room temp., 1 h; ii, a, POCl_3 , 0°C , 20 min, b, PhOH, 0°C , 3 h, c, H_2O , 0°C to room temp.; iii, a, **16**, $\text{Me}_3\text{C}\cdot\text{COCl}$, $\text{C}_5\text{H}_5\text{N}$, -20°C , 1 h, b, H_2O , $\text{C}_5\text{H}_5\text{N}$, -20°C to room temp.; iv, $(\text{CH}_3\text{COCH}_2\text{CH}_2\text{CO})_2\text{O}$, Et_3N , DMAP, CH_2Cl_2 , room temp.; v, HCl, CH_2Cl_2 , -50°C , 5 min; vi, pyrrole, $\text{Cl}_2\text{CHCO}_2\text{H}$, CH_2Cl_2 , room temp., 15 min.



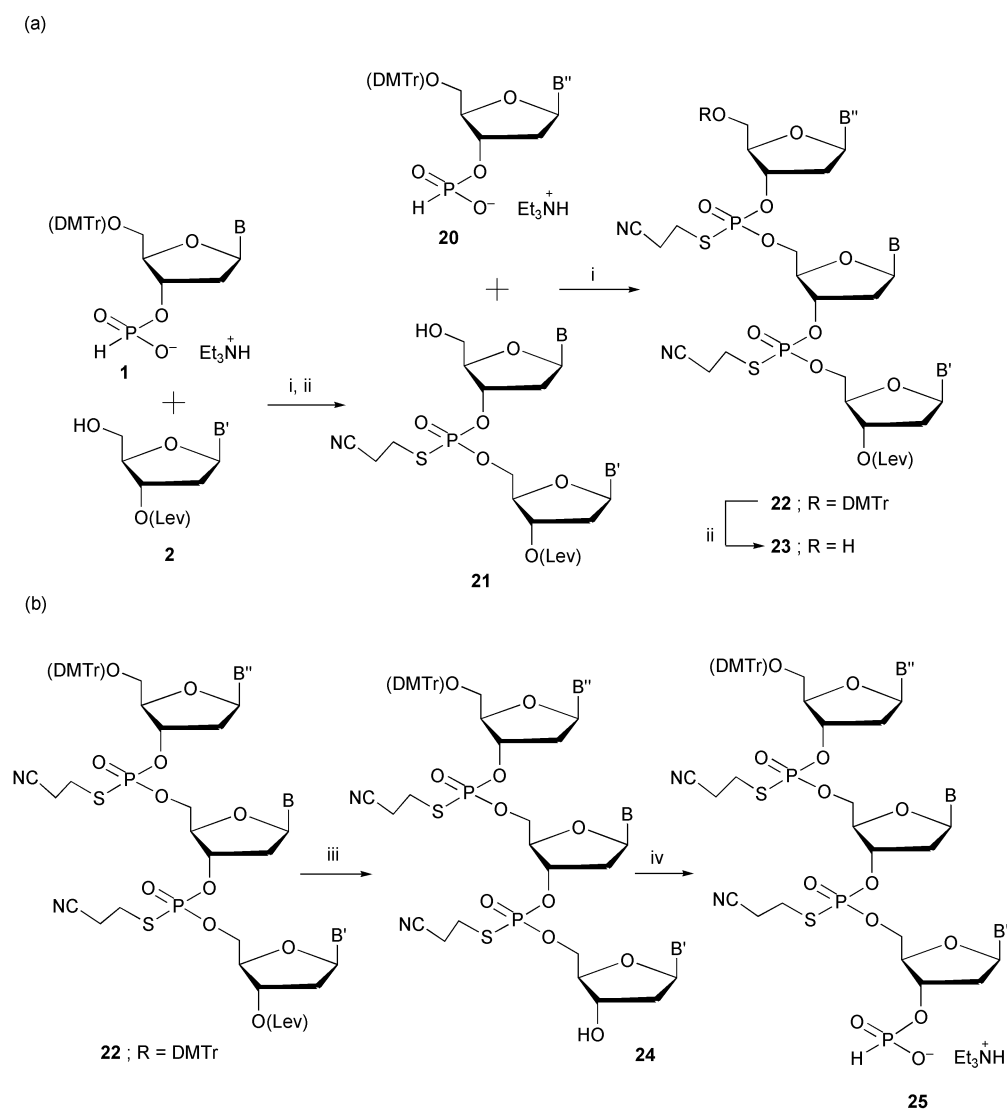
at a rate approximately an order of magnitude slower. However, the reaction involving the morpholine-3,5-dione reagent **8** proceeded much more rapidly in the presence of bis(2-chlorophenyl) phosphorochloridate **5a**. Fortunately, the four component coupling reactions appeared to proceed still more rapidly and high isolated yields of *S*-(2-cyanoethyl)-protected dinucleoside phosphorothioates **21** (Scheme 3a) were obtained (Table 1, entries 1–3) when only a 20% excess of the 3'H-phosphonate building block **1** was used in the presence of diphenyl phosphorochloridate **5b** as the coupling reagent

and *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** as the sulfur-transfer reagent. The four component reactions were all carried out in pyridine solution at room temperature. Diphenyl phosphorochloridate **5b** was used instead of bis(2-chlorophenyl) phosphorochloridate **5a** because it is commercially available and, as far as can be judged, equally effective. *N*-[(2-Cyanoethyl)sulfanyl]succinimide **19** has two advantages over the morpholine-3,5-dione sulfur-transfer reagent **8**. First, it is more soluble in pyridine and secondly, it appeared to react more selectively with the intermediate H-phosphonate diester in the presence of excess both of an H-phosphonate monoester **1** and the coupling reagent (e.g. **5a** or **5b**). It is convenient at this stage to use a system of abbreviations for protected oligonucleotide phosphorothioates^{6,7} in which nucleoside residues and internucleotide linkages are italicized if they are protected in a defined way. In the present context, *C*, *G*, and *T* represent 2'-deoxycytidine protected with a benzoyl group on *N*-4 of its cytosine residue (as in **10**), 2'-deoxyguanosine protected with an isobutyryl group on *N*-2 and a 2,5-dichlorophenyl group on *O*-6 of its guanine residue (as in **12**) and thymidine protected with a phenyl group on *O*-4 of its thymine residue (as in **14**), respectively; *p(s)* represents an *S*-(2-cyanoethyl)-protected phosphorothioate and p(H) (which is unprotected and therefore not italicized) represents an H-phosphonate monoester if it is attached to a monomer or if it is placed at the end of a sequence. Thus DMTr-Cp(H) and HO-Cp(*s*)/G-Lev

Table 1 Four component solution phase oligonucleotide synthesis^a

Entry no.	H-Phosphonate monomer (mol equiv.)	5'-OH Component	Coupling reagent 5b (mol equiv.)	Sulfur-transfer reagent 19 (mol equiv.)	Total reaction time/min	Product ^b	Isolated yield (%)
1	DMTr-Cp(H) (1.20)	HO-G-Lev	2.5	2.5	25	HO-Cp(s)G-Lev	95.6
2	DMTr-Tp(H) (1.20)	HO-T-Lev	2.5	2.5	25	HO-Tp(s)T-Lev	93.8
3	DMTr-Cp(H) (1.20)	HO-T-Lev	2.5	2.5	25	HO-Cp(s)T-Lev	96.7
4	DMTr-Gp(H) (1.40)	HO-Cp(s)G-Lev	2.5	2.5	25	HO-Gp(s)Cp(s)G-Lev	94.4
5	DMTr-Gp(H) (1.40)	HO-Cp(s)G-Lev	2.5	2.5	25	DMTr-Gp(s)Cp(s)G-OH	94.8
6	DMTr-Cp(H) (1.40)	HO-Tp(s)T-Lev	2.5	2.5	25	DMTr-Cp(s)Tp(s)T-OH	93.1
7	DMTr-Gp(H) (1.40)	HO-Cp(s)T-Lev	2.5	2.5	25	DMTr-Gp(s)Cp(s)T-OH	94.0
8	DMTr-Tp(H) (1.40)	HO-Tp(s)T-Lev	2.5	2.5	25	DMTr-Tp(s)Tp(s)T-OH	94.2

^a Reactions were carried out by adding a solution of diphenyl phosphorochloridate **5b** and *N*-(2-cyanoethyl)sulfanyl)succinimide **19** in anhydrous pyridine dropwise over a period of 5 min to a stirred solution of the H-phosphonate monomer and the 5'-OH component in anhydrous pyridine at room temperature. ^b The products indicated in entries nos. 1–4 were obtained by treating the initially-obtained four component reaction products with dichloroacetic acid and pyrrole in dichloromethane solution (see Scheme 3a and Experimental); the products indicated in entries nos. 5–8 were obtained by treating the initially-obtained four component reaction products with hydrazine hydrate in pyridine–acetic acid–water (see Scheme 3b and Experimental).



Scheme 3 Reagents and conditions: i, **5b**, **19**, C₅H₅N, room temp.; ii, pyrrole, Cl₂CHCO₂H, CH₂Cl₂, 0 °C, 10 min; iii, a, N₂H₄·H₂O, C₅H₅N, AcOH, H₂O, b, CH₂Ac₂; iv, **16**, Me₃C·COCl, –20 °C, 1 h, b, H₂O, C₅H₅N, –20 °C to room temp.

(Table 1, entry no. 1) represent the triethylammonium salt of 5'-*O*-(4,4'-dimethoxytrityl)-4-*N*-benzoyl-2'-deoxycytidine 3'H-phosphonate **1**; B = **10** and the partially-protected dinucleoside phosphorothioate **21**; B = **10**, B' = **12**, respectively.

Three partially-protected dinucleoside phosphorothioates, HO-Cp(s)G-Lev, HO-Tp(s)T-Lev and HO-Cp(s)T-Lev (Table 1, 1–3) were required for the synthesis of Vitravene. These

dimers **21** (Scheme 3a) were prepared in two steps and the average overall yield was ca. 95.4%. Following the four component coupling and sulfur-transfer step (step i), the 5'-terminal DMTr protecting groups were removed by treatment with dichloroacetic acid in the presence of pyrrole¹⁶ (step ii). The required trimer blocks [HO-Gp(s)Cp(s)G-Lev (and DMTr-Gp(s)Cp(s)G-OH), DMTr-Cp(s)Tp(s)T-OH, DMTr-

Table 2 Assembly of the fully-protected Vitravene sequence from trimer blocks^a

Entry	Trimer H-phosphonate	5'-OH Component	Me ₃ C-COCl (mol equiv.)	19/mmol	Pyridine ^b /cm ³	Yield ^c (%)
1	DMTr-Cp(s)Tp(s)Tp(H) 26 (4.24 g, 1.25 mol equiv.)	HO-Gp(s)Cp(s)G-Lev 27 (3.31 g, 2.00 mmol)	2.5	8.2	23 (3.05)	94.0 (91.5)
2	DMTr-Cp(s)Tp(s)Tp(H) 26 (6.77 g, 1.33 mol equiv.)	HO-Cp(s)Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev 29 (9.05 g, 3.0 mmol)	2.65	16.1	45 (2.84)	91.8 (90.4)
3	DMTr-Cp(s)Tp(s)Tp(H) 26 (6.26 g, 1.42 mol equiv.)	HO-[Cp(s)Tp(s)Tp(s)Tp(s)] ₂ Gp(s)Cp(s)G-Lev 31 (11.37 g, 2.60 mmol)	2.85	16.8	47 (2.67)	91.6 (88.0)
4	DMTr-Gp(s)Cp(s)Tp(H) 32 (5.46 g, 1.53 mol equiv.)	HO-[Cp(s)Tp(s)Tp(s)] ₃ Gp(s)Cp(s)G-Lev 33 (11.00 g, 1.92 mmol)	3.05	15.0	42 (2.55)	91.3 (86.5)
5	DMTr-Tp(s)Tp(s)Tp(H) 34 (4.45 g, 1.67 mol equiv.)	HO-Gp(s)Cp(s)Tp(s)Tp(s)[Cp(s)Tp(s)Tp(s)] ₃ Gp(s)Cp(s)G-Lev 35 (11.50 g, 1.58 mmol)	3.34	13.6	38 (2.38)	88.6 (84.0)
6	DMTr-Gp(s)Cp(s)Gp(H) 36 (4.65 g, 1.84 mol equiv.)	HO-Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)] ₃ Gp(s)Cp(s)G-Lev 37 (10.75 g, 1.25 mmol)	3.71	12.5	35 (2.27)	89.6

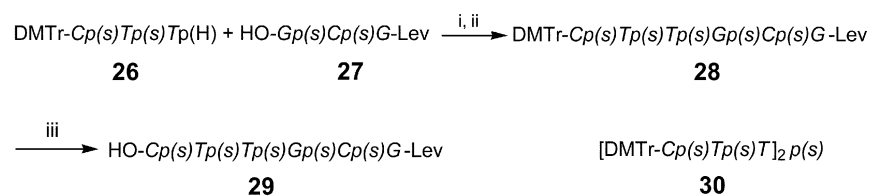
^a Pivaloyl chloride (2.5 mol equiv. with respect to the 5'-OH component) was added dropwise over a period of 30 s to a solution of trimer H-phosphonate and the 5'-OH component in anhydrous pyridine at 0 °C. After 30 min, a large enough excess of the sulfur-transfer reagent **19** was added to ensure saturation of the reaction solution, and the reactants were allowed to warm up to room temp. The products were worked up after a further period of 1 h [30 min in the preparation of DMTr-Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev **28** (entry no. 1)]. ^b The figures in parentheses represent the volumes of pyridine used to dissolve 1.0 g of total nucleotide material (i.e. trimer H-phosphonate and 5'-OH component combined). ^c The first figures relate to the isolated yields of fully protected oligonucleotide phosphorothioates obtained after coupling and sulfur-transfer; the figures in parentheses relate to the isolated yields obtained after the removal of the 5'-O-DMTr protecting groups by treatment with dichloroacetic acid and pyrrole in dichloromethane solution (see Experimental).

Gp(s)Cp(s)T-OH and DMTr-Tp(s)Tp(s)T-OH; Table 1, entries 4–8] were also prepared by four component synthesis (Scheme 3a). However, a larger excess (1.40 mol equiv.) of the 3'-H-phosphonate building block **20** was required in order to force the coupling process to completion. This is presumably because the 1 + 2 → 3 coupling reactions are slower than those involving two monomers (**1** and **2**), and therefore an increased proportion of the H-phosphonate monoester **20** will get converted¹² into phosphorothioate diester (corresponding to **17**). The partially-protected trimer HO-Gp(s)Cp(s)G-Lev (entry no. 4) was required (see below) as the 3'-terminal building block in the Vitravene synthesis. The other four partially-protected trimers **24** [DMTr-Gp(s)Cp(s)G-OH, DMTr-Cp(s)Tp(s)-T-OH, DMTr-Gp(s)Cp(s)T-OH and DMTr-Tp(s)Tp(s)-T-OH; entries 5–8] were prepared from the corresponding four component coupling products **22** [DMTr-Gp(s)Cp(s)-G-Lev, DMTr-Cp(s)Tp(s)T-Lev, DMTr-Gp(s)Cp(s)T-Lev and DMTr-Tp(s)Tp(s)T-Lev, respectively] by treatment with hydrazine hydrate in pyridine-acetic acid-water in the usual way¹⁷ (Scheme 3b, step iii). These trimers **24** were isolated in an average yield of ca. 94%; they were then phosphorylated by the triethylammonium *p*-tolyl H-phosphonate-pivaloyl chloride procedure¹⁴ (step iv) to give the corresponding H-phosphonate building blocks **25** in an average yield of ca. 92%.

3 Assembly of the fully-protected Vitravene sequence

As indicated above, the Vitravene sequence was prepared in six coupling steps, each of which involved a trimer H-phosphonate building block **25**. Four component synthesis was not attempted as it seemed possible that relatively large excesses of trimer H-phosphonates would then be required to drive the coupling reactions to completion, especially in the later stages. The procedure adopted for the first coupling reaction (i.e. 3 + 3 → 6; Table 2, entry no. 1) is illustrated in Scheme 4 (see also Table 2, footnote a). The trimer H-phosphonate **26** (1.25 mol equiv.) was first allowed to react with the 5'-OH component **27** (2.0 mmol, 1.0 mol equiv.) and pivaloyl chloride¹⁸ (2.5 mol equiv.) in anhydrous pyridine solution at 0 °C. After 30 min, in order to effect sulfur-transfer as rapidly as possible, sufficient *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** was added to saturate the reaction solution, which was then allowed to warm up to room temperature. After 30 min, the products were worked up and chromatographed to give the fully-protected hexamer **28** in 94% isolated yield (Table 2, entry 1). In addition, a small quantity (ca. 3%) of a less polar impurity, believed to be the symmetrical hexamer **30**, was obtained. The formation of this impurity, which was easily removed by chromatography, was probably mainly due to the H-phosphonate **26** being contaminated with a small quantity of the corresponding dephosphonylated material (i.e. DMTr-Cp(s)Tp(s)T-OH). The 5'-O-DMTr protecting group was then removed from the fully-protected hexamer **28** by treatment with dichloroacetic acid in the presence of pyrrole in dichloromethane solution to give the 5'-OH component **29**, required for the next coupling step (Table 2, entry no. 2), in 91.5% overall yield for the three steps (Scheme 4, steps i–iii).

A small proportion of each 5'-OH component was retained for complete deprotection (see below). The remainder of the material was coupled with the appropriate trimer H-phosphonate **26** and the resulting product was 'detritylated', as in the above preparation of the partially-protected hexamer **29** (Scheme 4), by treatment with dichloroacetic acid and pyrrole in dichloromethane solution. It can be seen from Table 2 that the excess of trimer H-phosphonate was increased by ca. 6–10% in each successive coupling step. Nevertheless the whole synthesis was relatively economical in trimer H-phosphonates in that the average excess used in the six coupling steps (Table 2, entries nos. 1–6) was only ca. 50%. This compares favourably with large scale phosphoramidite-based



Scheme 4 Reagents and conditions: i, $\text{Me}_3\text{C}\cdot\text{COCl}$, $\text{C}_5\text{H}_5\text{N}$, 0 °C, 30 min; ii, **19**, $\text{C}_5\text{H}_5\text{N}$, 0 °C to room temp., 30 min; iii, $\text{Cl}_2\text{CHCO}_2\text{H}$, pyrrole, CH_2Cl_2 , 0 °C, 30 min.

solid phase synthesis. The pivaloyl chloride/trimer H-phosphonate molar ratio was *ca.* 2.0 in each coupling step. As indicated above, sufficient sulfur-transfer reagent **19** was added to saturate the reaction solution. Thus the actual quantity used depended on the volume of pyridine used, which in turn, depended on the total weight of nucleotide material (*i.e.* the combined weight of the trimer H-phosphonate and the 5'-OH component). It can be seen from the penultimate column of Table 2 that, in order as far as possible to maintain the molar concentrations in each coupling reaction, the total nucleotide concentration by weight was increased as the synthesis progressed. Small quantities of by-products, believed to be symmetrical hexamers (*e.g.* **30**) were also isolated (see Experimental) from the products of the reactions leading to the fully-protected nonamer and pentadecamer (entries 2 and 4).

The scale of the coupling reactions with respect to the quantity of 5'-OH component used ranged from 3.0 mmol in the case of HO-Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev **29** (molecular weight, 3017.5 daltons; entry 2) to 1.25 mmol in the case of the partially-protected octadecamer **37** (molecular weight, 8616.5 daltons; entry 6). Following the latter coupling reaction (entry 6) and subsequent treatment with *N*-[(2-cyanoethyl)sulfanyl]succinimide **19**, 11.86 g (1.12 mmol) of fully-protected Vitravene, DMTr-Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)-Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev **38** (molecular weight, 10,608 daltons) was isolated from the products. The isolated yields of the fully-protected oligonucleotide phosphorothioates (Table 2, entries 1–6) averaged over 91% and the isolated overall yields of the 5'-OH components (entries 1–5) averaged over 88%. It should be emphasized that none of these reactions has yet been optimised.

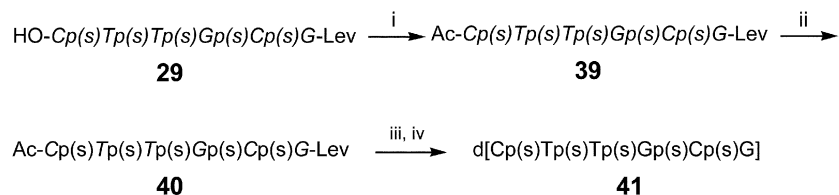
4 ¹H NMR spectra of the 5'-OH components

As they are complex mixtures of diastereoisomers (theoretically 32 for the hexamer **29**, 256 for the nonamer **31**, 2048 for the dodecamer **33**, 16384 for the pentadecamer **35** and 131072 for the octadecamer **37**), it is perhaps surprising that the ¹H NMR spectra of the 5'-OH components **29**, **31**, **33**, **35**, **37** (abbreviated to HO-6-Lev, HO-9-Lev, HO-12-Lev, HO-15-Lev and HO-18-Lev, respectively, in Table 3) are relatively simple, and indeed that their complexity does not appear to increase significantly with increasing molecular weight. Furthermore, the chemical shifts of the resonance signals assigned to specific protons in the base and sugar residues (Table 3, columns 1–14) vary only within very narrow bands for all five sequences. Although no attempt has been made to confirm the base sequences by NMR spectroscopy, it is very easy to confirm the base ratios. Thus, for all five sequences, the cytidine NH protons (Table 3, column 1) resonate at δ 11.25–11.27, almost 1 ppm downfield from the

guanine NH protons (column 2), and the relative integrals of the two sets of signals are in good accordance with the theoretical values. The dC : dG ratios can also be determined from the integrals of the H-8 (dG) and H-6 (dC) resonance signals (columns 3 and 4, respectively). It seems likely from the data in column 4 that the H-6 proton of a 5'-terminal dC residue resonates *ca.* 0.17 ppm downfield from that of a non-terminal dC residue. It is also noteworthy that the H-1' and H-3' protons of dG residues (columns 6 and 8, respectively) resonate *ca.* 0.25–0.3 ppm downfield from the H-1' and H-3' protons of dC and dT residues (columns 7 and 10, respectively). Thus the dG : (dC + dT) ratios may be determined. Despite the presence of large numbers of diastereoisomers, the 3'-terminal levulinyl methyl protons (column 12) invariably resonate as a sharp singlet at δ 2.12. The dT : dG ratios may be determined by dividing the integrals of the thymine 5-methyl protons resonance signals (at *ca.* δ 2.06; column 13) by one-half of the integrals of the guanine isobutyryl methyl protons resonance signals (at δ 0.93–0.94; column 14). Assignments have not been made in the relatively high field regions of the spectra where the resonance signals of the 2-cyanoethyl and levulinyl methylene protons are found. The ¹H NMR spectra of the fully-protected sequences (*i.e.* the 5'-O-DMTr and the 5'-O-acetyl derivatives of the 5'-OH components) are also relatively uncomplicated.

5 Unblocking of protected oligonucleotide phosphorothioates

The unblocking of the 5'-OH components involves four steps. This process, as exemplified by the unblocking of the hexamer, HO-Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev **29** is illustrated in Scheme 5. The 5'-hydroxy function is first acetylated (step i). This is a precaution to avoid the possible attack of the hydroxy function¹⁹ on the neighbouring phosphorothioate triester internucleotide linkage. Step ii involves the DBU-promoted removal of the *S*-(2-cyanoethyl) protecting groups from the internucleotide linkages. Chlorotrimethylsilane is added as a water scavenger in this step. Treatment with the conjugate base of *E*-2-nitrobenzaloxime **7** (step iii) then leads¹³ to the removal of the 6-*O*-(2,5-dichlorophenyl) and 4-*O*-phenyl protecting groups from the guanine and thymine residues, respectively. The remaining acyl protecting groups are then removed (step iv) from the cytosine and guanine base residues and the 3'- and 5'-terminal hydroxy functions by treatment with concentrated aqueous ammonia at 55 °C. 2-Mercaptoethanol is added²⁰ to the reaction solution in order to minimize the possibility of desulfurization. Finally, an aqueous solution of the fully-unblocked hexamer, d[Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G] **41** was passed through a column of Dowex 50 (Na^+ form) to convert it into its sodium salt. The other four 5'-OH components **31**, **33**, **35** and **37** were converted by the same four step process



Scheme 5 Reagents and conditions: i, Ac_2O , $\text{C}_5\text{H}_5\text{N}$, room temp., 16 h; ii, DBU, Me_3SiCl , room temp., 30 min; iii, *E*-2-nitrobenzaloxime **7**, DBU, MeCN, room temp., 12 h; iv, a, conc. aq. NH_3 (d 0.88), $\text{HSCH}_2\text{CH}_2\text{OH}$, 55 °C, 15 h, b, Dowex 50 (Na^+ form).

Table 3 ^1H NMR spectra of 5'-OH components

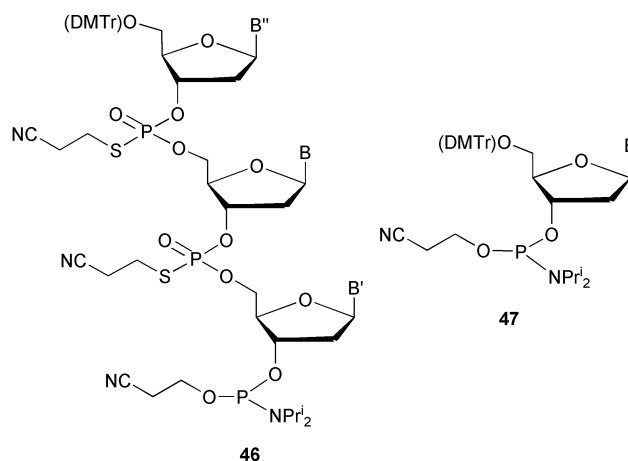
Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Assignment	4-NH (C)	2-NH (G)	H-8 (G)	H-6 (C)	Other aryl protons	H-1' (G)	H-1' (C, T)	H-3' (G)	5'-OH	H-3' (C, T)	H-4', H-5'	CH ₃ (Lev)	CH ₃ (T)	(CH ₃) ₂ C (G)
HO-6-Lev ^a 29	11.27 (2H)	10.32, 10.36 (1H), (1H)	8.50, 8.56 (1H), (1H)	8.17, 8.35 (1H), (1H)	7.1-8.0 (30H)	6.45 (2H)	6.18 (4H)	5.51 (2H)	5.27 (1H)	5.15 (4H)	4.27-4.45 (17H)	2.12 (3H)	2.07 (6H)	0.94 (12H)
HO-9-Lev ^a 31	11.27 (3H)	10.33, 10.37 (1H), (1H)	8.49, 8.56 (1H), (1H)	8.17, 8.34 (2H), (1H)	7.1-8.05 (48H)	6.45 (2H)	6.18 (7H)	5.51 (2H)	5.29 (1H)	5.17 (7H)	4.11-4.43 (27H)	2.12 (3H)	2.07 (12H)	0.94 (12H)
HO-12-Lev ^a 33	11.26 (4H)	10.32, 10.36 (1H), (1H)	8.49, 8.56 (1H), (1H)	8.17, 8.35 (3H), (1H)	7.1-8.05 (65H)	6.45 (2H)	6.19 (10H)	5.51 (2H)	5.28 (1H)	5.19 (10H)	4.26-4.43 (34H)	2.12 (3H)	2.06 (18H)	0.94 (12H)
HO-15-Lev ^a 35	11.25 (5H)	10.31, 10.36 (2H), (1H)	8.49, 8.56 (1H), (2H)	8.17 (5H)	7.1-8.0 (81H)	6.43 (3H)	6.18 (12H)	5.32, 5.51 (1H), (2H)	5.10 (1H)	5.19 (12H)	4.20-4.43 (43H)	2.12 (3H)	2.05 (21H)	0.93 (18H)
HO-18-Lev ^a 37	11.25 (5H)	10.31, 10.36 (2H), (1H)	8.49, 8.56 (2H), (1H)	8.16 (5H)	7.1-8.0 (99H)	6.45 (3H)	6.18 (15H)	5.51 (3H)	5.32 (1H)	5.17 (15H)	4.20-4.50 (52H)	2.12 (3H)	2.05 (30H)	0.93 (18H)

^a The abbreviations for the 5'-OH components (**29**, **31**, **33**, **35** and **37**; see Table 2) have been further abbreviated for convenience.

(Scheme 5) into the sodium salts of $d[\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{G}]$ **42**, $d[\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{G}]$ **43**, $d[\text{Gp}(s)\text{Cp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{G}]$ **44** and $d[\text{Tp}(s)\text{Tp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{G}]$ **45**. Relatively small quantities (*ca.* 0.01–0.02 mmol) of these five 5'-OH components were unblocked in this way and the overall yields of the isolated sodium salts all exceeded 80% (see Experimental). The capillary gel electrophoresis (CGE) profiles and the ^{31}P NMR spectra of the unblocked sequences are illustrated in Figs. 2 and 3 (see below), respectively; the molecular weights of these unblocked oligonucleotide phosphorothioates, as determined by electrospray ionization mass spectrometry, are listed in Table 4. Finally, the unblocking of fully-protected Vitravene (DMTr-*Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Tp(s)Tp(s)Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)G*-Lev **38** was carried out on a larger (*ca.* 0.1 mmol) scale. The 5'-*O*-DMTr group was first removed by the procedure described above (Scheme 4, step iii). The four-step unblocking process, outlined in Scheme 5, was then followed to give completely unblocked Vitravene ($d[\text{Gp}(s)\text{Cp}(s)\text{Gp}(s)\text{Tp}(s)\text{Tp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Cp}(s)\text{Tp}(s)\text{Tp}(s)\text{Gp}(s)\text{Cp}(s)\text{G}]$) as its sodium salt in 76% overall isolated yield. The CGE profile and ^{31}P NMR spectrum of Vitravene are illustrated in Figs. 2f and 3f (see below), respectively, and its molecular weight, as determined by electrospray ionization mass spectrometry, is included in Table 4. In addition, the ^1H NMR spectrum of Vitravene is illustrated in Fig. 4.

6 Conclusions

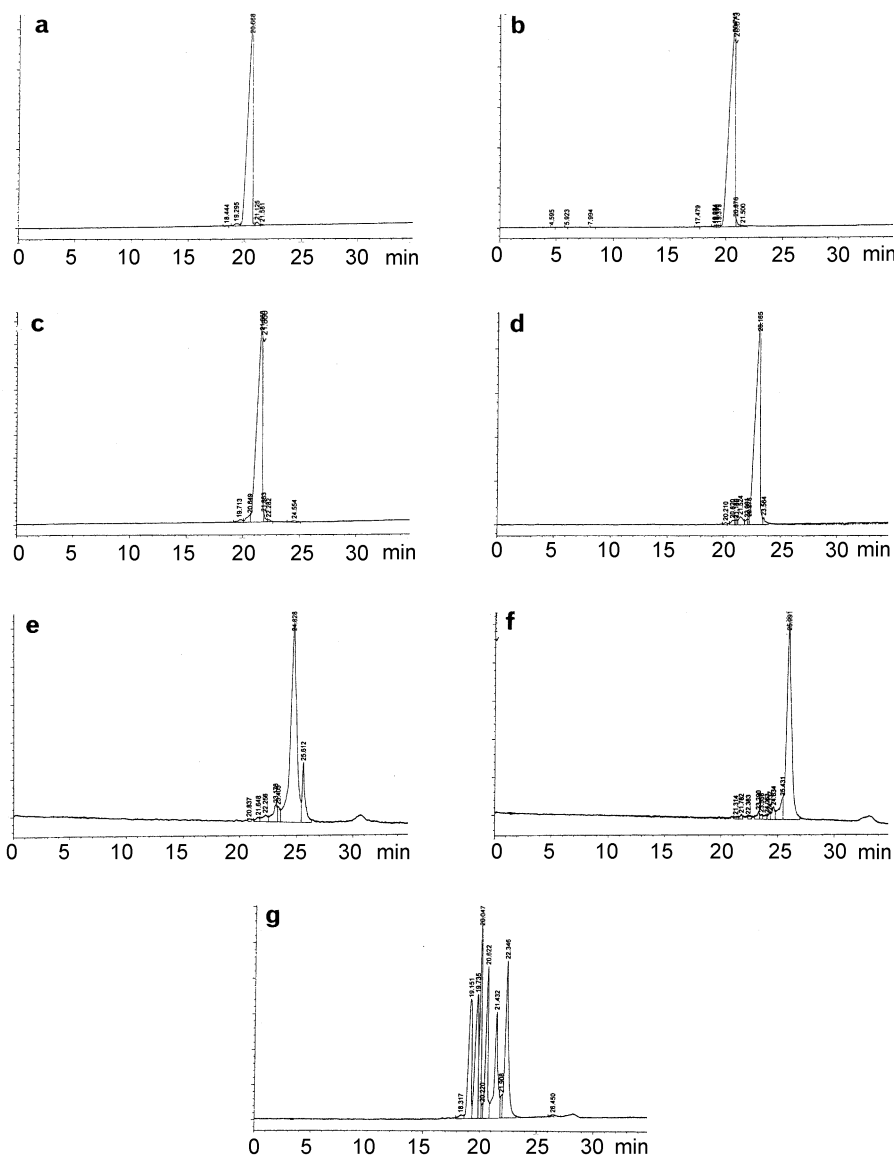
In our opinion, it may be concluded from the results of this study that the solution phase synthesis of oligonucleotide phosphorothioates by the modified H-phosphonate approach shows much promise. In the first place, it seems likely that it will be possible to carry out the four component synthesis of dimer and trimer (and perhaps tetramer) blocks on a very much larger scale. This could prove to be of considerable practical importance in solid phase as well as in solution phase synthesis. Thus building blocks such as trimer H-phosphonates **25** or the corresponding phosphoramidites **46** could be used instead of monomer H-phosphonates¹⁸ **1** or monomer phosphoramidites²¹ **47** in solid phase synthesis. This could introduce a strategic advantage when short (*e.g.* dimer or trimer) repeating sequences were present and would also avoid separation problems due to contamination with $n - 1$ and $n - 2$ sequences.



Although yields were, on the whole, good in the block coupling reactions (Table 2), we do not believe that this procedure has been optimized. Nevertheless, the CGE data (Fig. 2) suggest that the first four coupling steps (Table 2, entries 1–4) were very successful indeed. The least successful coupling step appears to be $3 + 15 \rightarrow 18$ (Fig. 2e and Table 2, entry 5);

Table 4 Electrospray ionization mass spectrometrically determined molecular weights (in daltons) of completely unblocked oligonucleotide phosphorothioates

Oligonucleotide	Molecular formula	Measured M.W.	Theoretical M.W.
6-mer 41	C ₅₈ H ₇₅ N ₂₀ O ₃₁ P ₅ S ₅	1863.38	1863.55
9-mer 42	C ₈₇ H ₁₁₃ N ₂₇ O ₄₈ P ₈ S ₈	2809.33	2809.33
12-mer 43	C ₁₁₆ H ₁₅₁ N ₃₄ O ₆₅ P ₁₁ S ₁₁	3755.44	3755.10
15-mer 44	C ₁₄₅ H ₁₈₈ N ₄₄ O ₈₁ P ₁₄ S ₁₄	4725.89	4725.89
18-mer 45	C ₁₇₅ H ₂₂₇ N ₅₀ O ₉₉ P ₁₇ S ₁₇	5686.75	5686.68
Vitravene	C ₂₀₄ H ₂₆₃ N ₆₃ O ₁₁₄ P ₂₀ S ₂₀	6683.13	6682.48

**Fig. 2** Capillary gel electrophoretic profiles of the fully-unblocked (a) 6-mer **41**, (b) 9-mer **42**, (c) 12-mer **43**, (d) 15-mer **44**, (e) 18-mer **45**, (f) 21-mer (Vitravene), (g) mixture of all six sequences.

the fact that this coupling appears to be less successful than $3 + 18 \rightarrow 21$ (Fig. 2f and Table 2, entry 6) suggests that the coupling efficiency does not necessarily fall off with increasing size of the 5'-hydroxy component. The block coupling reactions were carried out on a relatively large scale inasmuch as 11.86 g (*ca.* 1.12 mmol) of fully-protected Vitravene **38** was obtained. It must be emphasized that, following unblocking, *none of the oligonucleotide phosphorothioates was further purified at the phosphorothioate diester stage.*

Electrospray ionisation mass spectrometry provided satisfactory molecular weight values (Table 4) for all of the completely unblocked sequences. The ³¹P NMR spectra (in D₂O) of all six completely unblocked oligonucleotide phosphorothioate sequences (Fig. 3) display, as expected for phosphorothioate

diesters, resonance signals at *ca.* δ 56 ppm. In all six spectra, there are also very weak resonance signals at *ca.* δ 0, which indicate that not more than *ca.* 0.5% of the total internucleotide linkages are phosphodiester rather than phosphorothioate diesters. Although care was taken during the unblocking steps, it is believed that even this extent of phosphodiester contamination can be decreased. Finally, the ¹H NMR spectrum (in D₂O) of Vitravene is illustrated in Fig. 4. As Vitravene is theoretically a mixture of over 10^6 (*i.e.* 2²⁰) diastereoisomers and contains six differently situated 2'-deoxycytidine, five differently situated 2'-deoxyguanosine and ten differently situated thymidine residues, it is hardly surprising that the resonance signals are rather broad. The most downfield group of signals (δ 7.4–8.15, 21 H) is assigned to the H-6 resonances of the

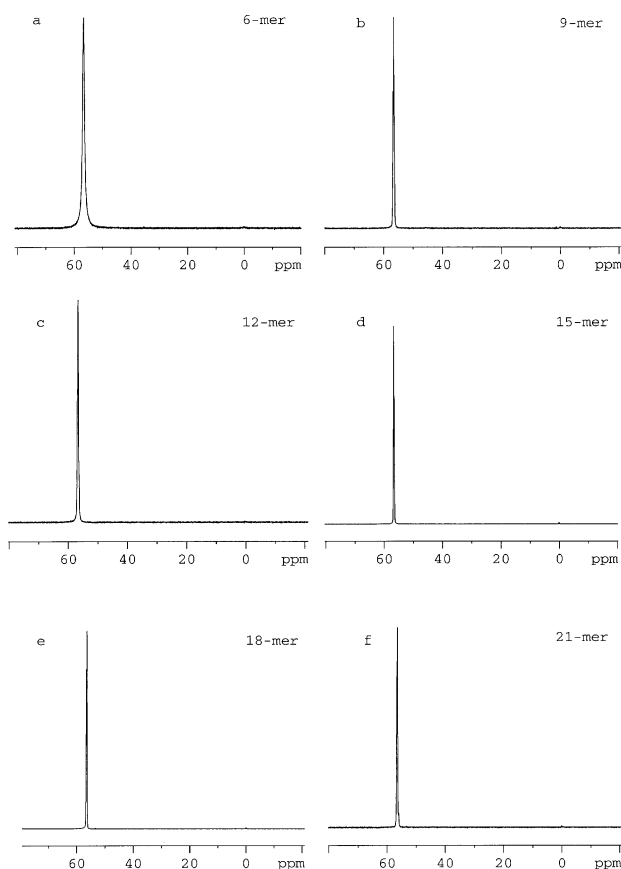


Fig. 3 ^{31}P NMR spectra (in D_2O) of the sodium salts of fully-unblocked (a) 6-mer **41**, (b) 9-mer **42**, (c) 12-mer **43**, (d) 15-mer **44**, (e) 18-mer **45**, (f) 21-mer (Vitravene).

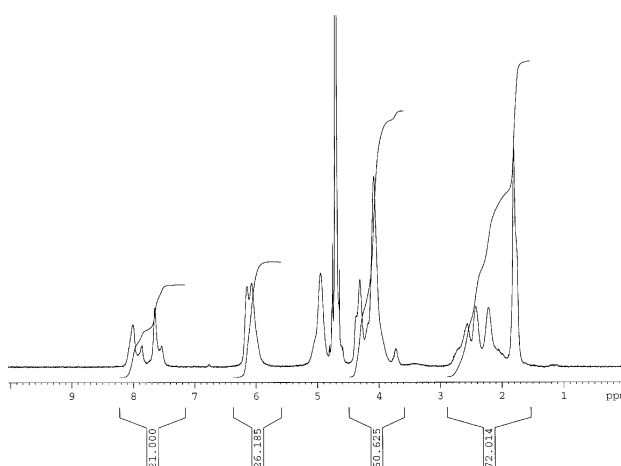


Fig. 4 ^1H NMR spectrum (in D_2O) of the sodium salt of fully-unblocked 21-mer (Vitravene).

cytosine, the H-8 resonances of the guanine and the H-6 resonances of the thymine residues. The next nearest group of signals (δ 5.8–6.25, 26.18 H) is assigned to all twenty-one H-1' resonances of the nucleoside residues and the H-5 resonances of the six cytosine residues; the integral is therefore 3% below the theoretical value (*i.e.* 27 H). However, the integral of the highest field group of signals (δ 1.65–2.85, 72 H), assigned to all forty-two H-2' resonances of the nucleoside residues and the 5-methyl proton resonances of the ten thymine residues is in accordance with the theoretical value.

Studies directed towards the further development of this methodology and its application to the synthesis of RNA sequences are now being undertaken in this laboratory.

Experimental

^1H NMR spectra were measured at 360 MHz with a Bruker AV 360 spectrometer; ^{13}C NMR spectra were measured at 90.6 MHz with the same spectrometer. Tetramethylsilane was used as an internal standard, and J values are given in Hz. ^{31}P NMR spectra were measured at 145.8 MHz with the same spectrometer; 85% orthophosphoric acid was used as an internal standard. Reverse phase high-performance liquid chromatography (HPLC) was carried out on a 250×4.6 mm Hypersil ODS 5μ column: the column was eluted with water–acetonitrile mixtures [programme A: linear gradient of water–acetonitrile (70 : 30 v/v to 20 : 80 v/v) over 10 min and then isocratic elution; programme B: linear gradient of water–acetonitrile (70 : 30 v/v to 10 : 90 v/v) over 10 min and then isocratic elution]. Capillary gel electrophoresis (CGE) was carried out by Mrs Joan Barrington at the Avecia Research Centre, Grangemouth with a Hewlett-Packard 3D CE instrument. Electrospray ionisation mass spectra were measured by Mr Brian Powell at the Avecia Research Centre, Manchester with a Micromass Quattro Ultima mass spectrometer. Merck silica gel 60 F_{254} TLC plates were developed in solvent systems A [dichloromethane–methanol (95 : 5 v/v)] and B [dichloromethane–methanol (93 : 7 v/v)]. Merck silica gel 60 (Art 7729 and 9385) were used for short column chromatography. Acetonitrile, 1-methylpyrrolidine, pyridine and triethylamine were dried by heating, under reflux, over calcium hydride and were then distilled. Dichloromethane and 1,2-dichloroethane were dried by heating, under reflux, over phosphorus pentoxide and were then distilled. 1,8-diazacyclo[5.4.0]undec-7-ene (DBU) and N^1, N^1, N^3, N^3 -tetramethylguanidine (TMG) were dried by heating with calcium hydride at 60 °C and were then distilled under reduced pressure. 5'-*O*-(4,4'-Dimethoxytrityl)-2'-deoxyribonucleoside derivatives were purchased from Cruachem Ltd., Glasgow.

N-(2-Cyanoethyl)sulfanyl)succinimide **19**

N-Bromosuccinimide (17.80 g, 0.10 mol) and bis(2-cyanoethyl) disulfide¹⁰ (17.20 g, 0.10 mol) were heated, under reflux, in dry 1,2-dichloroethane (30 cm^3) in an atmosphere of argon for 2 h. After the reaction mixture had been cooled down to room temperature, hexane (300 cm^3) was added and the mixture was stirred for 10 min. The upper layer was decanted and the oily residue was triturated with ethyl acetate (20 cm^3) for 10 min. The solid obtained was collected by filtration and washed with diethyl ether (70 cm^3) to give the *title compound* as an off-white solid (12.4 g, 67.3%) (found, in material recrystallized from absolute ethanol: C, 45.8; H, 4.3; N, 15.2. $\text{C}_7\text{H}_8\text{N}_2\text{O}_2\text{S}$ requires: C, 45.64; H, 4.38; N, 15.21%), mp 110–112 °C; δ_{H} [(CD_3) $_2\text{SO}$] 2.71 (4 H, s), 2.75 (2 H, t, J 6.9), 3.02 (2 H, t, J 6.9); δ_{C} [(CD_3) $_2\text{SO}$] 18.30, 29.07, 33.18, 119.62, 178.31.

5'-*O*-(4,4'-Dimethoxytrityl)-4-*O*-phenylthymidine **9**; **B** = **14**

1-Methylpyrrolidine (52.0 cm^3 , 0.50 mol) and chlorotrimethylsilane (12.7 cm^3 , 0.10 mol) were added to a stirred suspension of 5'-*O*-(4,4'-dimethoxytrityl)thymidine²² **9**; **B** = **13** (21.78 g, 40.0 mmol) in dry acetonitrile (200 cm^3) at room temperature. After 1 h, the reactants were cooled to 0 °C (ice–water bath) and stirred for 10 min. Phosphorus oxychloride (5.59 cm^3 , 60 mmol) was then added, followed after 20 min by phenol (22.59 g, 0.24 mol). After 3 h, water (10 cm^3) was added and the stirred products were allowed to warm up to room temperature. After 16 h, the products were concentrated under reduced pressure. The residue was dissolved in dichloromethane (200 cm^3) and the solution was washed with saturated aqueous sodium hydrogen carbonate (2×150 cm^3). The aqueous layer was back-extracted with dichloromethane (2×30 cm^3). The combined organic layers were dried (MgSO_4) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel. Appropriate fractions, which were

eluted with dichloromethane–methanol (99 : 1 v/v), were combined and evaporated under reduced pressure to give the *title compound* **9**; **B** = **14** (20.36 g, 82%) as a colourless glass; R_f 0.35 (system A) δ_H [(CD₃)₂SO] 1.73 (3 H, s), 2.18 (1 H, m), 2.30 (1 H, m), 3.26 (2 H, m), 3.75 (6 H, s), 3.97 (1 H, m), 4.34 (1 H, m), 5.37 (1 H, d, J 4.6), 6.15 (1 H, t, J 6.3), 6.92 (4 H, d, J 8.9), 7.13–7.48 (14 H, m), 7.99 (1 H, s); δ_C [(CD₃)₂SO] 11.89, 41.08, 55.26, 55.42, 63.72, 70.41, 86.07, 86.19, 86.27, 103.45, 113.65, 122.43, 126.08, 127.18, 128.02, 128.31, 129.85, 130.10, 135.60, 135.78, 142.24, 145.06, 152.18, 154.49, 158.52, 158.54, 170.14.

3'-*O*-Levulinyl-4-*O*-phenylthymidine **15**; **B** = **14**

Levulinic anhydride¹² (2.14 g, 10.0 mmol) was added to a stirred solution of 5'-*O*-(4,4'-dimethoxytrityl)-4-*O*-phenylthymidine **9**; **B** = **14** (3.16 g, 5.09 mmol), triethylamine (1.75 cm³, 12.6 mmol) and 4-(dimethylamino)pyridine (0.050 g, 0.41 mmol) in dry dichloromethane (25 cm³) at room temperature. After 1 h, the products were poured into saturated aqueous sodium hydrogen carbonate (30 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (3 × 20 cm³). The combined organic layers were dried (MgSO₄) and evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (50 cm³), and pyrrole (3.2 cm³, 46 mmol) and then dichloroacetic acid (2.5 cm³, 30 mmol) were added to the stirred solution at room temperature. After 15 min, the products were poured into saturated aqueous sodium hydrogen carbonate (30 cm³). The organic layer was separated and was again washed with saturated aqueous sodium hydrogen carbonate (30 cm³). The combined aqueous layers were back-extracted with dichloromethane (4 × 30 cm³). The combined organic layers were dried (MgSO₄) and evaporated to dryness under reduced pressure. The residue was triturated with ethyl acetate (20 cm³). The crystalline precipitate was collected by filtration and washed with cold ethyl acetate (3 cm³) to give the *title compound* **15**; **B** = **14** (1.74 g, 82%) (found, in material crystallized from dichloromethane–ethyl acetate: C, 60.3; H, 5.9; N 6.7. C₂₁H₂₄N₂O₇ requires: C, 60.57; H, 5.81; N, 6.73%), mp 184–185 °C; R_f 0.33 (system A); δ_H [(CD₃)₂SO] 2.09 (3 H, d, J 0.8), 2.12 (3 H, s), 2.24 (1 H, m), 2.37 (1 H, m), 2.51 (2 H, t, J 6.4), 2.75 (2 H, t, J 6.4), 3.67 (2 H, m), 4.05 (1 H, m), 5.22 (1 H, m), 5.28 (1 H, t, J 5.2), 6.15 (1 H, dd, J 5.8 and 8.2), 7.19 (2 H, m), 7.30 (1 H, m), 7.46 (2 H, m), 8.20 (1 H, s); δ_C [(CD₃)₂SO] 12.08, 27.69, 29.51, 37.40, 37.77, 61.12, 74.68, 85.20, 85.72, 103.32, 122.11, 125.75, 129.52, 142.25, 151.84, 154.26, 169.94, 172.01, 206.88.

Triethylammonium 5'-*O*-(4,4'-dimethoxytrityl)-4-*O*-phenylthymidine 3'-H-phosphonate **1**; **B** = **14**

A solution of ammonium 4-methylphenyl H-phosphonate¹⁴ (5.64 g, 29.8 mmol) and triethylamine (8.36 cm³, 60 mmol) in methanol (15 cm³) was evaporated under reduced pressure. The residue and 5'-*O*-(4,4'-dimethoxytrityl)-4-*O*-phenylthymidine **9**; **B** = **14** (6.21 g, 10.0 mmol) were dissolved in dry pyridine (20 cm³), and the solution was concentrated under reduced pressure. After the residue had been co-evaporated with dry pyridine (20 cm³), it was redissolved in pyridine (60 cm³) and the solution was cooled to –20 °C (industrial methylated spirits (IMS)–dry ice bath). Pivaloyl chloride (3.67 cm³, 29.8 mmol) was then added dropwise over a period of 5 min to the stirred solution. After a further period of 1 h, water (60 cm³) was added and the reaction mixture was allowed to warm up to room temperature. After 1 h, the products were partitioned between dichloromethane (300 cm³) and saturated aqueous sodium hydrogen carbonate (2 × 200 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (2 × 40 cm³). The combined organic layers were washed with 0.5 M triethylammonium phosphate buffer (pH 7.0; 2 × 100 cm³). The combined aqueous layers were back-extracted with dichloromethane (2 × 40 cm³). The combined

organic layers were then concentrated under reduced pressure to ca. 40 cm³ and toluene (50 cm³) was added. The resulting solution was applied to a short column of silica gel. Elution of the column and concentration of the combined appropriate fractions [eluted with dichloromethane–methanol (95 : 5 v/v)] gave the *title compound* **1**; **B** = **14** as a colourless glass (7.50 g, 95.4%); δ_H [(CD₃)₂SO] 1.14 (9 H, t, J 7.3), 1.68 (3 H, s), 2.25 (1 H, m), 2.44 (1 H, m), 2.98 (6 H, quart, J 7.2), 3.28 (2 H, m), 3.75 (6 H, s), 4.12 (1 H, s), 4.75 (1 H, s), 5.79 (0.5 H, s), 6.12 (1 H, t, J 6.3), 6.92 (2 H, d, J 8.9), 6.93 (2 H, d, J 8.9), 7.14–7.48 (14.5 H, m), 7.99 (1 H, s), 10.76 (1 H, br); δ_P [(CD₃)₂SO] 1.07 (d, J 585.9).

Four-component synthesis of partially-protected dinucleoside phosphorothioates

(a) **HO-Cp(s)G-Lev 21**; **B** = **10**, **B'** = **12**. A solution of triethylammonium 5'-*O*-(4,4'-dimethoxytrityl)-4-*N*-benzoyl-2'-deoxycytidine 3'-H-phosphonate⁷ **1**; **B** = **10** (5.753 g, 7.2 mmol) and 2'-deoxy-6-*O*-(2,5-dichlorophenyl)-2-*N*-isobutryl-3'-*O*-levulinylguanosine¹² **2**; **B'** = **12** (3.482 g, 6.0 mmol) in dry pyridine (15 cm³) was evaporated to dryness under reduced pressure and the residue was redissolved in dry pyridine (30 cm³). To this stirred solution at room temperature, a solution of *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** (2.763 g, 15.0 mmol) and diphenyl phosphorochloridate **5b** (3.1 cm³, 14.96 mmol) in dry pyridine (40 cm³) was added dropwise over a period of 10 min. After a further period of 10 min, water (5 cm³) was added. The reactants were stirred for 15 min more, and the products were then partitioned between dichloromethane (250 cm³) and saturated aqueous sodium hydrogen carbonate (250 cm³). The organic layer was separated and the aqueous layer was back-extracted with dichloromethane (2 × 20 cm³). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure. The residue was co-evaporated with dry toluene (3 × 50 cm³) and then dissolved in dichloromethane (80 cm³). The stirred solution was cooled to 0 °C (ice–water bath) and first pyrrole (4.16 cm³, 60.0 mmol) and then a solution of dichloroacetic acid (4.95 cm³, 60.0 mmol) in dichloromethane (40 cm³) were added. After 10 min, the products were cautiously poured into saturated aqueous sodium hydrogen carbonate (250 cm³). The organic layer was separated and was washed again with saturated aqueous sodium hydrogen carbonate (250 cm³); it was then dried (MgSO₄) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (97 : 3 v/v), were combined and evaporated under reduced pressure to give **HO-Cp(s)G-Lev 21**; **B** = **10**, **B'** = **12** (5.98 g, 95.6%) as a colourless glass; R_f 0.22 (system A); t_R 11.22 min (programme A); δ_H [(CD₃)₂SO] includes the following signals: 0.95 (6 H, m), 2.13 (3 H, s), 3.60 (2 H, m), 4.19 (1 H, m), 4.35 (1 H, m), 4.44 (2 H, m), 5.07 (1 H, m), 5.24 (1 H, m), 5.51 (1 H, m), 6.16 (1 H, t, J 6.7), 6.46 (1 H, t, J 7.0), 7.37–7.75 (7 H, m), 8.01 (2 H, d, J 7.4), 8.33 (1 H, m), 8.57 (1 H, m), 10.37 (1 H, br s), 11.29 (1 H, br); δ_P [(CD₃)₂SO] 27.20, 27.46.

(b) **HO-Tp(s)T-Lev 21**; **B** = **B'** = **14**. This partially-protected dinucleoside phosphorothioate was prepared from triethylammonium 5'-*O*-(4,4'-dimethoxytrityl)-4-*O*-phenylthymidine 3'-H-phosphonate **1**; **B** = **14** (7.08 g, 9.01 mmol) and 3'-*O*-levulinyl-4-*O*-phenylthymidine **2**; **B'** = **14** (3.126 g, 7.51 mmol), following the procedure and stoichiometry described in (a) above for the preparation of **HO-Cp(s)G-Lev 21**; **B** = **10**, **B'** = **12**. **HO-Tp(s)T-Lev 21**; **B** = **B'** = **14** (6.10 g, 93.8%) was isolated as a colourless glass following short column chromatography; R_f 0.26 (system A); t_R 11.35 min (programme A); δ_H [(CD₃)₂SO] includes the following signals: 2.10 (9 H, m), 2.74 (2 H, m), 2.94 (2 H, m), 3.15 (2 H, m), 3.69 (2 H, m), 4.22 (1 H, m), 4.28 (1 H, m), 4.36 (2 H, m), 5.13 (1 H, m), 5.24 (1 H, m),

5.32 (1 H, m), 6.17 (2 H, m), 7.18 (4 H, m), 7.29 (2 H, m), 7.44 (4 H, m), 8.01 (1 H, m), 8.15 (1 H, m); $\delta_{\text{p}} [(\text{CD}_3)_2\text{SO}]$ 27.78, 27.83.

(c) **HO-Cp(s)T-Lev 21; B = 10, B' = 14.** This partially-protected dinucleoside phosphorothioate was prepared from 2'-deoxy-(4,4'-dimethoxytrityl)-4-N-benzoylcytidine 3'-H-phosphonate **1**; **B = 10** (4.793 g, 6.00 mmol) and 3'-O-levulinyl-4-O-phenylthymidine **2**; **B' = 14** (2.082 g, 5.00 mmol), following the procedure and stoichiometry described in (a) above for the preparation of HO-Cp(s)G-Lev **21**; **B = 10, B' = 12.** HO-Cp(s)T-Lev **21**; **B = 10, B' = 14** (4.25 g, 96.7%) was isolated as a colourless glass following short column chromatography; R_{f} 0.24 (system A); t_{R} 9.21 min (programme A); $\delta_{\text{H}} [(\text{CD}_3)_2\text{SO}]$ 2.11 (6 H, m), 2.73 (3 H, m), 2.96 (2 H, m), 3.17 (2 H, m), 3.67 (2 H, m), 4.28 (2 H, m), 4.39 (2 H, m), 5.15 (1 H, m), 5.27 (2 H, m), 6.19 (2 H, m), 7.18 (2 H, d, J 8.0), 7.28 (1 H, m), 7.38–7.54 (5 H, m), 7.63 (1 H, m), 8.01 (3 H, m), 8.35 (1 H, m), 11.29 (1 H, br); $\delta_{\text{p}} [(\text{CD}_3)_2\text{SO}]$ 27.77, 27.84.

Four-component synthesis of partially-protected trinucleoside diphosphorothioates

(a) **HO-Gp(s)Cp(s)G-Lev 23; B' = B'' = 12; B = 10.** A solution of triethylammonium 2'-deoxy-6-O-(2,5-dichlorophenyl)-5'-O-(4,4'-dimethoxytrityl)-2-N-isobutyrylguanosine-3'-H-phosphonate **20**; **B'' = 12** (5.23 g, 5.51 mmol) and HO-Cp(s)G-Lev **21**; **B = 10, B' = 12** (4.101 g, 3.93 mmol) in dry pyridine (10 cm³) was evaporated to dryness under reduced pressure and the residue was redissolved in dry pyridine (19 cm³). To this stirred solution at room temperature, a solution of *N*-(2-cyanoethyl)sulfanyl)succinimide **19** (1.811 g, 9.83 mmol) and diphenyl phosphorochloridate (2.04 cm³, 9.84 mmol) in pyridine (27 cm³) was added dropwise over a period of 15 min. After a further period of 15 min, water (5 cm³) was added. The reactants were stirred for 15 min more, and the products were then partitioned between dichloromethane (150 cm³) and saturated aqueous sodium hydrogen carbonate (150 cm³). The organic layer was separated and the aqueous layer was back-extracted with dichloromethane (2 × 20 cm³). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure. The residue was co-evaporated with dry toluene (2 × 50 cm³) and was then redissolved in dichloromethane (60 cm³). The solution was cooled to 0 °C (ice-water bath) and first pyrrole (2.73 cm³, 39.3 mmol) and then a solution of dichloroacetic acid (3.24 cm³, 39.3 mmol) in dichloromethane (20 cm³) were added. After 15 min, the products were cautiously poured into saturated aqueous sodium hydrogen carbonate (150 cm³). The organic layer was separated and was washed again with saturated aqueous sodium hydrogen carbonate (150 cm³); it was then dried (MgSO₄) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane-methanol (97 : 3 v/v) were combined and evaporated under reduced pressure to give HO-Gp(s)Cp(s)G-Lev **23**; **B' = B'' = 12, B = 10** (6.15 g, 94.4%) as a colourless foam; R_{f} 0.17 (system A); t_{R} 13.7 min (programme A); $\delta_{\text{H}} [(\text{CD}_3)_2\text{SO}]$ includes the following signals: 0.95 (12 H, m), 2.13 (3 H, m), 3.61 (2 H, m), 4.23 (1 H, m), 4.41 (6 H, m), 5.10 (1 H, m), 5.18 (1 H, m), 5.33 (1 H, m), 5.51 (1 H, m), 6.19 (1 H, t, J 6.6), 6.44 (2 H, m), 7.36–7.51 (10 H, m), 7.98 (2 H, d, J 7.3), 8.21 (1 H, m), 8.57 (2 H, m), 10.32 (1 H, br s), 10.37 (1 H, br s), 11.29 (1 H, br); $\delta_{\text{p}} [(\text{CD}_3)_2\text{SO}]$ 27.85, 27.91, 27.98, 28.12.

(b) **DMTr-Cp(s)Tp(s)T-OH 24; B = B' = 14, B'' = 10.** A solution of triethylammonium 2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-4-N-benzoylcytidine-3'-H-phosphonate **20**; **B'' = 10** and HO-Tp(s)T-Lev **21**; **B = B' = 14** (5.40 g, 6.236 mmol) in dry pyridine (10 cm³) was evaporated to dryness under reduced pressure and the residue was redissolved in pyridine (30 cm³).

To this stirred solution at room temperature, a solution of *N*-(2-cyanoethyl)sulfanyl)succinimide **19** (2.872 g, 15.59 mmol) and diphenyl phosphorochloridate **5b** (3.23 cm³, 15.58 mmol) in pyridine (40 cm³) was added dropwise over a period of 15 min. After a further period of 15 min, water (5 cm³) was added. The reactants were stirred for 15 min more, and the products were then partitioned between dichloromethane (250 cm³) and saturated aqueous sodium hydrogen carbonate (250 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (2 × 30 cm³). The combined organic layers were dried (MgSO₄) and then concentrated under reduced pressure to ca. 20 cm³. The resulting solution was cooled to 0 °C (ice-water bath) and a mixture of hydrazine monohydrate (3.05 cm³, 62.9 mmol), water (6.2 cm³), glacial acetic acid (31 cm³) and pyridine (40 cm³) was added with stirring. After 15 min, pentane-2,4-dione (10 cm³, 97 mmol) was added and the reactants were allowed to warm up to room temperature. After 15 min, the products were cautiously poured into saturated aqueous sodium hydrogen carbonate (250 cm³). The layers were separated and the organic layer was again washed with saturated aqueous sodium hydrogen carbonate (2 × 200 cm³). The combined aqueous layers were back-extracted with dichloromethane (2 × 50 cm³). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure. Toluene (20 cm³) was added and the resulting solution was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane-methanol (96 : 4 v/v), were combined and evaporated under reduced pressure to give DMTr-Cp(s)Tp(s)T-OH **24**; **B = B' = 14, B'' = 10** (8.90 g, 93.1%) as a colourless foam; R_{f} 0.17 (system A); t_{R} 16.5 min (programme B); $\delta_{\text{H}} [(\text{CD}_3)_2\text{SO}]$ includes the following signals: 2.10 (7 H, m), 2.25 (1 H, m), 2.63 (1 H, m), 2.78 (1 H, m), 2.92 (4 H, m), 3.12 (4 H, m), 3.72 (6 H, m), 4.05 (1 H, m), 4.33 (7 H, m), 5.16 (2 H, m), 5.49 (1 H, d, J 4.4), 6.17 (3 H, m), 6.89 (4 H, m), 7.13–7.66 (23 H, m), 7.98 (4 H, m), 8.17 (1 H, m), 11.29 (1 H, br); $\delta_{\text{p}} [(\text{CD}_3)_2\text{SO}]$ 27.94, 27.99, 28.05, 28.12, 28.27, 28.30.

(c) **DMTr-Gp(s)Cp(s)T-OH 24; B = 10, B' = 14, B'' = 12.** This partially-protected trinucleoside diphosphorothioate was prepared from triethylammonium 2'-deoxy-6-O-(2,5-dichlorophenyl)-5'-O-(4,4'-dimethoxytrityl)-2-N-isobutyrylguanosine-3'-H-phosphonate **20**; **B'' = 12** (6.204 g, 6.53 mmol) and HO-Cp(s)T-Lev **21**; **B = 10, B' = 14** (4.10 g, 4.66 mmol), following the procedure and stoichiometry described in (b) above for the preparation of DMTr-Cp(s)Tp(s)T-OH **24**; **B = B' = 14, B'' = 10.** DMTr-Gp(s)Cp(s)T-OH **24**; **B = 10, B' = 14, B'' = 12** (7.45 g, 94%) was isolated as a colourless foam following short column chromatography; R_{f} 0.19 (system A); t_{R} 16.25 min (programme A); $\delta_{\text{H}} [(\text{CD}_3)_2\text{SO}]$ includes the following signals: 0.89 (6 H, m), 2.09 (3 H, s), 2.11 (1 H, m), 2.26 (1 H, m), 2.76 (3 H, m), 2.91 (4 H, m), 3.47 (1 H, m), 3.70 (6 H, s), 4.06 (1 H, m), 4.23–4.47 (7 H, m), 5.20 (1 H, m), 5.45 (1 H, m), 5.51 (1 H, d, J 4.2), 6.19 (2 H, m), 6.47 (1 H, t, J 6.6), 6.72 (4 H, m), 7.13–7.71 (21 H, m), 7.98 (3 H, m), 8.18 (1 H, m), 8.48 (1 H, s), 10.21 (1 H, br s), 11.29 (1 H, br); $\delta_{\text{p}} [(\text{CD}_3)_2\text{SO}]$ 27.67, 27.71, 27.73, 28.15, 28.27, 28.29.

(d) **DMTr-Tp(s)Tp(s)T-OH 24; B = B' = B'' = 14.** This partially-protected trinucleoside diphosphorothioate was prepared from triethylammonium 5'-O-(4,4'-dimethoxytrityl)-4-O-phenylthymidine-3'-H-phosphonate **20**; **B'' = 14** (4.57 g, 5.82 mmol) and HO-Tp(s)T-Lev **21**; **B = B' = 14** (3.60 g, 4.16 mmol), following the procedure and stoichiometry described in (b) above for the preparation of DMTr-Cp(s)Tp(s)T-OH **24**; **B = B' = 14, B'' = 10.** DMTr-Tp(s)Tp(s)T-OH **24**; **B = B' = B'' = 14** (5.95 g, 94.2%) was isolated as a colourless foam following short column chromatography; R_{f} 0.27 (system A); t_{R} 16.5 min (programme B); $\delta_{\text{H}} [(\text{CD}_3)_2\text{SO}]$ includes the following signals: 1.77 (3 H, s), 2.04 (3 H, s), 2.06 (3 H, m), 2.11 (2 H, m), 2.24

(1 H, m), 2.65 (2 H, m), 2.91 (4 H, m), 3.12 (4 H, m), 3.74 (6 H, s), 4.04 (1 H, m), 4.25–4.40 (7 H, m), 5.17 (2 H, m), 5.48 (1 H, d, J 4.4), 6.17 (3 H, m), 6.91 (4 H, m), 7.13–7.48 (24 H, m), 7.96 (3 H, m); δ_{P} [(CD₃)₂SO] 27.97, 28.03, 28.11, 28.26, 28.28.

(e) **DMTr-Gp(s)Cp(s)G-OH 24; B = 10, B' = B'' = 12.** This partially-protected trinucleoside diphosphorothioate was prepared from triethylammonium 2'-deoxy-6-*O*-(2,5-dichlorophenyl)-5'-*O*-(4,4'-dimethoxytrityl)-2-*N*-isobutrylguanosine 3'-H-phosphonate **20**; B'' = **12** (5.92 g, 6.23 mmol) and HO-Cp(s)G-Lev **21**; B = **10**; B' = **12** (4.64 g, 4.45 mmol), following the procedure and stoichiometry described in (b) above for the preparation of DMTr-Cp(s)Tp(s)T-OH **24**; B = B' = **14**, B'' = **10**. DMTr-Gp(s)Cp(s)G-OH **24**; B = **10**, B' = B'' = **12** (7.85 g, 94.8%) was isolated as a colourless foam following short column chromatography; R_{f} 0.22 (system A); t_{R} 17.1 min (programme B); δ_{H} [(CD₃)₂SO] includes the following signals: 0.90 (12 H, m), 3.69 (6 H, s), 4.11 (1 H, m), 4.22–4.40 (7 H, m), 4.65 (1 H, m), 5.14 (1 H, m), 5.49 (1 H, m), 5.56 (1 H, m), 6.16 (1 H, m), 6.45 (2 H, m), 6.71 (4 H, m), 7.13–7.74 (19 H, m), 7.98 (2 H, d, J 7.6), 8.15 (1 H, m), 8.48 (1 H, s), 8.53 (1 H, s), 10.20 (1 H, br s), 10.34 (1 H, br s), 11.30 (1 H, br); δ_{P} [(CD₃)₂SO] 27.65, 27.71, 27.83, 27.86.

Preparation of triethylammonium salts of trimer H-phosphonate building blocks

(a) **DMTr-Cp(s)Tp(s)Tp(H) 26.** A solution of ammonium 4-methylphenyl H-phosphonate (1.702 g, 9.0 mmol) and triethylamine (2.5 cm³, 17.9 mmol) in dry methanol (10 cm³) was evaporated under reduced pressure, and the residue was redissolved in dry pyridine (10 cm³). This solution was re-evaporated. The residue was dissolved in dry pyridine (10 cm³) and DMTr-Cp(s)Tp(s)T-OH **24**; B = B' = **14**, B'' = **10** (4.598 g, 3.0 mmol) was added. After the resulting solution had been concentrated under reduced pressure, the residue was redissolved in dry pyridine (10 cm³) and the solution was again evaporated. Finally, the residue was redissolved in dry pyridine (30 cm³) and the resulting solution was cooled to -20 °C (IMS-dry ice bath). Pivaloyl chloride (1.11 cm³, 9.0 mmol) was added over a period of 5 min to the stirred solution. After 1 h, water (10 cm³) was added and the products were allowed to warm up to room temperature over a period of 1 h. The products were partitioned between dichloromethane (150 cm³) and 1.0 M potassium phosphate buffer (pH 7.0, 80 cm³). The organic layer was separated and the aqueous layer was back-extracted with dichloromethane (2 × 15 cm³). The combined organic layers were washed with 0.5 M tetramethylguanidinium (TMGH⁺) phosphate buffer (pH 7.0, 2 × 60 cm³), and the combined aqueous layers were back-extracted with dichloromethane (2 × 15 cm³). Finally, the combined organic layers were extracted with 0.5 M triethylammonium phosphate buffer (pH 7.0, 80 cm³). After it had been separated and dried (MgSO₄), toluene (100 cm³) was added to the organic layer. The resulting solution was applied to a short column of silica gel. The column was eluted with dichloromethane-methanol mixtures: the appropriate fractions, which were eluted with dichloromethane-methanol (85 : 15 v/v), were combined and concentrated under reduced pressure to ca. 150 cm³. This solution was extracted with 0.5 M triethylammonium phosphate buffer (pH 7.0, 80 cm³). The organic layer was separated, dried (MgSO₄) and evaporated under reduced pressure to give triethylammonium DMTr-Cp(s)Tp(s)Tp(H) **26** (4.86 g, 95.4%) as a colourless glass; t_{R} 9.0 min (programme B); δ_{P} [(CD₃)₂SO] 1.48 (1 P, d, J 589.1), 27.99 (2 P, s).

(b) **DMTr-Gp(s)Cp(s)Tp(H) 32.** This trimer H-phosphonate was prepared from DMTr-Gp(s)Cp(s)T-OH **24**; B = **10**, B' = **14**, B'' = **12** (7.648 g, 4.50 mmol), following the procedure and stoichiometry described in (a) above for the preparation of the

triethylammonium salt of DMTr-Cp(s)Tp(s)Tp(H) **26**. The triethylammonium salt of DMTr-Gp(s)Cp(s)Tp(H) **32** (7.62 g, 91.0%) was isolated as a colourless glass following short column chromatography of the products; t_{R} 9.4, 9.8 min (programme B); δ_{P} [(CD₃)₂SO] 1.04 (1 P, d, J 584.0), 27.53, 27.60, 27.66, 28.01, 28.15 (2 P, m).

(c) **DMTr-Tp(s)Tp(s)Tp(H) 34.** This trimer H-phosphonate was prepared from DMTr-Tp(s)Tp(s)T-OH **24**; B = B' = B'' = **14**, (5.536 g, 3.64 mmol), following the procedure and stoichiometry described in (a) above for the preparation of the triethylammonium salt of DMTr-Cp(s)Tp(s)Tp(H) **26**. The triethylammonium salt DMTr-Tp(s)Tp(s)Tp(H) **34** (5.60 g, 91.2%) was isolated as a colourless glass following short column chromatography of the products; t_{R} 11.6 min (programme B); δ_{P} [(CD₃)₂SO] 1.23 (1 P, d, J 582.5), 27.91, 27.98, 28.01, 28.08 (2 P, m).

(d) **DMTr-Gp(s)Cp(s)Gp(H) 36.** This trimer H-phosphonate was prepared from DMTr-Gp(s)Cp(s)G-OH **24**; B = **10**; B' = B'' = **12** (7.44 g, 4.0 mmol), following the procedure and stoichiometry described in (a) above for the preparation of the triethylammonium salt of DMTr-Cp(s)Tp(s)Tp(H) **26**. The triethylammonium salt DMTr-Gp(s)Cp(s)Gp(H) **36** (7.36 g, 90.8%) was isolated as a colourless glass following short column chromatography of the products; t_{R} 9.5, 10.2 min (programme B); δ_{P} [(CD₃)₂SO] 1.12 (1 P, d, J 590.0), 27.48, 27.63, 27.76 (2 P, m).

HO-Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev 29

A solution of the triethylammonium salt of DMTr-Cp(s)Tp(s)Tp(H) **26** (4.24 g, 2.50 mmol, 1.25 mol equiv.) and HO-Gp(s)Cp(s)G-Lev **27** (3.31 g, 2.00 mmol, 1.00 mol equiv.) in dry pyridine (5 cm³) was evaporated under reduced pressure. The residue was redissolved in dry pyridine (23 cm³) and the solution was cooled to 0 °C (ice-water bath). Pivaloyl chloride (0.62 cm³, 5.0 mmol, 2.5 mol equiv.) was added dropwise over a period of 30 s to the stirred solution. After a further period of 30 min, finely powdered *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** (1.513 g, 8.2 mmol, 4.1 mol equiv.) was added. The reactants were allowed to warm up to room temperature over a period of 30 min. The products were then partitioned between dichloromethane (100 cm³) and saturated aqueous sodium hydrogen carbonate (100 cm³). The organic layer was separated, and the aqueous layer was back-extracted with dichloromethane (20 cm³). The combined organic layers were dried (MgSO₄) and concentrated under reduced pressure. The products were fractionated by short column chromatography on silica gel: elution of the column with dichloromethane-methanol (96 : 4 v/v) yielded an impurity [0.20 g, R_{f} 0.42 (system B)]; elution with dichloromethane-methanol (94 : 6 v/v) and evaporation of the combined appropriate fractions gave a colourless glass (6.25 g), R_{f} 0.37 (system B). This material (6.20 g) was dissolved in dichloromethane (19 cm³) and the stirred solution was cooled to 0 °C (ice-water bath). Pyrrole (2.6 cm³, 37.5 mmol) and dichloroacetic acid (2.0 cm³, 24.2 mmol) were then added. After 30 min, the products were partitioned between dichloromethane (50 cm³) and saturated aqueous sodium hydrogen carbonate (80 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (10 cm³). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane-methanol (91 : 9 v/v) were combined and evaporated under reduced pressure to give HO-Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev **29** (5.48 g, 91.5%) as a colourless glass; R_{f} 0.33 (system B); δ_{H} [(CD₃)₂SO] includes the following signals: 0.94 (12 H, m), 2.07 (6 H, m), 2.12 (3 H, s), 3.66 (2 H, m), 4.27–4.45 (17 H, m), 5.15 (4 H, m), 5.27 (1 H,

m), 5.51 (2 H, m), 6.18 (4 H, m), 6.45 (2 H, m), 7.13–7.74 (24 H, m), 7.97 (6 H, m), 8.17 (1 H, d, *J* 7.6), 8.35 (1 H, m), 8.50 (1 H, m), 8.56 (1 H, m), 10.32 (1 H, br), 10.36 (1 H, br s), 11.27 (2 H, br); δ_{P} [(CD₃)₂SO] 27.76, 27.81, 27.88, 27.96, 27.98, 28.04, 28.09, 28.16, 28.27, 28.35.

HO-[Cp(s)Tp(s)Tp(s)]₂Gp(s)Cp(s)G-Lev 31

The triethylammonium salt of DMTr-Cp(s)Tp(s)Tp(H) **26** (6.77 g, 3.99 mmol, 1.33 mol equiv.) and HO-Cp(s)Tp(s)-Tp(s)Gp(s)Cp(s)G-Lev **29** (9.05 g, 3.00 mmol, 1.00 mol equiv.) were coupled together in the presence of pivaloyl chloride (0.98 cm³, 7.96 mmol, 2.65 mol equiv.) in pyridine (45 cm³) at 0 °C as in the above preparation of the hexamer **29**. After 30 min, finely powdered *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** (2.96 g, 16.1 mmol, 5.36 mol equiv.) was added. The reaction was allowed to proceed for 1 h and the products were worked up as in the above preparation of the hexamer **29**. The products were then fractionated by short column chromatography on silica gel: elution of the column with dichloromethane–methanol (96 : 4 v/v) yielded an impurity [0.25 g, *R_f* 0.42 (system B)]; elution with dichloromethane–methanol (94 : 6 v/v) and evaporation of the combined appropriate fractions gave a colourless glass (12.90 g), *R_f* 0.36 (system B). Pyrrole (3.76 cm³, 54.2 mmol) and dichloroacetic acid (3.58 cm³, 43.4 mmol) were added to a stirred solution of this material (12.70 g) in dichloromethane (27 cm³) at 0 °C (ice–water bath) and the reaction was allowed to proceed for 40 min before it was worked up according to the procedure described above in the preparation of the partially-protected hexamer **29**. The products were fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (90 : 10 v/v), were combined and evaporated under reduced pressure to give HO-[Cp(s)Tp(s)Tp(s)]₂Gp(s)Cp(s)G-Lev **31** (11.70 g, 90.4%) as a colourless glass; *R_f* 0.30 (system B); δ_{H} [(CD₃)₂SO] includes the following signals: 0.94 (12 H, m), 2.07 (12 H, m), 2.12 (3 H, s), 4.11–4.43 (27 H, m), 5.23 (8 H, m), 5.51 (2 H, m), 6.18 (7 H, m), 6.45 (2 H, m), 7.13–7.74 (38 H, m), 7.97 (10 H, m), 8.17 (2 H, m), 8.34 (1 H, m), 8.49 (1 H, m), 8.56 (1 H, m), 10.33 (1 H, br), 10.37 (1 H, br. s), 11.27 (3 H, br); δ_{P} [(CD₃)₂SO] 27.77, 27.83, 27.89, 27.97, 28.03, 28.10, 28.18, 28.24, 28.30, 28.35.

HO-[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 33

The triethylammonium salt of DMTr-Cp(s)Tp(s)Tp(H) **26** (6.26 g, 3.69 mmol, 1.42 mol equiv.) and HO-[Cp(s)Tp(s)-Tp(s)]₂Gp(s)Cp(s)G-Lev **31** (11.37 g, 2.60 mmol, 1.00 mol equiv.) were coupled together in the presence of pivaloyl chloride (0.91 cm³, 7.4 mmol, 2.85 mol equiv.) in pyridine (47 cm³) solution at 0 °C as in the above preparation of the hexamer **29**. After 30 min, finely powdered *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** (3.09 g, 16.8 mmol, 6.47 mol equiv.) was added. The reaction was allowed to proceed for 1 h and the products were worked up as in the above preparation of the hexamer **29**. The products were then fractionated by short column chromatography on silica gel: elution with dichloromethane–methanol (93 : 7 v/v) and evaporation of the combined appropriate fractions gave a colourless glass (14.37 g), *R_f* 0.29 (system B). Pyrrole (3.17 cm³, 45.7 mmol) and dichloroacetic acid (3.80 cm³, 46.1 mmol) were added to a stirred solution of this material (13.80 g) in dichloromethane (23 cm³) at 0 °C (ice–water bath), and the reaction was allowed to proceed for 1 h before it was worked up according to the procedure described above in the preparation of the partially-protected hexamer **29**. The products were fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (90 : 10 v/v) were combined and evaporated under reduced pressure to give HO-[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev **33** (12.58 g, 88%) as a colourless glass; *R_f* 0.26 (system B); δ_{H} [(CD₃)₂SO] includes the

following signals: 0.94 (12 H, m), 2.06 (18 H, m), 2.12 (3 H, s), 3.66 (2 H, m), 4.26–4.43 (34 H, m), 5.19 (10 H, m), 5.28 (1 H, m), 5.51 (2 H, m), 6.19 (10 H, m), 6.45 (2 H, m), 7.11–7.74 (51 H, m), 7.98 (14 H, m), 8.17 (3 H, m), 8.34 (1 H, m), 8.49 (1 H, m), 8.56 (1 H, m), 10.32 (1 H, br), 10.36 (1 H, br s), 11.26 (4 H, br); δ_{P} [(CD₃)₂SO] 27.76, 27.83, 27.89, 27.96, 28.02, 28.10, 28.18, 28.25, 28.29, 28.35.

HO-Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 35

The triethylammonium salt of DMTr-Gp(s)Cp(s)Tp(H) **32** (5.457 g, 2.93 mmol, 1.53 mol equiv.) and HO-[Cp(s)Tp(s)-Tp(s)]₃Gp(s)Cp(s)G-Lev **33** (11.00 g, 1.92 mmol, 1.00 mol equiv.) were coupled together in the presence of pivaloyl chloride (0.72 cm³, 5.84 mmol, 3.05 mol equiv.) in pyridine (42 cm³) solution at 0 °C as in the above preparation of the hexamer **29**. After 30 min, finely powdered *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** (2.763 g, 15.0 mmol, 7.83 mol equiv.) was added. The reaction was allowed to proceed for 1 h and the products were worked up as in the above preparation of the hexamer **29**. The products were then fractionated by short column chromatography on silica gel: elution of the column with dichloromethane–methanol (96 : 4 v/v) yielded an impurity [0.35 g, *R_f* 0.43 (system B)]; elution with dichloromethane–methanol (93 : 7 v/v) and evaporation of the combined appropriate fractions gave a colourless glass (13.24 g), *R_f* 0.26 (system B). Pyrrole (2.38 cm³, 34.3 mmol) and dichloroacetic acid (2.86 cm³, 34.7 mmol) were added to a stirred solution of this material (13.00 g) in dichloromethane (17 cm³) at 0 °C (ice–water bath) and the reaction was allowed to proceed for 1 h before it was worked up according to the procedure described above in the preparation of the partially-protected hexamer **29**. The products were fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (90 : 10 v/v) were combined and evaporated under reduced pressure to give HO-Gp(s)Cp(s)Tp(s)[Cp(s)-Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev **35** (11.817 g, 86.5%) as a colourless glass; *R_f* 0.25 (system B); δ_{H} [(CD₃)₂SO] includes the following signals: 0.93 (18 H, m), 2.05 (21 H, m), 2.12 (3 H, s), 3.63 (2 H, m), 4.20–4.43 (43 H, m), 5.09–5.20 (12 H, m), 5.32 (1 H, m), 5.51 (2 H, m), 6.18 (12 H, m), 6.43 (3 H, m), 7.10–7.74 (64 H, m), 7.96 (17 H, m), 8.17 (5 H, m), 8.49 (1 H, m), 8.56 (2 H, m), 10.31 (2 H, br), 10.36 (1 H, s), 11.25 (5 H, br); δ_{P} [(CD₃)₂SO] 27.76, 27.86, 27.97, 28.02, 28.10, 28.26, 28.33, 28.36.

HO-Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃-Gp(s)Cp(s)G-Lev 37

The triethylammonium salt of DMTr-Tp(s)Tp(s)Tp(H) **34** (4.45 g, 2.64 mmol, 1.67 mol equiv.) and HO-Gp(s)Cp(s)-Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev **35** (11.50 g, 1.58 mmol, 1.00 mol equiv.) were coupled together in the presence of pivaloyl chloride (0.65 cm³, 5.28 mmol, 3.34 mol equiv.) in pyridine (38 cm³) solution at 0 °C as in the above preparation of the hexamer **29**. After 30 min, finely powdered *N*-[(2-cyanoethyl)sulfanyl]succinimide **19** (2.502 g, 13.58 mmol, 8.59 mol equiv.) was added. The reaction was allowed to proceed for 1 h and the products were worked up as in the above preparation of the hexamer **29**. The products were then fractionated by short column chromatography on silica gel: elution of the column with dichloromethane–methanol (93 : 7 v/v) and evaporation of the combined appropriate fractions gave a colourless glass (12.50 g), *R_f* 0.26 (system B). Pyrrole (1.83 cm³, 26.4 mmol) and dichloroacetic acid (2.20 cm³, 26.7 mmol) were added to a stirred solution of this material (12.00 g) in dichloromethane (14 cm³) at 0 °C (ice–water bath) and the reaction was allowed to proceed for 1 h before it was worked up according to the procedure described above in the preparation of the partially-protected hexamer **29**. The products were fractionated by short

column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (90 : 10 v/v) were combined and evaporated under reduced pressure to give HO-*Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 37* (11.01 g, 84%) as a colourless glass; R_f 0.26 (system B); δ_H [(CD₃)₂SO] includes the following signals: 0.93 (18 H, m), 2.05 (30 H, m), 2.12 (3 H, s), 3.66 (2 H, m), 4.20–4.50 (52 H, m), 5.17 (15 H, m), 5.32 (1 H, m), 5.51 (3 H, m), 6.18 (15 H, m), 6.45 (3 H, m), 7.10–7.74 (80 H, m), 7.94 (19 H, m), 8.16 (5 H, m), 8.49 (2 H, m), 8.56 (1 H, m), 10.31 (2 H, br), 10.36 (1 H, br. s), 11.25 (5 H, br); δ_P [(CD₃)₂SO] 27.77, 27.87, 27.93, 27.97, 28.02, 28.09, 28.26, 28.32.

DMTr-*Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)-[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 38*

The triethylammonium salt of DMTr-*Gp(s)Cp(s)Gp(H) 36* (4.65 g, 2.30 mmol, 1.84 mol equiv.) and HO-*Tp(s)Tp(s)Tp(s)-Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 37* (10.75 g, 1.25 mmol, 1.00 mol equiv.) were coupled together in the presence of pivaloyl chloride (0.57 cm³, 4.63 mmol, 3.71 mol equiv.) in pyridine (35 cm³) solution at 0 °C as in the above preparation of the hexamer **29**. After 30 min, finely powdered *N*-(2-cyanoethyl)sulfanyl succinimide **19** (2.303 g, 12.50 mmol, 10.0 mol equiv.) was added. The reaction was allowed to proceed for 1 h and the products were worked up as in the above preparation of the hexamer **29**. The products were then fractionated by short column chromatography on silica gel: elution of the column with dichloromethane–methanol (92 : 8 v/v) and evaporation of the combined appropriate fractions gave DMTr-*Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 38* as a colourless glass (11.86 g, 89.6%), R_f 0.27 (system B). δ_H [(CD₃)₂SO] includes the following signals: 0.85–0.95 (30 H, m), 2.05 (30 H, m), 2.12 (3 H, s), 3.68 (6 H, s), 4.2–4.6 (61 H, m), 5.19 (16 H, m), 5.5 (5 H, m), 6.18 (16 H, m), 6.46 (5 H, m), 6.69 (4 H, m), 7.12–7.74 (98 H, m), 7.96 (22 H, m), 8.17 (6 H, m), 8.48 (4 H, m), 8.56 (1 H, m), 10.18 (1 H, br. s), 10.31 (2 H, br), 10.36 (2 H, br), 11.25 (6 H, br); δ_P [(CD₃)₂SO] 27.60, 27.66, 27.76, 27.82, 27.97, 28.02, 28.10, 28.26, 28.31.

Acetylation of partially-protected oligonucleotide phosphorothioates with free 5'-hydroxy functions

Substrate (e.g. **29**; ca. 0.02–0.06 mmol) was dissolved in dry pyridine (1 cm³) and the solution was evaporated under reduced pressure. After this procedure had been repeated, the residue was dissolved in pyridine (2 cm³) and acetic anhydride (10–25 mol equiv. with respect to substrate) was added to the stirred solution at room temperature. After 16 h, methanol (0.3 cm³) was added and, after 10 min, the products were partitioned between dichloromethane (15 cm³) and saturated aqueous sodium hydrogen carbonate (15 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (5 cm³). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (93 : 7 to 92 : 8 v/v) were combined and evaporated under reduced pressure.

(a) HO-*Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev 29* (0.150 g, 0.05 mmol) was treated with acetic anhydride (0.047 cm³, 0.50 mmol) to give Ac-*Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev 39* (0.141 g, 93%) as a colourless froth.

(b) HO-*[Cp(s)Tp(s)Tp(s)]₂Gp(s)Cp(s)G-Lev 31* (0.250 g, 0.057 mmol) was treated with acetic anhydride (0.054 cm³, 0.57 mmol) to give Ac-*[Cp(s)Tp(s)Tp(s)]₂Gp(s)Cp(s)G-Lev 32* (0.240 g, 95%) as a colourless froth.

(c) HO-*[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 33* (0.200 g, 0.035 mmol) was treated with acetic anhydride (0.066 cm³, 0.70

mmol) to give Ac-*[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 34* (0.189 g, 94%) as a colourless froth.

(d) HO-*Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 35* (0.200 g, 0.028 mmol) was treated with acetic anhydride (0.052 cm³, 0.55 mmol) to give Ac-*Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 36* (0.188 g, 93.5%) as a colourless froth.

(e) HO-*Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 37* (0.200 g, 0.023 mmol) was treated with acetic anhydride (0.044 cm³, 0.47 mmol) to give Ac-*Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 38* (0.185 g, 92%) as a colourless froth.

Complete unblocking of 5'-acetylated fully-protected oligonucleotide phosphorothioates

Substrate (e.g. **39**; ca. 0.005–0.02 mmol) was dissolved in dry pyridine (1 cm³) and the solution was evaporated under reduced pressure. After this procedure had been repeated once more, dry acetonitrile (1.0 cm³) was added to the residue. Chlorotrimethylsilane (ca. 0.025 cm³) and DBU (9 mol equiv. per phosphorothioate triester linkage) were then added and the resulting solution was stirred at room temperature. After 30 min, the reaction solution was concentrated under reduced pressure. The residue was dissolved in acetonitrile (1.5–2.0 cm³) at room temperature and *E*-2-nitrobenzaloxime **7** (ca. 5 mol equiv. per phosphorothioate triester linkage) was added to the stirred solution. After 12 h, the products were evaporated under reduced pressure. Concentrated aqueous ammonia (*d* 0.88, 1.1–1.2 cm³) and 2-mercaptoethanol (0.10 cm³) were added and the resulting mixture was heated at 55 °C for 15 h. The cooled products were evaporated under reduced pressure and the residue was co-evaporated with ethanol (2 × 2 cm³). The residue was then dissolved in methanol (2 cm³) and ethyl acetate (30 cm³) was added. After centrifugation, the supernatant was discarded. This process was repeated once more and the precipitate was kept *in vacuo* to remove the retained solvent. The precipitate was dissolved in water (2 cm³) and the solution was applied to a column (5 cm × 2 cm diameter) of Dowex 50 HCR W2 (Na⁺ form) cation-exchange resin. The column was eluted with water, and the appropriate fractions were combined and concentrated (to ca. 3 cm³) under reduced pressure. The concentrated solution was freeze-dried to give the sodium salt of the fully-unblocked oligonucleotide phosphorothioate.

(a) Ac-*Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G-Lev 39* (0.050 g, 0.016 mmol) was converted into the sodium salt of d[Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G] **41** (0.027 g, 84%); δ_H [D₂O] includes the following signals: 1.7–2.7 (18 H, m), 3.65–4.3 (18 H, m), 5.75–6.2 (8 H, m), 7.45–7.95 (6 H, m); δ_P [D₂O] 56.70.

(b) Ac-*[Cp(s)Tp(s)Tp(s)]₂Gp(s)Cp(s)G-Lev 31* (0.050 g, 0.011 mmol) was converted into the sodium salt of d[Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G] **42** (0.029 g, 86%); δ_H [D₂O] includes the following signals: 1.75–2.8 (30 H, m), 3.7–4.3 (27 H, m), 5.85–6.2 (12 H, m), 7.45–7.95 (9 H, m); δ_P [D₂O] 56.30, 56.39, 56.47, 56.64, 56.77, 56.89.

(c) Ac-*[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 33* (0.060 g, 0.010 mmol) was converted into the sodium salt of d[Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Gp(s)Cp(s)G] **43** (0.037 g, 89%); δ_H [D₂O] includes the following signals: 1.7–2.8 (42 H, m), 3.7–4.4 (36 H, m), 5.8–6.3 (16 H, m), 7.45–8.0 (12 H, m); δ_P [D₂O] 56.58, 56.83.

(d) Ac-*Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 35* (0.050 g, 0.0068 mmol) was converted into the sodium salt of d[Gp(s)Cp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Cp(s)G] **44** (0.029 g, 84%); δ_H [D₂O] includes the following signals: 1.7–2.8 (51 H, m), 3.7–4.4 (45 H, m), 5.75–6.25 (20 H, m), 7.45–8.0 (15 H, m); δ_P [D₂O] 56.50, 56.56, 56.58, 56.71, 56.80.

(e) Ac-*Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev 37* (0.050 g, 0.0058 mmol) was

converted into the sodium salt of d[Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)Tp(s)Tp(s)Gp(s)Cp(s)G] **45** (0.029 g, 83%); δ_{H} [D₂O] includes the following signals: 1.65–2.8 (66 H, m), 3.65–4.4 (54 H, m), 5.7–6.25 (23 H, m), 7.45–8.05 (18 H, m); δ_{P} [D₂O] 56.30, 56.39, 56.51, 56.61.

Complete unblocking of fully-protected Vitravene **38**

Pyrrole (0.29 cm³, 4.18 mmol) and dichloroacetic acid (0.18 cm³, 2.18 mmol) were added to a cooled (ice–water bath), stirred solution of DMTr-Gp(s)Cp(s)Gp(s)Tp(s)Tp(s)Tp(s)-Gp(s)Cp(s)Tp(s)[Cp(s)Tp(s)Tp(s)]₃Gp(s)Cp(s)G-Lev **38** (1.500 g, 0.141 mmol) in dichloromethane (5 cm³). After 1 h, the products were partitioned between dichloromethane (35 cm³) and saturated aqueous sodium hydrogen carbonate (30 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (10 cm³). The organic layers were combined, dried (MgSO₄) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (91 : 9 v/v), were combined and evaporated under reduced pressure to give a colourless glass (1.383 g). A solution of this material (1.30 g) in dry pyridine (3 cm³) was evaporated under reduced pressure. After this procedure had been repeated once more, the residue was dissolved in dry pyridine (5 cm³). Acetic anhydride (0.12 cm³, 1.3 mmol) was then added to the stirred solution at room temperature. After 16 h, methanol (2 cm³) was added and, after a further period of 10 min, the products were partitioned between dichloromethane (40 cm³) and saturated aqueous sodium hydrogen carbonate (40 cm³). The layers were separated and the aqueous layer was back-extracted with dichloromethane (10 cm³). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–methanol (91 : 9 v/v) were pooled and concentrated under reduced pressure to give a colourless froth (1.186 g).

A solution of this material (1.00 g) in dry pyridine (5 cm³) was evaporated under reduced pressure. After this procedure had been repeated once more, dry acetonitrile (15 cm³) was added to the residue. Chlorotrimethylsilane (0.52 cm³, 4.1 mmol) and then DBU (2.60 cm³, 17.4 mmol) were added to the stirred mixture at room temperature. After 30 min, the products were concentrated under reduced pressure. The residue was dissolved in dry acetonitrile (15 cm³) at room temperature and *E*-2-nitrobenzaloxime **7** (3.211 g, 19.3 mmol) was added to the stirred solution. After 15 h, the products were evaporated under reduced pressure. A mixture of the residue, 2-mercaptoethanol (2.0 cm³) and concentrated aqueous ammonia (*d* 0.88, 18 cm³) was heated at 55 °C. After 15 h, the products were cooled and evaporated under reduced pressure. Absolute ethanol (5 cm³) was added and the resulting mixture was evaporated to dryness. After this procedure had been repeated twice more, the residue was dissolved with the aid of sonification in methanol (20 cm³)

and ethyl acetate (200 cm³) was added to the solution. The resulting precipitate was collected by centrifugation. This precipitation procedure was repeated once more and the solid obtained was dried *in vacuo*. A solution of this material in water (15 cm³) was passed through a column (6 cm × 4 cm diameter) of Dowex 50 HCR W2 (Na⁺ form) cation exchange resin. The appropriate fractions were pooled and concentrated under reduced pressure to *ca.* 15 cm³. The resulting solution was freeze-dried to give the sodium salt of d[Gp(s)Cp(s)Gp(s)Tp(s)-Tp(s)Tp(s)Gp(s)Cp(s)Tp(s)Cp(s)Tp(s)Tp(s)Cp(s)Tp(s)-Tp(s)-Cp(s)Tp(s)Tp(s)Gp(s)Cp(s)G] (Vitravene) as an off-white froth (0.61 g, 76% overall yield); δ_{H} [D₂O] includes the following signals: 1.65–2.85 (72 H, m), 3.65–4.2 (61 H, m), 5.8–6.25 (26 H, m), 7.4–8.15 (21 H, m); δ_{P} [D₂O] 55.99, 56.44, 56.69.

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