Synthesis of analogues of oligonucleotides; synthesis of unprotected *C*-linked di- and tri-nucleotides

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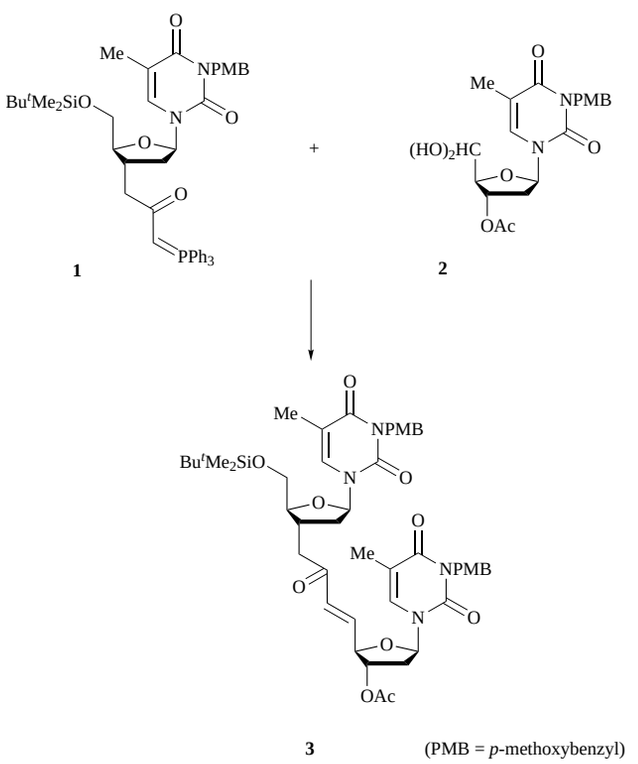
The Wittig reaction between the *N*-benzyloxymethylthymidine-derived ylide 25 and the aldehyde 16 followed by hydrogenolysis gives access to the unprotected *C*-linked dinucleotide 20 on a multi-gram scale. Oxidation of the dinucleotide gives the aldehyde 27 which is condensed with the ylide 25 to give the unprotected bis-*C*-linked trinucleotide 29 after hydrogenolysis. The mono-*C*-linked trinucleotide 44 is prepared by oxidation of the dinucleotide ester 34 to the aldehyde 36 which is condensed with the ylide 25 followed by hydrogenolysis to give the mono-*C*-linked trinucleotide ester 38. This intermediate is also prepared from the *C*-linked dinucleotide 20 by conversion into the phosphoramidite 41 which is coupled with 3-acetylthymidine 15 to give the mono-*C*-linked trinucleotide phosphite 42. Oxidation and deprotection give the phosphate triester 38. Treatment of 38 with methanolic ammonia gives the fully unprotected mono-*C*-linked trinucleotide 44.

The synthesis of non-polar analogues of oligonucleotides is an area of considerable interest at present with analogues in which the phosphate residues have been replaced by isosteric fragments which are more stable to nuclease enzymes being of particular interest in the context of the development of anti-sense oligonucleotides for the control of gene transcription.[1] In the preceding paper,[2] a synthesis of the *N*-protected *C*-linked dinucleotide **3** using the Wittig reaction between the thymidine-derived, stabilized ylide **1** and the 5′-aldehyde hydrate **2**, is described. This reaction provides efficient access to the *C*-linked dinucleotide but attempts to remove the *p*-methoxybenzyl groups were unsuccessful.[3] We now report further developments of this chemistry which have led to the synthesis of unprotected *C*-linked di- and tri-nucleotides.

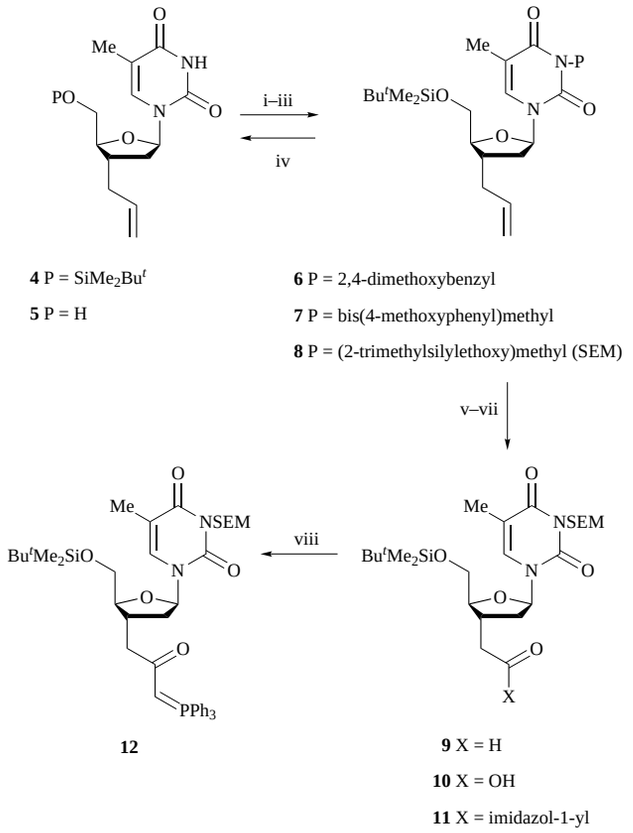


Results and discussion

The first objective was the development of an *N*-protecting group for the 3′-deoxythymidine-derived ylide which could be efficiently removed after the Wittig reaction. To this end the 3′-propenyl-3′-deoxythymidine **4**[4] was converted into its 2,4-dimethoxybenzyl-, 4,4′-dimethoxybenzhydryl- and (2-trimethylsilylethoxy)methyl derivatives **6**–**8** (Scheme 1).[5,6] Oxidative cleavage of the 2,4-dimethoxybenzyl derivative **6** using dichlorodicyanoquinone or ceric ammonium nitrate was unsuccessful and the dimethoxybenzhydryl derivative **7** was found to be somewhat unstable to handling. However, the (2-trimethylsilylethoxy)methyl compound **8** gave 3′-propenyl-3′-deoxythymidine **5** on treatment with tetrabutylammonium fluoride, and was taken through to the ylide **12** by oxidative cleavage using osmium tetraoxide[7] and sodium periodate which gave the aldehyde **9**. Further oxidation then gave the acid **10** which was converted into the ylide **12** by reaction of its imidazolide **11** with an excess of methylene(triphenyl)phosphorane.[2]

The literature precedent would suggest that it is not necessary to *N*-protect the thymidine-5′-aldehyde before the Wittig reaction.[8] To check this before proceeding with the synthesis of a *C*-linked dinucleotide, the 5′-aldehyde **16** was generated by oxidation of 3′-acetylthymidine **15** under Pfizner–Moffatt conditions[9] and treated without purification with the ylide **14** prepared from the imidazolide of 2-methylpropanoic acid (Scheme 2). A good yield (82%) of the (*E*)-unsaturated ketone **17** was isolated so confirming that *N*-protection of the thymidine-derived aldehyde is not necessary for the Wittig step.

The ylide **12** was then added to the aldehyde **16** which had been freshly prepared but not purified, and the reaction mixture was stirred at room temperature for 28 h. This procedure gave the *C*-linked dinucleotide **18** in a 78% yield based on 3′-acetylthymidine **15**. However, attempts to remove the (2-trimethylsilyloxy)methyl substituent from the Wittig product **18** using tetrabutylammonium fluoride, following the procedure which had been successful for the mononucleoside **8**, led to decomposition, perhaps because of susceptibility of the α,β-unsaturated ketone towards nucleophilic attack. The double bond was therefore removed by hydrogenation which was accompanied, perhaps surprisingly, by cleavage of the 5′-*tert*-butyldimethylsilyl group, to give the saturated *C*-linked dinucleotide **19**. Attempts to remove the *N*-(2-trimethylsilylethoxy)methyl substituent from **19** using tetrabutylammonium fluoride were also unsuccessful with the starting material being recovered, but treatment with dilute aqueous

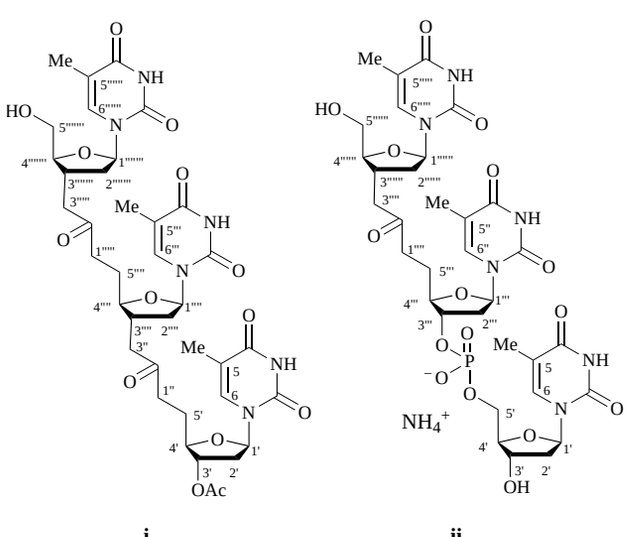


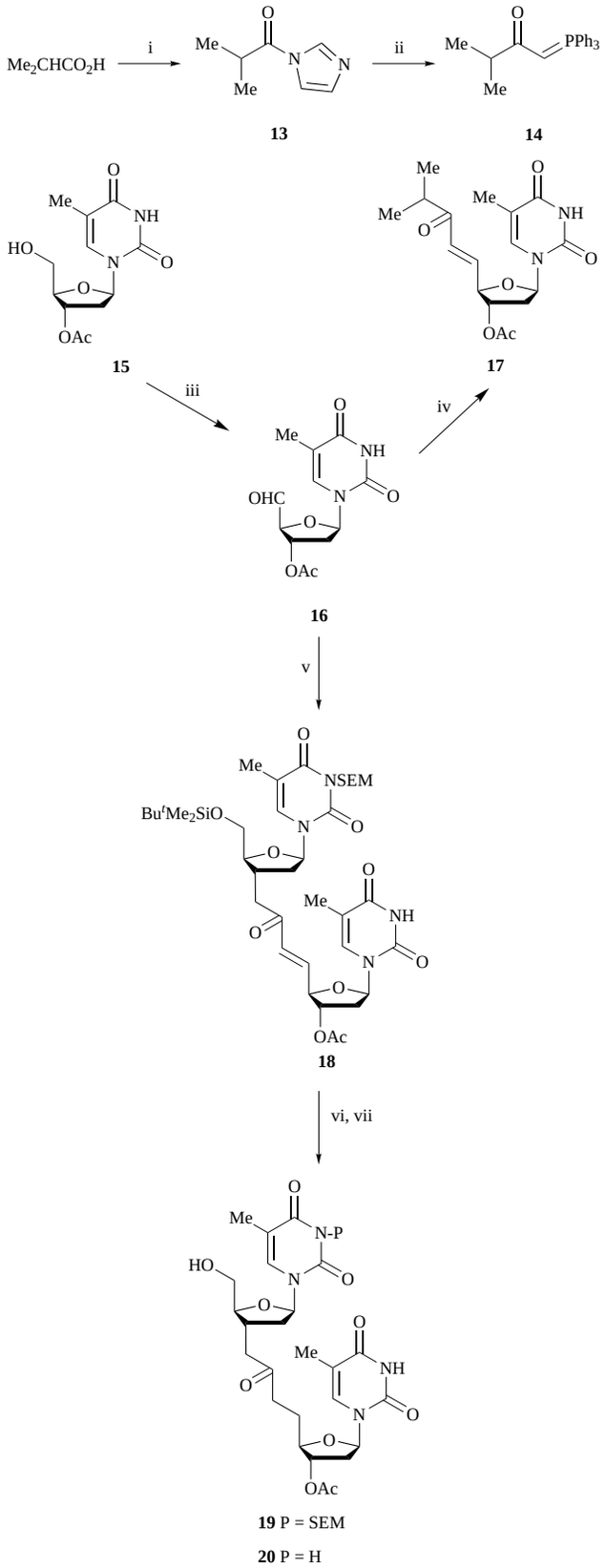
Scheme 1 *Reagents and conditions*: i, 2,4-$(MeO)_2C_6H_3CH_2OH$, Ph_3P, $EtO_2CN{=}NCO_2Et$, dioxane (43%); ii, $(4\text{-}MeOC_6H_4)_2CHOH$, Ph_3P, $EtO_2CN{=}NCO_2Et$, dioxane (55%); iii, $Me_3SiCH_2CH_2OCH_2Cl$, Pr^i_2NEt, CH_2Cl_2 (55%); iv, Bu_4NF, tetrahydrofuran; v, osmium tetraoxide (cat.), sodium periodate, dioxane (56%); vi, 2-methylbut-2-ene, sodium chlorite, sodium dihydrogen orthophosphate, *tert*-butyl alcohol, water (100%); vii, $CO(imid.)_2$, tetrahydrofuran (100%); viii, $Ph_3P{=}CH_2$, tetrahydrofuran (25%)

hydrogen fluoride gave the required deprotected *C*-linked dinucleotide 3′-acetate **20** in an excellent yield. This compound was fully characterized spectroscopically, an accurate mass measurement confirming its molecular weight.†

The reactions outlined in Scheme 2 had achieved our object-

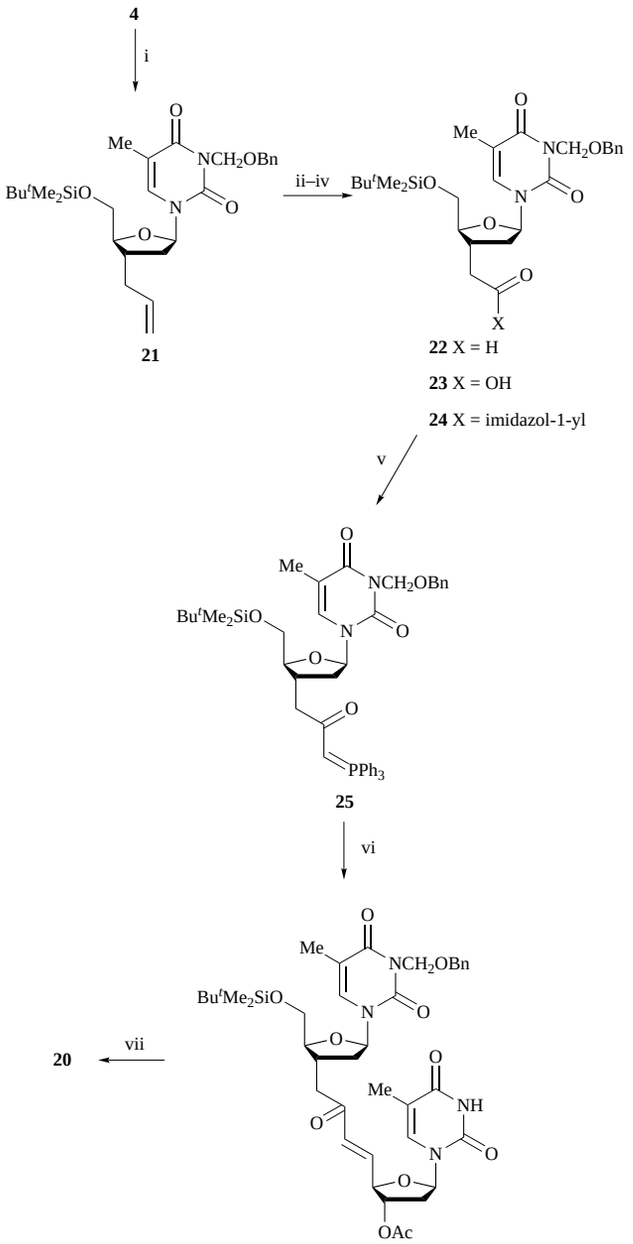
† The *C*-linked di- and tri-nucleotides prepared in this paper were named as thymidine derivatives to illustrate their structural homology with parent oligodeoxyribonucleotides. The numbering scheme used for the *C*-linked dinucleotides is outlined in the previous paper (see ref. 2). That used for numbering the mono- and bis-*C*-linked trinucleotides is illustrated in **i** and **ii** below.





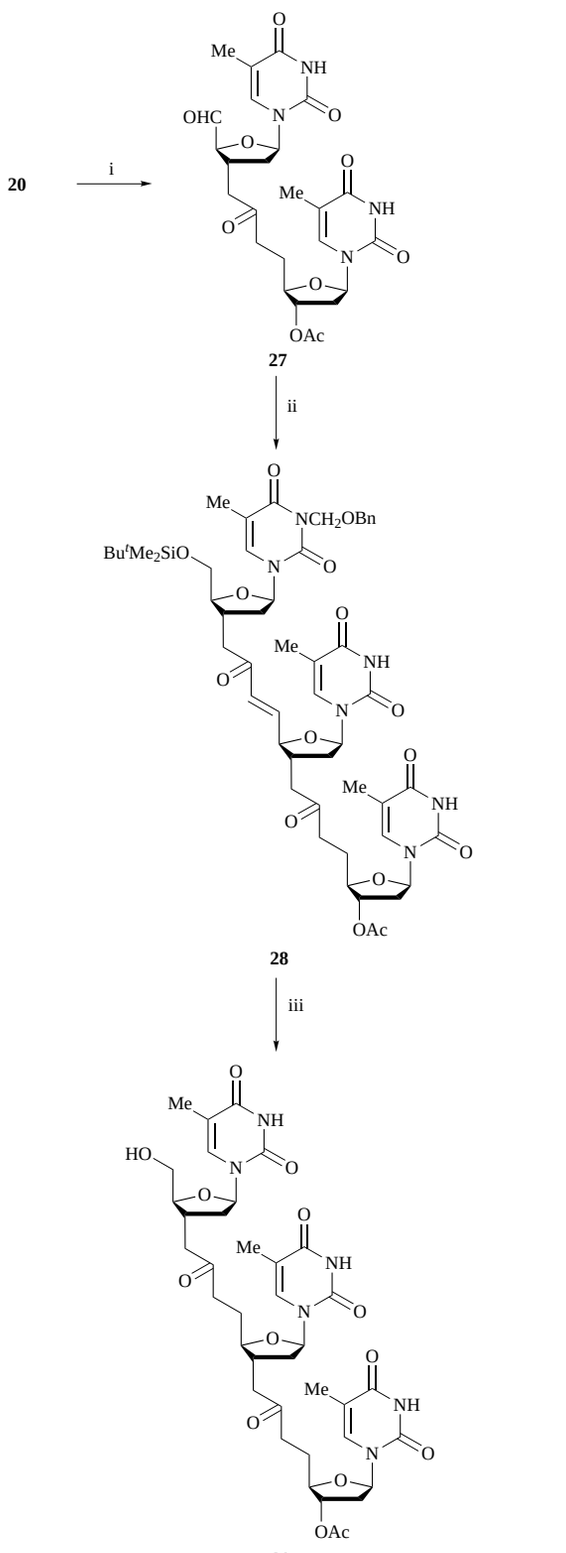
Scheme 2 *Reagents and conditions*: i, $CO(imid.)_2$, tetrahydrofuran (100%); ii, $Ph_3P{=}CH_2$, tetrahydrofuran (72%); iii, dicyclohexylcarbodiimide, Cl_2CHCO_2H, dimethyl sulfoxide; iv, **14**, pyridine, 48 h, rt (82% from **15**); v, **12**, pyridine, 28 h, rt (78% from **15**); vi, 10% Pd/C, methanol, H_2, 18 h (76%); vii, aq. HF, MeCN, 5 h, rt (90%)

ive of preparing the *N*-deprotected *C*-linked dinucleotide **20**. However, in view of the difficulties experienced during the removal of the *N*-(2-trimethylsilylethoxy)methyl substituent, it was decided to investigate alternative *N*-protecting groups before proceeding with syntheses of more complex systems.



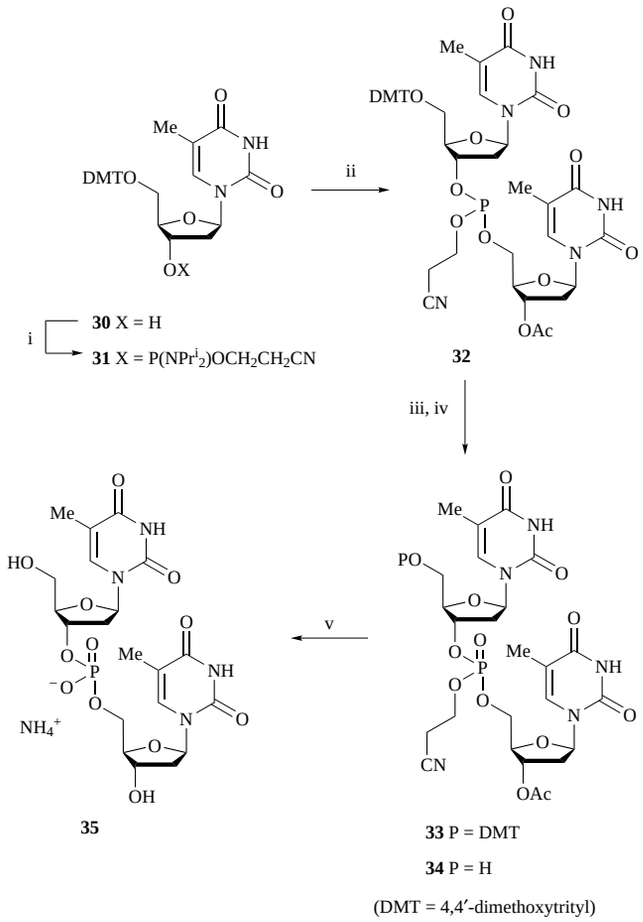
Scheme 3 *Reagents and conditions*: i, Pr^i_2NEt, $BnOCH_2Cl$, CH_2Cl_2, 16 h, rt (100%); ii, osmium tetraoxide (cat.), sodium periodate, aq. dioxane (46%); iii, 2-methylbut-2-ene, sodium chlorite, sodium dihydrogen orthophosphate, *tert*-butyl alcohol, water (100%); iv, $CO(imid.)_2$, tetrahydrofuran (100%); v, $Ph_3P{=}CH_2$, tetrahydrofuran (41%); vi, **16**, Cl_2CHCO_2H, dicyclohexylcarbodiimide, dimethyl sulfoxide, 1 h, then **25**, tetrahydrofuran, pyridine, 28 h, rt (67%); vii, 20% palladium hydroxide on carbon, H_2, methanol (60%)

The 3′-propenyl-3′-deoxythymidine **4** was converted into its *N*-benzyloxymethyl derivative **21** (Scheme 3). This was converted into the ylide **25** by oxidative cleavage to the aldehyde **22**, further oxidation to the carboxylic acid **23**, and treatment of the corresponding acyl imidazolide **24** with two mole equivalents of methylene(triphenyl)phosphorane.[2] The ylide **25** was obtained in an overall yield of 41% from the aldehyde **22** and gave a good yield of the *C*-linked dinucleotide **26** on coupling with the 5′-aldehyde **16**. Moreover, hydrogenation of **26** was accompanied by concomitant hydrogenolysis of the benzyloxymethyl group together with cleavage of the *tert*-butyldimethylsilyl group and gave the 3′-acetyl deprotected *C*-linked dinucleotide **20** in a single deprotection step. As gram quantities of the *C*-linked dinucleotide **20** were now available, it was possible to study aspects of its chemistry.

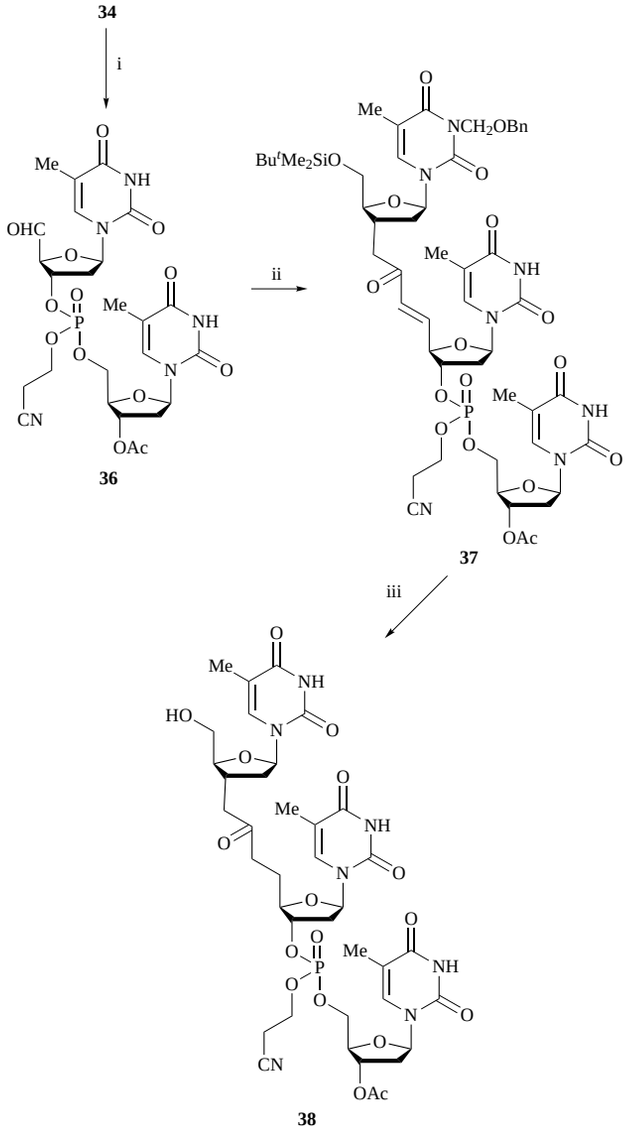


Scheme 4 *Reagents and conditions*: i, Cl_2CHCO_2H, dicyclohexylcarbodiimide, dimethyl sulfoxide; ii, **25**, pyridine, tetrahydrofuran, 24 h, rt (51% from **20**); iii, 20% palladium hydroxide on carbon, H_2, methanol (51%)

To show that the Wittig procedure could be used to prepare analogues of higher nucleotides, the *C*-linked dinucleotide **20** was oxidized to the aldehyde **27** (Scheme 4). This was coupled with the ylide **25** to give the bis-*C*-linked trinucleotide **28**. As before, hydrogenation, hydrogenolysis and desilylation using Pearlman's catalyst under an atmosphere of hydrogen gave the 3′-acetyl bis-*C*-linked trinucleotide **29** in a single deprotection step. The trinucleotides **28** and **29** were fully characterized by



Scheme 5 *Reagents and conditions*: i, Pr^i_2NEt, tetrazole, $(Pr^i_2N)_2POCH_2CH_2CN$, CH_2Cl_2, 16 h, rt (95%); ii, **15**, tetrazole, tetrahydrofuran (87%); iii, iodine, tetrahydrofuran, pyridine, water (98%); iv, Cl_2CHCO_2H, CH_2Cl_2, 2 h, rt (76%); v, NH_3, methanol (100%)



Scheme 6 *Reagents and conditions*: i, dicyclohexylcarbodiimide, Cl_2CHCO_2H, dimethyl sulfoxide; ii, **25**, pyridine, tetrahydrofuran, 24 h, rt (62% from **34**); iii, 20% palladium hydroxide on carbon, H_2, methanol

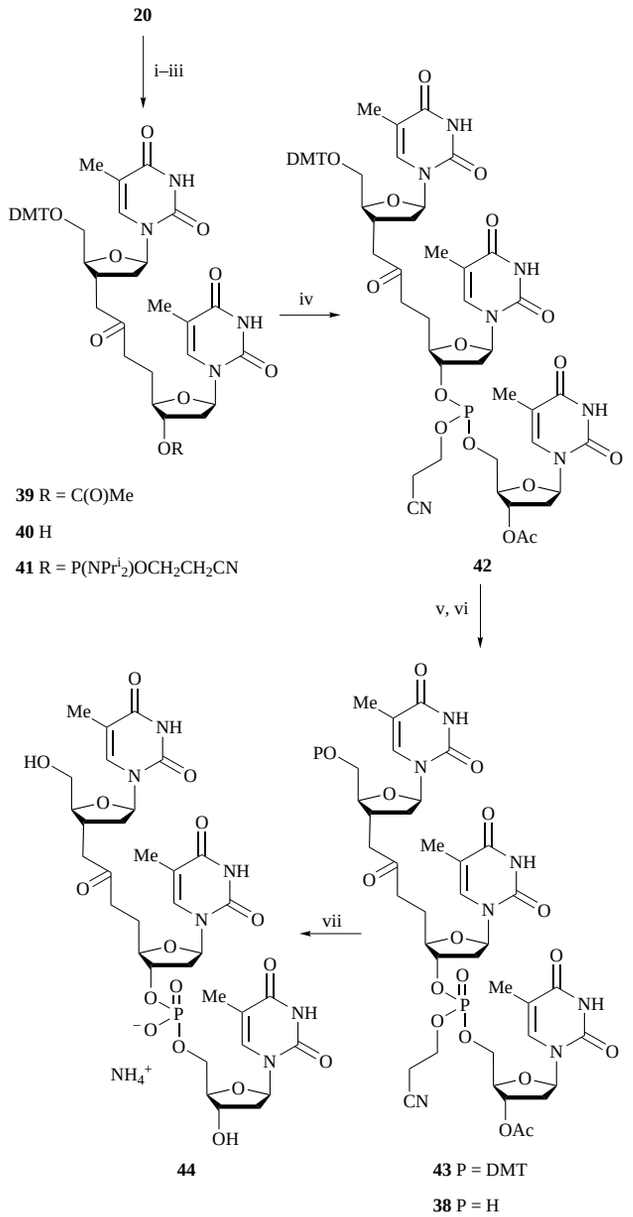
spectroscopic methods with NMR assignments being made by comparison with spectra of simpler compounds.†

It was decided to study the synthesis of a trinucleotide containing one phosphate link and one carbon link between the nucleosides in anticipation of the incorporation of *C*-linked nucleotides into oligonucleotide chains. Two strategies were envisaged for the synthesis of the mixed phosphate-*C*-linked trinucleotide, namely the conversion of a *C*-linked dinucleotide into a monophosphate containing trinucleotide using conventional methods for nucleotide synthesis, and the conversion of a dinucleotide derivative into its 5′-aldehyde followed by a Wittig condensation with the ylide **25**. Both of these approaches were studied using solution phase chemistry with the products fully characterized by spectroscopic methods.† However, it may well be that the methods developed will also be suitable for solid-phase synthesis of oligonucleotide analogues.

Using standard conditions, 5′-dimethoxytritylthymidine **30** was converted into the phosphoramidite **31** which was isolated as a mixture of epimers at phosphorus (Scheme 5).[10,11] The phosphoramidite was condensed with 3′-acetylthymidine **15** to give the dinucleotide phosphite triester **32** which was oxidized using iodine to give the phosphate triester **33**. Treatment with dichloroacetic acid then removed the dimethoxytrityl group to give the dinucleotide triester **34**. The ^{1}H NMR spectra of the phosphite and phosphate triesters **32**–**34** were complicated by the presence of two diastereoisomers due to the chirality at phosphorus. However, ^{1}H–^{13}C HETCOR NMR spectroscopy enabled assignments to be made to the signals due to the 3′- and 3‴-protons in the two deoxyribose rings, and the remaining assignments followed from the application of 2D-NMR techniques. Treatment with methanolic ammonia gave the dinucleotide **35** which had a considerably simplified ^{1}H NMR spectrum due to the loss of chirality at phosphorus.

Oxidation of the phosphate triester **34** using Pfizner–Moffatt conditions gave the aldehyde **36** (Scheme 6). This was separated from the dicyclohexylurea formed during the oxidation by filtration and added to the ylide **25** without any further purification. Following this procedure, the protected mono-*C*-linked trinucleotide **37** was isolated in a 62% yield based on the alcohol **34**. This result shows that the Wittig procedure is compatible with the presence of phosphate triester functionality in the aldehyde component and so it should be possible to add a *C*-linked dinucleotide unit to the 5′-end of a growing oligonucleotide chain. Simultaneous hydrogenation, hydrogenolysis and desilylation gave the 3′-acetyl mono-*C*-linked trinucleotide ester **38**.

The mono-*C*-linked trinucleotide **38** was also prepared from the *C*-linked dinucleotide **20** which was protected as its dimethoxytrityl derivative **39** and saponified to the 3′-alcohol **40** (Scheme 7). This was converted into the phosphoramidite **41** which was coupled with 3′-acetylthymidine **15** to give the phosphite **42**. Oxidation then gave phosphate **43** which was treated with dichloroacetic acid to remove the dimethoxytrityl group to give the mono-*C*-linked trinucleotide ester **38** which had spectroscopic and chromatographic properties identical to



Scheme 7 *Reagents and conditions*: i, Pr^i_2NEt, 4,4′-dimethoxytrityl chloride, 4-dimethylaminopyridine, CH_2Cl_2, 4 h, rt (95%); ii, NH_3, methanol (81%); iii, Pr^i_2NEt, tetrazole, $(Pr^iN)_2POCH_2CH_2CN$, CH_2Cl_2 (90%); iv, **15**, tetrazole, CH_2Cl_2, 18 h, rt (86%); v, iodine, tetrahydrofuran, pyridine, water, 2 h (90%); vi, Cl_2CHCO_2H, CH_2Cl_2 (73%); vii, NH_3, methanol (100%)

the sample prepared by the deprotection of the Wittig product **37**.

The acetyl and cyanoethyl substituents in **38** were then cleaved using methanolic ammonia to give the fully unprotected mono-*C*-linked trinucleotide **44**. The structure of this product was fully supported by spectroscopic data. Stereochemical assignments were made on the basis of the known structures of the starting materials.[2] At no point in this work was any proliferation of stereoisomers observed, *e.g.* due to the epimerisation of the ribose fragments at C(1).†

Conclusions

This work has shown that the Wittig reaction between a 5′-nucleoside aldehyde and a 3′-ketophosphorane provides efficient and flexible access to *C*-linked oligodeoxyribonucleotides.[12] This chemistry can be used for the preparation of fully deprotected *C*-linked oligonucleotides on multi-gram scales using benzyloxycarbonyl groups for *N*-protection of the thymine rings. It has been shown that the Wittig reaction can be carried out on a 5′-aldehyde prepared from a preformed dinucleotide and so it should be possible to incorporate all-carbon linkages into growing oligonucleotide chains. Conversely, it has been shown that a *C*-linked dinucleotide can be incorporated into the phosphotriester approach to oligonucleotide synthesis. Further work will develop procedures for the incorporation of nucleosides other than thymidine into *C*-linked oligodeoxyribonucleotides for hybridization and other biological investigations.

Experimental

For general experimental details see the preceding paper. The acyl imidazolide **13** (750 mg, 100%) was prepared by treatment of 2-methylpropanoic acid (0.5 cm^3, 5.4 mmol) with carbonyl diimidazole. Conversion to 3-methyl-1-(triphenylphosphoranylidene)butan-2-one **14**,[13] mp 172 °C (lit.,[13] 172–175 °C) was effected using an excess of methylene(triphenyl)phosphorane generated in tetrahydrofuran from methyl(triphenyl)phosphonium bromide and *n*-butyllithium.

(3′*S*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3-(2,4-dimethoxybenzyl)-3′-(prop-2-enyl)-3′-deoxythymidine 6

2,4-Dimethoxybenzyl alcohol (56 mg, 0.33 mmol) in dioxane (1 cm^3), triphenylphosphine (87 mg, 0.33 mmol) and diethyl azodicarboxylate (0.041 cm^3, 0.33 mmol) were added to a solution of the 3′-propenylnucleoside **4**[4] (106 mg, 0.27 mmol) in dioxane (1 cm^3). The reaction mixture was stirred for 15 h, then concentrated under reduced pressure. Chromatography of the residue using light petroleum–ethyl acetate (8:1) as eluent gave the *title compound* **6** (63 mg, 43%), $[a]_D$ +17.7 (*c* 0.88 in $CHCl_3$) (Found: $M^+ + H$, 531.2875. $C_{28}H_{43}N_2O_6Si$ requires *M*, 531.2890); ν_{max}/cm^{-1} 1704, 1667, 1646, 1615, 1591, 1466, 1261, 1210, 1158 and 836; δ_H 0.16 (6 H, s, Me_2Si), 0.97 (9 H, s, $SiCMe_3$), 1.99 (3 H, s, 5-Me), 2.10–2.40 (5 H, overlapping m, 2′-H_2, 3′-H and 1″-H_2), 3.80 (5 H, m, OMe, 4′-H and 5′-H), 3.85 (3 H, s, OMe), 4.05 (1 H, dd, *J* 3, 11, 5′-H′), 5.10 (4 H, m, 3″-H_2 and 3-CH_2), 5.80 (1 H, m, 2″-H), 6.15 (1 H, t, *J* 5.5, 1′-H), 6.45 (2 H, m, ArH), 6.9 (1 H, m, ArH) and 7.6 (1 H, s, 6-H); *m*/*z* (CI) 531 ($M^+ + 1$, 100%).

(3′*S*)-3-[Bis(4-methoxyphenyl)methyl]-5′-*O*-(*tert*-butyldimethylsilyl)-3′-(prop-2-enyl)-3′-deoxythymidine 7

4,4′-Dimethoxybenzhydrol (60 mg, 0.25 mmol) in dioxane (1 cm^3), triphenylphosphine (64 mg, 0.25 mmol) and diethyl azodicarboxylate (0.03 cm^3, 0.25 mmol) were added to a solution of the 3′-propenylnucleoside **4** (77.7 mg, 0.2 mmol) in dioxane (1 cm^3). After being stirred for 24 h, the reaction mixture was concentrated under reduced pressure. Chromatography of the residue using light petroleum–ethyl acetate (8:1) as eluent gave the *title compound* **7** (68 mg, 55%), $[a]_D$ +18.5 (*c* 0.33 in $CHCl_3$) (Found: $M^+ + H$, 607.3181. $C_{34}H_{47}N_2O_6Si$ requires *M*, 607.3125); ν_{max}/cm^{-1} 1704, 1667, 1646, 1611, 1514, 1463, 1443, 1250, 1178, 1110, 1036 and 837; δ_H 0.15 (6 H, s, Me_2Si), 0.95 (9 H, s, $SiCMe_3$), 1.95 (3 H, s, 5-Me), 2.10–2.40 (5 H, overlapping m, 2′-H_2, 3′-H and 1″-H_2), 3.75 (2 H, m, 4′-H and 5′-H), 3.82 (6 H, s, 2 × OMe), 4.0 (1 H, dd, *J* 3, 11, 5′-H′), 4.30 (1 H, s, 3-CH), 5.10 (2 H, m, 3″-H_2), 5.8 (1 H, m, 2″-H), 6.10 (1 H, t, *J* 6, 1′-H), 6.85 (4 H, m, ArH), 7.30 (4 H, m, ArH) and 7.60 (1 H, s, 6-H); *m*/*z* (CI) 607 ($M^+ + 1$, 10%) and 227 (100).

(3′*S*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3′-(prop-2-enyl)-3-[(2-trimethylsilylethoxy)methyl]-3′-deoxythymidine 8

Diisopropylethylamine (2.5 cm^3, 14.1 mmol) and 2-(trimethylsilylethoxy)methyl chloride (1 cm^3, 6.4 mmol) were added to a solution of the 3′-propenylnucleoside **4** (1.8 g, 4.7 mmol) in dichloromethane (5 cm^3). After 15 h, the reaction was diluted with dichloromethane and washed with water and brine, then dried ($MgSO_4$) and concentrated under reduced pressure.

Chromatography of the residue using light petroleum–ethyl acetate (7:3) as eluent gave the *title compound* **8** (1.18 g, 50%) (Found: $M^+ + H$, 511.3017. $C_{25}H_{47}N_2O_5Si_2$ requires *M*, 511.3024); ν_{max}/cm^{-1} 3077, 1708, 1664, 1466, 1362, 1251, 1194, 1093, 1012, 838 and 777; δ_H 0.0 (9 H, s, Me_3Si), 0.1 (6 H, s, Me_2Si), 0.95 (11 H, m, Me_3CSi and CH_2Si), 1.95 (3 H, s, 5-Me), 2.05–2.45 (5 H, overlapping m, 2′-H_2, 3′-H and 1″-H_2), 3.68–3.85 (4 H, overlapping m, 4′-H, 5′-H and OCH_2), 4.05 (1 H, dd, *J* 2, 11, 5′-H′), 5.10 (2 H, m, 3″-H_2), 5.43 (2 H, s, 3-CH_2), 5.77 (1 H, m, 2″-H), 6.12 (1 H, t, *J* 5.5, 1′-H) and 7.56 (1 H, s, 6-H); *m*/*z* (CI) 511 ($M^+ + 1$, 100%).

Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.4 cm^3, 0.4 mmol) was added to the (2-trimethylsilylethoxy)methyl derivative **8** (20 mg, 0.04 mmol) in tetrahydrofuran (1 cm^3) and the mixture heated to 45 °C. After 24 h, the reaction had gone to 50% completion. Chromatography using ethyl acetate as eluent gave the 3′-propenyl-3′-deoxythymidine **5**;[4] $[\alpha]_D$ +31 (*c* 0.3 in $CHCl_3$) (Found: $M^+ + H$, 267.1343. $C_{13}H_{19}O_4N_2$ requires *M*, 267.1345).

(3′*R*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3′-(formylmethyl)-3-[(2-trimethylsilylethoxy)methyl]-3′-deoxythymidine 9

Osmium tetraoxide (1% in water, 0.1 mol equiv.) was added to the alkene **8** (600 mg, 1.17 mmol) in aqueous dioxane (75%, 10 cm^3) and sodium periodate (527 mg, 2.45 mmol) was added after 15 min. The mixture was stirred vigorously for 3 h, diluted with ethyl acetate and stirred for 30 min. The precipitate was filtered off and washed with ethyl acetate. The filtrate was dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography of the residue using light petroleum–ethyl acetate (2:1) gave the *title compound* **9** (338 mg, 56%), $[\alpha]_D$ +16.0 (*c* 1.56 in $CHCl_3$) (Found: $M^+ - C_4H_9$, 455.2036. $C_{20}H_{35}N_2O_6Si_2$ requires *M*, 455.2034); ν_{max}/cm^{-1} 1708, 1662, 1466, 1251, 1088, 837 and 777; δ_H 0.0 (9 H, s, Me_3Si), 0.12 (6 H, s, Me_2Si), 0.95 (11 H, m, Me_3CSi and CH_2Si), 1.95 (3 H, s, 5-Me), 2.10 (1 H, ddd, *J* 7, 7, 14, 2′-H), 2.32 (1 H, ddd, *J* 5, 7, 14, 2′-H′), 2.57 (1 H, m, 1″-H), 2.75 (2 H, m, 1″-H′ and 3′-H), 3.67 (2 H, m, OCH_2), 3.80 (2 H, m, 4′-H and 5′-H), 4.0 (1 H, dd, *J* 2.5, 11, 5′-H′), 5.36 and 5.37 (each 1 H, d, *J* 11, 3-CH), 6.15 (1 H, dd, *J* 5, 7, 1′-H), 7.55 (1 H, s, 6-H) and 9.79 (1 H, s, CHO); δ_C −5.4, −1.4, 13.4, 18.1, 18.5, 26.0, 32.1, 38.8, 46.7, 63.2, 67.5, 70.1, 85.5, 109.8, 134.1, 151.0, 163.5 and 199.8; *m*/*z* (EI) 455 ($M^+ - 57$, 11%), 397 (24) and 257 (100).

(3′*R*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3′-(carboxymethyl)-3-[(2-trimethylsilylethoxy)methyl]-3′-deoxythymidine 10

2-Methylbut-2-ene (8 cm^3), sodium chlorite (794 mg, 7 mmol) and sodium dihydrogen orthophosphate (876 mg, 5.6 mmol) in water (2 cm^3) were added to a solution of the aldehyde **9** (360 mg, 0.7 mmol) in *tert*-butyl alcohol (6 cm^3). The mixture was stirred vigorously for 5 h, then concentrated under reduced pressure and the residue diluted with ethyl acetate. The organic solution was washed with water and brine, dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* **10** (372 mg, 100%), $[\alpha]_D$ +11.6 (*c* 1.20 in $CHCl_3$) (Found: $M^+ + H$, 529.2738. $C_{24}H_{45}N_2O_7Si_2$ requires *M*, 529.2765); ν_{max}/cm^{-1} 3200br, 1708, 1664, 1471, 1251, 1093, 838 and 778; δ_H 0.0 (9 H, s, Me_3Si), 0.12 (6 H, s, Me_2Si), 0.95 (11 H, m, Me_3CSi and CH_2Si), 1.97 (3 H, s, 5-Me), 2.20–2.45 (3 H, overlapping m, 2′-H_2 and 1″-H), 2.70 (2 H, m, 1″-H′ and 3′-H), 3.70 (2 H, m, OCH_2), 3.82 (2 H, m, 4′-H and 5′-H), 4.03 (1 H, dd, *J* 2.5, 11, 5′-H′), 5.43 and 5.45 (each 1 H, d, *J* 11, 3-CH), 6.17 (1 H, dd, *J* 5, 7, 1′-H) and 7.6 (1 H, s, 6-H); *m*/*z* (FAB) ($M^+ + 1$, 33%) and 199 (100).

(3′*R*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3′-(3-triphenylphosphoranylidene-2-oxopropyl)-3-[(2-trimethylsilylethoxy)methyl]-3′-deoxythymidine 12

Carbonyldiimidazole (245 mg, 1.5 mmol) was added to a solution of the acid **10** (266 mg, 0.5 mmol) in tetrahydrofuran (3 cm^3). After 16 h, the mixture was diluted with ice-cold ether and washed with ice-cold water and brine. The organic extract was dried (Na_2SO_4), concentrated under reduced pressure, and azeotropically dried with benzene to afford the *compound* **11** (291 mg, 100%); ν_{max}/cm^{-1} 3091, 3072, 3036, 1815, 1708, 1667, 1466, 1250, 1088, 837, 777 and 677; δ_H 0.0 (9 H, s, Me_3Si), 0.12 (6 H, s, Me_2Si), 0.95 (11 H, overlapping m, Me_3CSi and CH_2Si), 1.95 (3 H, s, 5-Me), 2.25 (2 H, m, 2′-H_2), 2.92 (2 H, m, 1″-H and 3′-H), 3.20 (1 H, m, 1″-H′), 3.60 (2 H, m, OCH_2), 3.9 (3 H, m, 4′-H and 5′-H_2), 5.39 and 5.40 (each 1 H, d, *J* 11, 3-CH), 6.23 (1 H, t, *J* 6.5, 1′-H), 7.05 (1 H, s, imid. H), 7.45 (1 H, s, imid. H), 7.5 (1 H, s, 6-H) and 8.1 (1 H, s, imid. H); *m*/*z* (CI) 579 (M^+, 15%).

n-Butyllithium (1.8 cm^3, 2.7 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (950 mg, 2.7 mmol) in tetrahydrofuran (20 cm^3) and the solution stirred for 20 min. The acyl imidazolide **11** (735 mg, 1.3 mmol) prepared as outlined above, was added dropwise and the reaction mixture was stirred for 6 h at 20 °C, filtered through Celite and washed with ethyl acetate. The organic extract was washed with saturated aqueous sodium hydrogen carbonate, water, brine, then dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using ethyl acetate as eluent gave the *title compound* **12** (250 mg, 25%), $[\alpha]_D$ +7.1 (*c* 0.41 in $CHCl_3$) (Found: $M^+ + H$, 788.3766. $C_{43}H_{62}N_2O_6PSi_2$ requires *M*, 788.3806); ν_{max}/cm^{-1} 3055, 1708, 1662, 1514, 1466, 1438, 1262, 1106, 837 and 778; δ_H 0.02 (9 H, s, Me_3Si), 0.10 (6 H, s, Me_2Si), 0.95 (11 H, m, Me_3CSi and CH_2Si), 1.94 (3 H, s, 5-Me), 2.20 (3 H, m, 1″-H and 2′-H_2), 2.63 (2 H, m, 1″-H′ and 3′-H), 3.75 (6 H, m, OCH_2, 3″-H, 4′-H and 5′-H_2), 5.46 and 5.47 (each 1 H, d, *J* 11, 3-CH), 6.20 (1 H, dd, *J* 5, 7, 1′-H), 7.50–7.70 (16 H, m, ArH and 6-H); *m*/*z* (FAB) 788 ($M^+ + 1$, 20%) and 199 (100).

(*E*)-3′-*O*-Acetyl-5′-(3-methyl-2-oxobutylidene)-5′-deoxythymidine 17

Dicyclohexylcarbodiimide (1.31 g, 6.36 mmol) and dichloroacetic acid (0.09 cm^3, 1.06 mmol) were added to a solution of 3′-*O*-acetylthymidine **15** (0.6 g, 2.12 mmol) in dimethyl sulfoxide. After 60 min, the mixture was filtered and the precipitate washed with ethyl acetate. The ketophosphorane **14**[12] (0.8 g, 2.3 mmol) and pyridine (0.2 cm^3, 2.5 mmol) were added to the filtrate and the mixture stirred at room temperature for 48 h. The reaction mixture was then diluted with ethyl acetate, washed with water, brine, dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using ethyl acetate as eluent gave the *title compound* **17** (350 mg, 82%) as a foam, $[\alpha]_D$ +15.9 (*c* 0.54 in $CHCl_3$) (Found: M^+, 350.1475. $C_{17}H_{22}N_2O_6$ requires *M*, 350.1478); ν_{max}/cm^{-1} 1695, 1468, 1375, 1234 and 1050; δ_H 1.20 (6 H, d, *J* 7, 2 × Me), 1.98 (3 H, s, 5-Me), 2.17 (3 H, s, $MeCO_2$), 2.30 (1 H, m, 2′-H), 2.45 (1 H, ddd, *J* 2.5, 6 and 14, 2′-H′), 2.85 (1 H, septet, *J* 7, 3″-H), 4.65 (1 H, m, 4′-H), 5.20 (1 H, m, 3′-H), 6.43 (1 H, dd, *J* 6, 9, 1′-H), 6.55 (1 H, dd, *J* 1.5, 15.5, 1″-H), 6.95 (1 H, dd, *J* 5, 15.5, 5′-H), 7.16 (1 H, s, 6-H) and 8.96 (1 H, br s, NH); δ_C 12.7, 18.0, 18.2, 20.9, 36.3, 39.7, 83.0, 84.8, 112.0, 128.7, 134.5, 140.1, 150.3, 163.3, 170.4 and 202.9; *m*/*z* (CI) 368 ($M^+ + 18$, 90%).

(*E*)-3′-*O*-Acetyl-5′-(3-{(3′*R*)-5′-*O*-(*tert*-butyldimethylsilyl)-3-[(2-trimethylsilylethoxy)methyl]-3′-deoxythymidin-3′-yl}-2-oxopropylidene)-5′-deoxythymidine 18

Dichloroacetic acid (0.011 cm^3, 0.13 mmol) was added to a solution of 3′-*O*-acetylthymidine **15**[7] (90 mg, 0.32 mmol) and dicyclohexylcarbodiimide (164 mg, 0.8 mmol) in dimethyl sulfoxide (2 cm^3) and the reaction mixture was stirred for 1 h then filtered. The precipitate was washed with ethyl acetate and the washings and filtrate concentrated under reduced pressure. Tetrahydrofuran (5 cm^3), the ketophosphorane **12** (208 mg, 0.26 mmol) and pyridine (0.065 cm^3, 0.78 mmol) were added and the reaction mixture stirred at room temperature for 28 h. After concentration under reduced pressure, the residue was

dissolved in ethyl acetate. This solution was washed with water and brine, dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using ethyl acetate as eluent then gave the *title compound* **18** (160 mg, 78%), as a foam (Found: $M^+ + H$, 791.3703. $C_{37}H_{59}N_4O_{11}Si_2$ requires *M*, 791.3719); ν_{max}/cm^{-1} 1705, 1665, 1466, 1363, 1249, 1088, 837 and 784; δ_H 0.00 (9 H, s, Me_3Si), 0.10 (6 H, s, Me_2Si), 0.9 (9 H, s, Me_3CSi), 0.95 (2 H, m, CH_2Si), 1.94 (3 H, s, 5-Me), 1.97 (3 H, s, 5‴-Me), 2.05 (1 H, m, 2⁗-H), 2.1 (3 H, s, $MeCO_2$), 2.25 (2 H, m, 2′-H and 2⁗-H′), 2.40 (1 H, ddd, *J* 3, 6 and 14, 2′-H′), 2.68 (1 H, dd, *J* 6, 17, 3″-H), 2.75 (1 H, m, 3⁗-H), 2.85 (1 H, dd, *J* 6, 17, 3″-H′), 3.64 (2 H, m, OCH_2), 3.77 (1 H, m, 4⁗-H), 3.8 and 3.94 (each 1 H, dd, *J* 3, 11, 5⁗-H), 4.47 (1 H, m, 4′-H), 5.06 (1 H, m, 3′-H), 5.37 and 5.39 (each 1 H, d, *J* 11, 3‴-CH), 6.15 (1 H, t, *J* 7, 1⁗-H), 6.30 (1 H, dd, *J* 5, 7, 1′-H), 6.38 (1 H, dd, *J* 1.5, 16, 1″-H), 6.90 (1 H, dd, *J* 5, 16, 5′-H), 7.1 (1 H, s, 6-H), 7.55 (1 H, s, 6‴-H) and 9.1 (1 H, br s, NH); *m*/*z* (CI) 791 ($M^+ + 1$, 5%).

3′-*O*-Acetyl-5′-(3-{(3′*R*)-3-[(2-trimethylsilylethoxy)methyl]-3′-deoxythymidin-3′-yl}-2-oxopropyl)-5′-deoxythymidine 19

The unsaturated *C*-linked dinucleotide **18** (185 mg, 0.24 mmol) was stirred in methanol (2 cm^3) with 10% palladium on carbon (240 mg) under an atmosphere of hydrogen for 18 h. The reaction mixture was then filtered through Celite, the Celite washed with ethyl acetate and the filtrate and washings concentrated under reduced pressure to give the *title compound* **19** (120 mg, 76%) as a foam; ν_{max}/cm^{-1} 3467, 3076, 1703, 1470, 1368, 1250, 1083, 917, 862, 838 and 732; δ_H 0.0 (9 H, s, Me_3Si), 0.95 (2 H, m, CH_2Si), 1.9 (6 H, s, 2 × Me), 2.0 (2 H, m, 5′-H_2), 2.05 (3 H, s, $MeCO_2$), 2.2–2.35 (4 H, overlapping m, 2′-H_2 and 2⁗-H_2), 2.62 (5 H, m, 1″-H_2, 3″-H_2 and 3⁗-H), 2.90 (1 H, br s, OH), 3.60 (4 H, m, CH_2O, 4⁗-H and 5⁗-H), 3.95 (2 H, m, 4′-H and 5⁗-H′), 4.95 (1 H, m, 3′-H), 5.35 and 5.37 (each 1 H, d, *J* 10, 3‴-CH), 6.05 (1 H, dd, *J* 3, 6, 1⁗-H), 6.15 (1 H, dd, *J* 6, 8, 1′-H), 7.06 (1 H, s, 6-H), 7.72 (1 H, s, 6‴-H) and 9.15 (1 H, br s, NH); *m*/*z* (CI) 679 ($M^+ + 1$, 5%) and 225 (100).

3′-*O*-Acetyl-5′-{3-[(3′*R*)-3′-deoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidine 20

Aqueous hydrogen fluoride in acetonitrile (10%, 1 cm^3) was added to the saturated *C*-linked dinucleotide **19** (23 mg, 0.036 mmol). After 5 h, saturated aqueous sodium hydrogen carbonate and ethyl acetate were added, and the organic layer washed with water and brine. The organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using methanol (4%) in dichloromethane gave the *title compound* **20** (18 mg, 90%) as a white foam, $[\alpha]_D$ +5.8 (*c* 0.69 in $CHCl_3$) (Found: $M^+ + NH_4$, 566.2455. $C_{25}H_{36}N_5O_{10}$ requires *M*, 566.2462); ν_{max}/cm^{-1} 3400br, 3100, 1700, 1669, 1471, 1363, 1235, 1093, 838, 776, 738 and 700; δ_H 1.89 (3 H, s, 5-Me), 1.91 (3 H, s, 5‴-Me), 2.0 (2 H, m, 5′-H_2), 2.08 (3 H, s, $MeCO_2$), 2.24 (3 H, m, 2⁗-H_2 and 2′-H), 2.33 (1 H, ddd, *J* 2, 6 and 14, 2′-H′), 2.61 (5 H, m, 1″-H_2, 3″-H_2 and 3⁗-H), 2.84 (1 H, br s, OH), 3.65 (2 H, m, 5⁗-H_2), 3.9 (2 H, m, 4′-H and 4⁗-H), 4.88 (1 H, m, 3′-H), 6.05 (1 H, dd, *J* 4, 8, 1⁗-H), 6.15 (1 H, dd, *J* 6, 8, 1′-H), 7.05 (1 H, s, 6-H), 7.70 (1 H, s, 6‴-H) and 8.80 and 9.0 (each 1 H, br s, NH); *m*/*z* (CI) 566 ($M^+ + 18$, 40%) and 549 ($M^+ + 1$, 35).

(3′*S*)-3-(Benzyloxymethyl)-5′-*O*-(*tert*-butyldimethylsilyl)-3′-(prop-2-enyl)-3′-deoxythymidine 21

Diisopropylethylamine (18.3 cm^3, 0.105 mol) and benzyloxymethyl chloride (10.5 cm^3, 78.44 mmol) were added to a solution of the 3′-propenylnucleoside **4**[4] (20.0 g, 52.49 mmol) in dichloromethane (150 cm^3). After 16 h, the reaction mixture was diluted with more dichloromethane and washed with water and brine, then dried ($MgSO_4$) and concentrated under reduced pressure to afford the *title compound* **21** (26.3 g, 100%), $[\alpha]_D$ +19.0 (*c* 1.33, $CHCl_3$) (Found: $M^+ + H$, 501.2782. $C_{27}H_{41}N_2O_5Si$ requires *M*, 501.2784); ν_{max}/cm^{-1} 1762, 1709, 1663, 1466, 1258, 1094 and 837; δ_H 0.16 (6 H, s, Me_2Si), 0.97 (9 H, s, Me_3CSi), 1.96 (3 H, s, 5-Me), 2.10–2.40 (5 H, overlapping m, 2′-H_2, 3′-H and 1″-H_2), 3.78 (2 H, m, 4′-H and 5′-H), 4.06 (1 H, m, 5′-H′), 4.74 (2 H, s, CH_2Ph), 5.13 (2 H, m, 3″-H_2), 5.53 (2 H, s, 3-CH_2), 5.8 (1 H, m, 2″-H), 6.10 (1 H, t, *J* 5.5, 1′-H), 7.4 (5 H, m, ArH) and 7.61 (1 H, s, 6-H); δ_C −5.1, 13.5, 16.5, 18.6, 26.1, 36.5, 36.8, 39.0, 63.0, 70.4, 72.2, 85.5, 85.9, 109.3, 117.0, 127.5, 128.1, 134.3, 135.2, 137.9, 150.8 and 163.4; *m*/*z* (CI) 518 ($M^+ + 18$, 18%), 501 ($M^+ + 1$, 100).

(3′*R*)-3-(Benzyloxymethyl)-5′-*O*-(*tert*-butyldimethylsilyl)-3′-(formylmethyl)-3′-deoxythymidine 22

Osmium tetraoxide (4% aqueous solution, 8.2 cm^3) was added to a solution of the 3′-alkene **21** (26.0 g, 52.48 mmol) in aqueous dioxane (75%, 200 cm^3) and the mixture stirred for 15 min. Sodium periodate (24.0 g, 0.112 mol) was added portionwise, with cooling, over 1 h. The reaction mixture was then stirred vigorously for 16 h, diluted with ethyl acetate and stirred vigorously for a further 30 min until the precipitate formed during the reaction was uniformly dispersed throughout the mixture. The precipitate was filtered off and washed with ethyl acetate. The filtrate was dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography using light petroleum–ethyl acetate (2:1) as eluent gave the *title compound* **22** (12.0 g, 46%) (Found: $M^+ + H$, 503.2575. $C_{26}H_{39}N_2O_6Si$ requires *M*, 503.2577); ν_{max}/cm^{-1} 1708, 1661, 1466, 1273, 1093, 838 and 776; δ_H 0.15 (6 H, s, Me_2Si), 0.95 (9 H, s, Me_3CSi), 1.97 (3 H, s, 5-Me), 2.11 (1 H, m, 2′-H), 2.30 (1 H, ddd, *J* 5, 7 and 14, 2′-H′), 2.64 (1 H, m, 1″-H), 2.77 (2 H, m, 3′-H and 1″-H′), 3.80 (2 H, m, 4′-H and 5′-H), 4.08 (1 H, dd, *J* 2.5, 11, 5′-H′), 4.73 (2 H, s, CH_2Ph), 5.52 (2 H, s, 3-CH_2), 6.16 (1 H, dd, *J* 5, 7, 1′-H), 7.38 (5 H, m, ArH), 7.54 (1 H, s, 6-H) and 9.83 (1 H, s, CHO); δ_C −5.3, 13.3, 14.2, 18.5, 26.0, 32.2, 38.8, 46.7, 63.2, 70.5, 72.2, 85.4, 85.5, 109.8, 127.7, 128.3, 134.2, 138.1, 151.0, 163.5 and 199.7; *m*/*z* (CI) 503 ($M^+ + 1$, 15%) and 247 (100).

(3′*R*)-3-(Benzyloxymethyl)-5′-*O*-(*tert*-butyldimethylsilyl)-3′-(carboxymethyl)-3′-deoxythymidine 23

2-Methylbut-2-ene (100 cm^3) and a solution of sodium chlorite (27.0 g, 0.239 mol) and sodium dihydrogen orthophosphate (30.0 g, 0.191 mmol) in water (25 cm^3) were added to a solution of the aldehyde **22** (12.0 g, 23.86 mmol) in *tert*-butyl alcohol (150 cm^3). The reaction mixture was stirred vigorously for 5 h, then concentrated under reduced pressure and the residue diluted with ethyl acetate. The organic phase was washed with water and brine, then dried ($MgSO_4$) and concentrated under reduced pressure to afford the *title compound* **23** (12.4 g, 100%) (Found: $M^+ + H$, 519.2515. $C_{26}H_{39}N_2O_7Si$ requires *M*, 519.2526); $[\alpha]_D$ +13.9 (*c* 1.53, $CHCl_3$); ν_{max}/cm^{-1} 3200br, 1708, 1664, 1468, 1259, 1095, 838 and 777; δ_H 0.15 (6 H, s, Me_2Si), 0.95 (9 H, s, Me_3CSi), 1.95 (3 H, s, 5-Me), 2.13–2.42 (3 H, overlapping m, 2′-H_2 and 1″-H), 2.53 (2 H, m, 3′-H and 1″-H′), 3.80 (2 H, m, 4′-H and 5′-H), 3.95 (1 H, dd, *J* 2.5, 11, 5′-H′), 4.70 (2 H, s, CH_2Ph), 5.50 (2 H, s, 3-CH_2), 6.10 (1 H, dd, *J* 5, 7, 1′-H), 7.3 (5 H, m, ArH) and 7.49 (1 H, s, 6-H); *m*/*z* (CI) 519 ($M^+ + 1$, 100%).

(3′*R*)-3-(Benzyloxymethyl)-5′-*O*-(*tert*-butyldimethylsilyl)-3′-[3-(triphenylphosphoranylidene)-2-oxopropyl]-3′-deoxythymidine 25

N,*N*′-Carbonyldiimidazole (11.6 g, 71.58 mmol) was added to a solution of the acid **23** (12.4 g, 23.86 mmol) in tetrahydrofuran (100 cm^3). After 16 h, the mixture was diluted with ice-cold ether, then washed with ice-cold water and brine. The organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and azeotropically dried with benzene to give the *imidazolide* **24** (13.6 g, 100%); ν_{max}/cm^{-1} 3124, 1738, 1706, 1658, 1468, 1242, 1092 and 837; δ_H 0.12 (6 H, s, Me_2Si), 0.92 (9 H, s, Me_3CSi), 1.94 (3 H, s, 5-Me), 2.27 (2 H, m, 2′-H_2), 3.05 (3 H,

overlapping m, 3′-H and 1″-H_2), 3.90 (3 H, overlapping m, 4′-H and 5′-H_2), 4.70 (2 H, s, CH_2Ph), 5.50 (2 H, s, 3-CH_2), 6.15 (1 H, t, *J* 6, 1′-H), 7.05 (1 H, m, imid. H), 7.20–7.60 (7 H, m, imid. H, ArH and 6-H) and 8.15 (1 H, s, imid. H); *m*/*z* (CI) 569 (M^+ + 1, 100%).

n-Butyllithium (1.6 M in hexane, 32.0 cm^3) was added dropwise to a suspension of methyl(triphenyl)phosphonium bromide (18.8 g, 52.68 mmol) in tetrahydrofuran (200 cm^3) at 0 °C, and the solution stirred for 20 min. The imidazolide **24** (13.6 g, 23.94 mmol) was then added dropwise and immediate precipitation was observed. The reaction mixture was stirred for 6 h at 20 °C, filtered through Celite and the retained solids washed with ethyl acetate. The filtrate was washed with saturated aqueous sodium hydrogen carbonate, water and brine, dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 3% methanol in dichloromethane as eluent gave the *title compound* **25** (7.6 g, 41%), $[\alpha]_D$ +9.7 (*c* 1.52, $CHCl_3$) (Found: M^+ + H, 777.3493. $C_{45}H_{54}N_2O_6PSi$ requires *M*, 777.3489); ν_{max}/cm^{-1} 1707, 1661, 1542, 1466, 1438, 1266, 1198, 1119, 837, 721 and 695; δ_H 0.15 and 0.16 (each 3 H, s, MeSi), 0.94 (9 H, s, Me_3CSi), 1.95 (3 H, s, 5-Me), 2.20–2.80 (5 H, overlapping m, 2′-H_2, 3′-H and 1″-H_2), 3.75 (1 H, dd, *J* 4, 11, 5′-H), 3.93 (2 H, m, 4′-H and 3″-H), 4.0 (1 H, dd, *J* 11, 2, 5′-H′), 4.75 (2 H, s, CH_2Ph), 5.56 (2 H, s, 3-CH_2), 6.20 (1 H, dd, *J* 5, 7, 1′-H), 7.30–7.75 (21 H, m, ArH and 6-H); *m*/*z* (CI) 777 (M^+ + 1, 2.5%), 531 (2.5) and 263 (100).

(*E*)-3′-*O*-Acetyl-5′-{3-[(3′*R*)-3-benzyloxymethyl-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxythymidin-3′-yl]-2-oxopropylidene}-5′-deoxythymidine 26

Dichloroacetic acid (0.2 cm^3, 1.91 mmol) was added to a solution of 3′-*O*-acetylthymidine **15**[7] (1.40 g, 4.95 mmol) and dicyclohexylcarbodiimide (2.35 g, 11.44 mmol) in dimethyl sulfoxide (5 cm^3). After 1 h, the precipitate was removed by filtration and washed with ethyl acetate, and the filtrate concentrated under reduced pressure. Tetrahydrofuran (5 cm^3), the ylide **25** (3.0 g, 3.8 mmol) and pyridine (0.5 cm^3) were added to the residue and the mixture stirred at room temperature for 28 h before being concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the solution washed with water and brine, dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using ethyl acetate as eluent gave the *title compound* **26** (2.0 g, 67%) as a foam (Found: M^+ + H, 781.3463. $C_{39}H_{53}N_4O_{11}Si$ requires *M*, 781.3480); ν_{max}/cm^{-1} 1702, 1660, 1468, 1365, 1236, 1091, 838, 777 and 737; δ_H 0.15 (6 H, s, Me_2Si), 0.96 (9 H, s, Me_3CSi), 1.97 and 2.00 (each 3 H, s, 5-Me, 5‴-Me), 2.1 (1 H, m, 2‴-H), 2.17 (3 H, s, $MeCO_2$), 2.30–2.55 (3 H, m, 2′-H_2 and 2‴-H′), 2.65–3.0 (3 H, m, 3″-H_2 and 3‴-H), 3.85 (2 H, m, 4‴-H and 5‴-H), 4.00 (1 H, dd, *J* 2, 11, 5‴-H′), 4.60 (1 H, m, 4′-H), 4.73 (2 H, s, CH_2Ph), 5.18 (1 H, m, 3′-H), 5.53 (2 H, s, 3‴-CH_2), 6.20 (1 H, t, *J* 6, 1‴-H), 6.32 (1 H, dd, *J* 6, 8, 1′-H), 6.42 (1 H, dd, *J* 1.5, 16, 1″-H), 6.95 (1 H, dd, *J* 5, 16, 5′-H), 7.13 (1 H, s, 6-H), 7.3 (5 H, m, ArH), 7.56 (1 H, s, 6‴-H) and 8.60 (1 H, br, NH); δ_C −5.3, 12.7, 13.3, 18.5, 20.9, 26.0, 33.9, 36.1, 38.7, 43.8, 63.8, 70.5, 72.2, 76.3, 77.2, 82.7, 85.5, 85.6, 109.9, 112.0, 127.6, 128.0, 128.4, 130.1, 134.3, 135.0, 138.1, 141.2, 150.1, 151.0, 163.1, 163.5, 170.4 and 197.3; *m*/*z* (FAB) 781 (M^+ + 1, 25%).

The unsaturated *C*-linked dinucleotide **26** (2.0 g, 2.56 mmol) was stirred in methanol (15 cm^3) with palladium hydroxide on carbon (20%, 500 mg) under an atmosphere of hydrogen. After 16 h, the reaction mixture was filtered through Celite, the retained solids washed with methanol and the filtrate concentrated under reduced pressure. Chromatography of the residue using 4% methanol in ethyl acetate as eluent gave the saturated *C*-linked dinucleotide **20** (850 mg, 60%) as a white foam with spectroscopic data identical to those of the samples prepared from the SEM-protected system **19**.

(*E*)-3′-*O*-Acetyl-5′-{3-[(3′*R*)-5′-(3-[(3′*R*)-3-benzyloxymethyl-5′-*O*-*tert*-butyldimethylsilyl-3′-deoxythymidin-3′-yl]-2-oxopropylidene)-3′,5′-dideoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidine 28

Dichloroacetic acid (0.006 cm^3, 0.07 mmol) was added to a solution of the *C*-linked dinucleotide **20** (80 mg, 0.14 mmol) and dicyclohexylcarbodiimide (80 mg, 0.44 mmol) in dimethyl sulfoxide (1 cm^3). The reaction mixture was stirred at room temperature for 1 h, then filtered and the retained solids washed with one portion of tetrahydrofuran (1 cm^3). Pyridine (0.05 cm^3, 0.62 mmol) and the ylide **25** (400 mg, 0.51 mmol) were added to the filtrate. The reaction mixture was stirred at room temperature for 24 h, then diluted with ethyl acetate and washed with water and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 2% methanol in dichloromethane as eluent gave the *title compound* **28** (77 mg, 51%) as a white foam, $[\alpha]_D$ +2.2 (*c* 0.41, $CHCl_3$) (Found: M^+ + H, 1045.4633. $C_{52}H_{69}N_6O_{15}Si$ requires *M*, 1045.4590); ν_{max}/cm^{-1} 1692, 1468, 1369, 1270, 1074 and 732; δ_H 0.10 (6 H, s, Me_2Si), 0.90 (9 H, s, Me_3CSi), 1.90 and 1.93 (each 3 H, d, *J* 1, Me), 2.02 (4 H, m, 5′-H_2, 2‴″-H and 2‴″″-H), 2.02 (3 H, d, *J* 1, Me), 2.07 (3 H, s, $MeCO_2$), 2.15–2.35 (4 H, m, 2′-H_2, 2‴-H′ and 2‴″″-H′), 2.48–2.90 (8 H, m, 1″-H_2, 3″-H_2, 3‴-H, 3‴″-H_2 and 3‴″″-H), 3.76 (1 H, m, 4‴″″-H), 3.79 (1 H, dd, *J* 2, 11, 5‴″″-H), 3.89 (1 H, m, 4′-H), 3.93 (1 H, dd, *J* 2, 11, 5‴″″-H′), 4.14 (1 H, m, 4‴-H), 4.67 (2 H, s, CH_2Ph), 4.96 (1 H, m, 3′-H), 5.45 (2 H, s, 3‴″″-CH_2), 6.13 (2 H, m, 1‴-H and 1‴″″-H), 6.20 (1 H, dd, *J* 6, 8, 1′-H), 6.32 (1 H, dd, *J* 2, 16, 1‴″-H), 6.80 (1 H, dd, *J* 6, 16, 5‴-H), 7.06 and 7.09 (each 1 H, m, 6-H and 6‴-H), 7.29 (5 H, m, ArH), 7.52 (1 H, m, 6‴″-H) and 9.15 and 9.25 (each 1 H, s, NH); *m*/*z* (FAB) 1045 (M^+ + 1, 5%).

3′-*O*-Acetyl-5′-{3-[(3′*R*)-5′-(3-[(3′*R*)-3′-deoxythymidin-3′-yl]-2-oxopropyl)-3′,5′-dideoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidine 29

The unsaturated *C*-linked trinucleotide **28** (45 mg, 0.043 mmol) was stirred in methanol (1 cm^3) with palladium hydroxide (20%) on carbon (30 mg) under an atmosphere of hydrogen. After 16 h, the reaction mixture was filtered through Celite and the retained solids washed with methanol. The filtrate was concentrated under reduced pressure and chromatography of the residue using 4% methanol in dichloromethane gave the *title compound* **29** (18 mg, 51%) as a foam (Found: M^+ + H, 813.3304. $C_{38}H_{49}N_6O_{14}$ requires *M*, 813.3307); ν_{max}/cm^{-1} 3433br, 1690, 1471, 1270 and 1049; δ_H([$^2H_4$]methanol) 1.97, 1.99 and 2.00 (each 3 H, d, *J* 1, Me), 2.08 (6 H, m, 5′-H_2, 2‴-H, 5‴-H_2 and 2‴″″-H), 2.17 (3 H, s, $MeCO_2$), 2.30–2.50 (5 H, m, 2′-H_2, 2‴-H′, 3‴-H and 2‴″″-H′), 2.60–2.90 (9 H, m, 1″-H_2, 3″-H_2, 1‴″-H_2, 3‴″-H_2 and 3‴″″-H), 3.68 (1 H, dt, *J* 8.5, 3.5, 4‴″-H), 3.79 (2 H, m, 4‴″″-H and 5‴″″-H), 3.97 (1 H, m, 5‴″″-H′), 4.04 (1 H, dt, *J* 7.5, 3, 4′-H), 5.15 (1 H, m, 3′-H), 6.05 (1 H, dd, *J* 4, 7.5, 1‴″″-H), 6.11 (1 H, dd, *J* 3.5, 7, 1‴″-H), 6.26 (1 H, dd, *J* 6, 8.5, 1′-H) and 7.47, 7.49 and 8.05 (each 1 H, m, 6-H, 6‴-H and 6‴″″-H); *m*/*z* (FAB) 835 (5%) and 813 (M^+ + 1, 2).

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)thymidine 31

Diisopropylethylamine (0.96 cm^3, 5.51 mmol), tetrazole (386 mg, 5.51 mmol) and bis(diisopropylamino)(2-cyanoethoxy)-phosphine (1.52 cm^3, 4.78 mmol) were added to a solution of the (4,4′-dimethoxytrityl)thymidine **30** (2.0 g, 3.68 mmol) in dichloromethane (5 cm^3). After 16 h, the reaction mixture was diluted with dichloromethane and washed with saturated aqueous sodium carbonate, water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was then dissolved in dichloromethane (2 cm^3) and added dropwise to rapidly stirred hexane at 0 °C. The resultant precipitate was filtered off and washed with ice-cold hexane, then dried under reduced pressure to give the *title compound* **31**

(2.6 g, 95%) as an off-white powder which was stored at below 0 °C (Found: M^- − H, 743.3210. $C_{40}H_{48}N_4O_8P$ requires *M*, 743.3210); ν_{max}/cm^{-1} 3181, 3057, 3038, 1692, 1607, 1509, 1465, 1251, 1179, 1035, 979, 912, 830 and 733; δ_H 1.22 and 1.24 (each 6 H, d, *J* 7, 2 × *Me*CH), 1.46 and 1.47 (each 1.5 H, s, 5-Me), 2.33–2.63 (4 H, m, 2′-H_2 and CH_2CN), 3.35–4.00 (6 H, m, OCH_2, 5′-H_2, 2 × Me_2CH), 4.20 (1 H, m, 3′-H), 4.72 (1 H, m, 4′-H), 6.48 (1 H, m, 1′-H), 6.87 (4 H, m, ArH), 7.35 (9 H, m, ArH), 7.63 and 7.68 (each 0.5 H, s, 6-H) and 9.1 (1 H, br s, NH); δ_P(122 MHz) 149.03 and 149.45; *m*/*z* (−FAB) 743 (M^- − 1, 100%).

(3′-*O*-Acetylthymidin-5′-*O*-yl)(2-cyanoethoxy)[5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-*O*-yl]phosphane 32

3′-*O*-Acetylthymidine **15** (1.08 g, 3.82 mmol) and tetrazole (100 mg) were added to a solution of the phosphoramidite **31** (2.58 g, 3.77 mmol) in tetrahydrofuran (10 cm^3). After 2 h at room temperature, the reaction mixture was diluted with dichloromethane and washed with 2% aqueous sodium carbonate and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 4% methanol and 1% triethylamine in dichloromethane as eluent gave the *title compound* **32** (2.8 g, 87%) as a white foam, $[\alpha]_D$ +3.0 (*c* 1.00, $CHCl_3$) (Found: M^- − H, 926.2971. $C_{46}H_{49}N_5O_{14}P$ requires *M*, 926.3014); ν_{max}/cm^{-1} 3184, 3051, 2252, 1691, 1609, 1509, 1466, 1250, 1178, 1032 and 729; δ_H 1.43 and 1.44 (each 1.5 H, s, 5-Me), 1.88 (3 H, s, 5″-Me), 2.07 and 2.08 (each 1.5 H, s, $MeCO_2$), 2.17 (1 H, m, 2‴-H), 2.35 (2 H, m, 2′-H and 2‴-H′), 2.58 (1 H, m, 2′-H′), 2.55 and 2.59 (each 1 H, t, *J* 6.5, CH_2CN), 3.32 (1 H, m, 5‴-H), 3.50 (1 H, m, 5‴-H′), 3.76 (6 H, s, 2 × OMe), 3.90–4.15 (6 H, m, 4′-H, 5′-H_2, 4‴-H and OCH_2), 4.90 and 4.96 (each 0.5 H, m, 3′-H), 5.18 and 5.24 (each 0.5 H, m, 3‴-H), 6.29 (1 H, m, 1‴-H), 6.36 (1 H, m, 1′-H), 6.82 (4 H, m, ArH), 7.20–7.35 (10 H, m, ArH and 6-H), 7.55 (1 H, s, 6″-H) and 9.1 (2 H, br s, 2 × NH); δ_P(122 MHz) 139.75 and 139.95; *m*/*z* (−FAB) 926 (M^- − 1, 100%).

2-Cyanoethyl (3′-*O*-acetylthymidin-5′-*O*-yl)[5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-*O*-yl]phosphinate 33

The phosphite triester **32** (2.80 g, 3.02 mmol) was stirred in a solution of iodine in tetrahydrofuran–pyridine–water (ratio 40 : 20 : 1, 0.1 M, 36 cm^3). After 30 min, the reaction mixture was diluted with dichloromethane and washed with saturated aqueous sodium thiosulfate, water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 4% methanol and 1% triethylamine in dichloromethane as eluent gave the *title compound* **33** (2.8 g, 98%) as a white foam (Found: M^-, 943.3040. $C_{46}H_{50}N_5O_{15}P$ requires *M*, 943.3041); ν_{max}/cm^{-1} 3184, 3066, 2252, 1692, 1608, 1509, 1467, 1251, 1029 and 729; δ_H 1.38 and 1.39 (each 1.5 H, s, 5-Me), 1.88 (3 H, s, 5″-Me), 2.07 and 2.08 (each 1.5 H, s, $MeCO_2$), 2.25 (1 H, m, 2′-H), 2.40 (2 H, m, 2′-H′ and 2‴-H), 2.68 (2 H, m, 2‴-H′ and CH_2CN), 2.75 (1 H, m, CH_2CN), 3.38 (1 H, m, 5‴-H), 3.50 (1 H, m, 5‴-H′), 3.8 (6 H, s, 2 × OMe), 4.10–4.40 (6 H, m, 4′-H, 5′-H_2, 4‴-H and OCH_2), 5.18 (1.5 H, m, 3′-H and 3‴-H), 5.27 (0.5 H, m, 3′-H), 6.28 (1 H, m, 1′-H), 6.40 (1 H, m, 1‴-H), 6.80 (4 H, m, ArH), 7.20–7.35 (10 H, m, ArH and 6″-H), 7.51 and 7.53 (each 0.5 H, s, 6-H), 8.99 (0.5 H, s, NH), 9.02 (1 H, s, NH) and 9.1 (0.5 H, s, NH); δ_P(122 MHz) −2.01; *m*/*z* (−FAB) 943 (M^-, 40%), 890 (50) and 416 (100).

2-Cyanoethyl (3′-*O*-acetylthymidin-5′-*O*-ylthymidin-3′-*O*-yl)-phosphinate 34

Dichloroacetic acid (0.5 cm^3, 8.2 mmol) was added to a solution of the dimethoxytrityl ether **33** (2.80 g, 2.97 mmol) in dichloromethane (25 cm^3). After 2 h at room temperature, no starting material was observable by TLC, and triethylamine (2 cm^3) was added. The reaction mixture was diluted with dichloromethane and washed with 2% aqueous sodium carbonate and brine. The combined aqueous extracts then extracted repeatedly with dichloromethane. The organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 8% methanol in dichloromethane gave the *title compound* **34** (1.4 g, 76%) as a white powder, $[\alpha]_D$ +5.2 (*c* 0.67, $CHCl_3$) (Found: M^+ + Na, 664.1643. $C_{25}H_{32}N_5O_{13}PNa$ requires *M*, 664.1632); ν_{max}/cm^{-1} 3405br, 3199br, 3056, 2253, 1690, 1472, 1370, 1276, 1245 and 1024; δ_H([$^2H_4$]methanol) 2.06 (3 H, s, Me), 2.10 (3 H, d, *J* 1, Me), 2.28 and 2.29 (each 1.5 H, s, $MeCO_2$), 2.62 (3 H, m, 2′-H_2 and 2‴-H), 2.76 (1 H, m, 2‴-H′), 3.20 (2 H, t, *J* 5.5, CH_2CN), 3.99 (2 H, m, 5‴-H_2), 4.43 (2 H, m, 4′-H and 4‴-H), 4.54 (2 H, m, CH_2O), 4.62 (2 H, m, 5′-H_2), 5.35 (1 H, m, 3‴-H), 5.54 (1 H, m, 3′-H), 6.44 (1 H, dd, *J* 6.5, 7.5, 1′-H), 6.49 (1 H, m, 1‴-H), 7.73 and 7.75 (each 1.5 H, d, *J* 1, 6-H) and 7.98 (1 H, s, 6″-H); δ_C([$^2H_4$]methanol) 12.6, 12.7, 20.1, 20.2, 20.9, 37.4, 39.5, 62.5, 64.6, 64.7, 69.1, 69.2, 69.8, 75.0, 75.1, 80.8, 80.9, 81.0, 81.1, 83.8, 83.9, 86.1, 86.7, 86.9, 87.0, 111.9, 112.2, 118.6, 137.7, 137.9, 152.2, 152.3, 166.2, 166.3, 171.4 and 172.3; δ_P([$^2H_4$]methanol) −1.55 and −1.62; *m*/*z* (FAB) 664 (M^+ + 23, 10%), 642 (M^+ + 1, 2) and 239 (100).

Ammonia gas was bubbled through a solution of the phosphate **34** (20 mg, 0.031 mmol) in methanol (1 cm^3) for 10 min. The flask was then sealed and left at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with methanol–water (1 : 1) and then with anhydrous methanol to give thymidin-5′-*O*-yl)thymidin-3′-*O*-yl)phosphinate **35** (19 mg, 100%) as a colourless glass (Found: M^-, 545.1263. $C_{20}H_{26}N_4O_{12}P$ requires *M*, 545.1285); ν_{max}(KBr)/cm^{-1} 3395br, 1702, 1690, 1667, 1475, 1374, 1276 and 1083; $\delta_H(D_2O)$ 1.69 and 1.71 (each 3 H, d, *J* 1, Me), 2.18 (3 H, m, 2′-H_2 and 2‴-H), 2.38 (1 H, ddd, *J* 3.5, 6.5 and 14, 2‴-H′), 3.60 and 3.64 (each 1 H, dd, *J* 3.5, 12.5, 5‴-H), 3.95 (4 H, m, 4′-H, 4‴-H and 5′-H_2), 4.40 (1 H, m, 3′-H), 4.58 (1 H, m, 3‴-H), 6.02 (1 H, t, *J* 6.5, 1′-H), 6.13 (1 H, t, *J* 6.5, 1‴-H), 7.48 (1 H, d, *J* 1, 6-H) and 7.50 (1 H, d, *J* 1, 6″-H); $\delta_C(D_2O)$ 12.6, 38.5, 39.6, 61.6, 65.7, 69.4, 71.9, 75.8, 75.9, 85.7, 85.8, 85.9, 86.0, 86.5, 86.6, 112.4, 138.2, 138.3, 152.3, 152.5, 167.0, 167.2 and 171.9; $\delta_P(D_2O)$ −0.24; *m*/*z* (−FAB) 545 (M^-, 100%).

2-Cyanoethyl (5′-{(*E*)-3-[(3′*R*)-3-benzyloxymethyl-5′-*O*-*tert*-butyldimethylsilyl-3′-deoxythymidin-3′-yl]-2-oxopropylidene}-5′-deoxythymidin-3′-*O*-yl)(3′-acetylthymidin-5′-*O*-yl)phosphinate 37

Dicyclohexylcarbodiimide (123 mg, 0.6 mmol) and dichloroacetic acid (0.007 cm^3, 0.086 mmol) were added to a solution of the dinucleotide derivative **34** (110 mg, 0.171 mmol) in dimethyl sulfoxide (1 cm^3). After 1 h at room temperature, the reaction mixture was filtered and the retained solids washed with tetrahydrofuran (0.5 cm^3). The ylide **25** (500 mg, 0.635 mmol) and pyridine (0.1 cm^3, 1.2 mmol) were then added to the filtrate. The reaction mixture was stirred at room temperature for 24 h, diluted with dichloromethane and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 1% methanol in dichloromethane gave the *title compound* **37** (120 mg, 62%) as an oil; ν_{max}/cm^{-1} 3170, 2253, 1704, 1667, 1468, 1275, 1027, 837 and 753; δ_H 0.10 (6 H, s, Me_2Si), 0.90 (9 H, s, Me_3CSi), 1.90 (9 H, s, 3 × Me), 2.05 (1 H, m, 2‴″-H), 2.08 (3 H, s, $MeCO_2$), 2.23 (1 H, m, 2‴″-H′), 2.35 (2 H, m, 2′-H_2), 2.5 and 2.58 (each 1 H, m, 2‴-H), 2.76 (5 H, overlapping m, 3‴-H_2, 3‴″-H and CH_2CN), 3.74 (1 H, m, 4‴″-H), 3.76 and 3.82 (each 1 H, dd, *J* 2, 11, 5‴″-H), 4.15 (1 H, m, 4′-H), 4.32 (4 H, m, CH_2O and 5′-H_2), 4.65 (3 H, m, CH_2Ph and 4‴-H), 5.02 (1 H, m, 3‴-H), 5.23 and 5.26 (each 0.5 H, s, 3′-H), 5.45 (2 H, s, 3‴″-CH_2), 6.11 (2 H, m, 1‴-H and 1‴″-H), 6.25 (1 H, q, *J* 7, 1′-H), 6.34 and 6.38 (each 0.5 H, dd, *J* 1.5, 16, 1‴″-H), 6.85 and 6.87 (each 0.5 H, dd, *J* 16, 4, 5‴-H), 7.02 and 7.04 (each 0.5 H, s, 6-H), 7.28 (6 H, m, ArH and 6″-H), 7.52 (1 H, s, 6‴″-H) and 9.70 (2 H, m, 2 × NH); δ_P −1.83 and −2.01; *m*/*z* (−ES) 1137 (M^-, 30%).

3′-*O*-Acetyl-5′-{3-[(3′*R*)-5′-(4,4′-dimethoxytrityl)-3′-deoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidine 39

Diisopropylethylamine (0.12 cm^3, 0.68 mmol), 4,4′-dimethoxytrityl chloride (180 mg, 0.55 mmol) and 4-dimethylaminopyridine (5 mg) were added to a solution of the *C*-linked dinucleotide **20** (250 mg, 0.46 mmol) in dichloromethane (5 cm^3). After 4 h, the reaction mixture was diluted with dichloromethane, washed with water and brine and dried (Na_2SO_4). After concentration under reduced pressure, chromatography of the residue using 3% methanol and 1% triethylamine in dichloromethane as eluent gave the *title compound* **39** (370 mg, 95%) as a white foam, $[\alpha]_D$ +4.1 (*c* 1.02, $CHCl_3$) (Found: M^-, 850.3396. $C_{46}H_{50}N_4O_{12}$ requires *M*, 850.3425); ν_{max}/cm^{-1} 3207, 3058, 1692, 1607, 1509, 1466, 1250, 1177 and 1033; δ_H 1.50 (3 H, s, 5-Me), 1.90 (3 H, s, 5‴-Me), 1.94 (3 H, m, 5′-H_2 and 2⁗-H), 2.06 (3 H, s, $MeCO_2$), 2.17 (1 H, m, 2′-H), 2.30–2.60 (6 H, overlapping m, 2′-H′, 1″-H_2, 3″-H_2 and 2⁗-H′), 2.76 (1 H, m, 3⁗-H), 3.24 (1 H, dd, *J* 4, 11, 5⁗-H), 3.47 (1 H, dd, *J* 3, 11, 5⁗-H′), 3.75 (7 H, m, 2 × OMe and 4⁗-H), 3.87 (1 H, m, 4′-H), 4.95 (1 H, m, 3′-H), 6.08 (1 H, dd, *J* 4, 7, 1‴-H), 6.20 (1 H, dd, *J* 5, 8, 1′-H), 6.80 (4 H, m, ArH), 7.05 (1 H, s, 6-H), 7.25 (7 H, m, ArH), 7.42 (2 H, m, ArH) and 7.57 (1 H, s, 6‴-H); *m*/*z* (−FAB) 850 (M^-, 75%) and 547 (40).

5′-{3-[(3′*R*)-5′-(4,4′-Dimethoxytrityl)-3′-deoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidine 40

Ammonia gas was bubbled through a solution of the acetate **39** (260 mg, 0.31 mmol) in methanol (15 cm^3) at room temperature for 10 min. The solution was warmed to 40 °C for 5 h, then concentrated under reduced pressure. The residue was dissolved in dichloromethane, washed with water and brine, then dried (Na_2SO_4). Concentration under reduced pressure gave the *title compound* **40** (200 mg, 81%) as a white foam, $[\alpha]_D$ +4.0 (*c* 0.69, $CHCl_3$) (Found: M^-, 808.3347. $C_{44}H_{48}N_4O_{11}$ requires *M*, 808.3320); ν_{max}/cm^{-1} 3452, 3184, 3057, 1653, 1608, 1509, 1469, 1255, 1178, 1035 and 733; δ_H 1.50 (3 H, s, 5-Me), 1.87 (3 H, s, 5‴-Me), 1.95 (3 H, m, 5′-H_2 and 2⁗-H), 2.15 (1 H, m, 2′-H), 2.30 (2 H, m, 2′-H′ and 2⁗-H′), 2.44 (2 H, m, 3″-H_2), 2.46 and 2.57 (each 1 H, m, 1″-H), 2.75 (1 H, m, 3⁗-H), 3.23 (1 H, dd, *J* 4, 11, 5⁗-H), 3.44 (1 H, dd, *J* 3, 11, 5⁗-H′), 3.75 (8 H, overlapping m, 4′-H, 4⁗-H and 2 × OMe), 4.14 (1 H, m, 3′-H), 6.05 (1 H, dd, *J* 4, 6, 1⁗-H), 6.12 (1 H, t, *J* 7, 1′-H), 6.80 (4 H, m, ArH), 7.05 (1 H, s, 6-H), 7.24 (7 H, m, ArH), 7.38 (2 H, m, ArH) and 7.57 (1 H, s, 6‴-H); *m*/*z* (−FAB) 808 (M^-, 40%).

5′-{3-[(3′*R*)-5′-(4,4′-Dimethoxytrityl)-3′-deoxythymidin-3′-yl]-2-oxopropyl}-3′-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5′-deoxythymidine 41

Diisopropylethylamine (0.02 cm^3, 0.12 mmol), tetrazole (7 mg, 0.12 mmol), and bis(diisopropylamino)-2-cyanoethoxyphosphine (0.08 cm^3, 0.50 mmol) were added to a solution of the *C*-linked dinucleotide **40** (200 mg, 0.25 mmol) in dichloromethane (3 cm^3). After 16 h, the reaction mixture was diluted with dichloromethane and extracted with saturated aqueous sodium carbonate and brine. The two aqueous layers were extracted with dichloromethane and the organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 4% methanol and 1% triethylamine in dichloromethane as eluent gave the *title compound* **41** (225 mg, 90%) as a white foam, $[\alpha]_D$ +3.0 (*c* 0.96, $CHCl_3$); ν_{max}/cm^{-1} 3200br, 1689, 1510, 1467, 1252, 1179 and 1034; δ_H 1.15 (6 H, d, *J* 7, 2 × CH*Me*), 1.22 and 1.23 (each 3 H, d, *J* 7, CH*Me*), 1.87 (3 H, s, 5-Me), 1.95 (2 H, m, 5′-H_2), 2.10–2.8 (14 H, overlapping m, 2′-H_2, 1″-H_2, 3″-H_2, 2⁗-H_2, 3⁗-H, CH_2CN and 5‴-Me), 3.20–3.88 (13 H, overlapping m, OCH_2, 4⁗-H, 5⁗-H_2, 2 × Me_2CH and 2 × OMe), 4.17 (2 H, m, 3′-H and 4′-H), 6.08 (1 H, dd, *J* 3.5, 6.5, 1⁗-H), 6.17 (1 H, m, 1′-H), 6.79 (4 H, m, ArH), 7.2 (10 H, m, ArH and 6-H) and 7.56 (1 H, s, 6‴-H); δ_P 149.30; *m*/*z* (−FAB) 954 (M^- − 54, 100%).

2-Cyanoethyl (5′-{3-[(3′*R*)-3′-deoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidin-3′-yl)(3′-acetylthymidin-5′-*O*-yl)-phosphinate 38

3′-*O*-Acetylthymidine **15** (153 mg, 0.542 mmol) and tetrazole (5 mg) were added to a solution of phosphoramidite **41** (420 mg, 0.417 mmol) in dichloromethane (5 cm^3). The reaction mixture was stirred at room temperature for 18 h, then diluted with dichloromethane and washed with 2% aqueous sodium carbonate, water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 2% methanol in dichloromethane gave the phosphite **42** (427 mg, 86%) as a white foam; *m*/*z* (−FAB) 1190 (M^-, 95%) and 970 (100); δ_P 175.27 and 176.68.

The phosphite **42** (100 mg, 0.084 mmol) was stirred in a solution of iodine in tetrahydrofuran–pyridine–water (40 : 20 : 1, 0.1 M, 5 cm^3) for 2 h. The reaction mixture was then diluted with dichloromethane and washed with saturated aqueous sodium thiosulfate, water and brine. The organic extract was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 3% methanol and 1% triethylamine in dichloromethane as eluent gave the phosphate **43** (91 mg, 90%) as a white foam; δ_P 8.45 and 8.64.

Dichloroacetic acid (0.1 cm^3, 1.21 mmol) was added to a solution of the phosphate **43** (90 mg, 0.075 mmol) in dichloromethane (5 cm^3). After 2 h, triethylamine (0.2 cm^3) was added and the reaction mixture diluted with dichloromethane, and washed with 2% aqueous sodium carbonate and brine. The aqueous extracts were exhaustively extracted with dichloromethane and the organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue using 8% methanol in dichloromethane gave the *title compound* **38** (49 mg, 73%) as a colourless glass, $[\alpha]_D$ +2.1 (*c* 0.42, MeOH) (Found: $M^+ + H$, 906.2942. $C_{38}H_{49}N_7O_{17}P$ requires *M*, 906.2923); ν_{max}/cm^{-1} 3416br, 3193br, 2250, 1693, 1470, 1274, 1031, 1006 and 756; δ_H([$^2H_4$]methanol) 1.97 (6 H, s, 2 × Me), 1.99 (3 H, s, Me), 2.1 (3 H, m, 2⁗-H and 5‴-H_2), 2.18 and 2.19 (each 1.5 H, s, $MeCO_2$), 2.36 (1 H, m, 2⁗-H′), 2.50–2.80 (8 H, overlapping m, 2′-H_2, 2‴-H, 1⁗-H_2, 3⁗-H_2 and 3⁗-H), 2.86 (1 H, dd, *J* 4.5, 14, 2‴-H′), 3.02 (2 H, m, CH_2CN), 3.79 (2 H, m, 4⁗-H and 5⁗-H), 3.96 (1 H, m, 5⁗-H′), 4.16 (1 H, m, 4‴-H), 4.34 (1 H, m, 4′-H), 4.42 (2 H, m, OCH_2), 4.51 (2 H, m, 5′-H_2), 5.02 (1 H, m, 3‴-H), 5.42 (1 H, m, 3′-H), 6.12 (1 H, dd, *J* 4, 7, 1⁗-H), 6.24 (1 H, m, 1‴-H), 6.35 (1 H, t, *J* 7.5, 1′-H), 7.46 and 7.47 (each 0.5 H, s, 6″-H), 7.63 and 7.64 (each 0.5 H, s, 6-H) and 8.05 (1 H, s, 6⁗-H); δ_C([$^2H_4$]methanol) 12.4, 12.5, 12.6, 20.1, 20.2, 20.8, 27.9, 30.7, 33.7, 37.3, 38.0, 39.4, 39.8, 45.5, 62.0, 64.5, 64.6, 69.1, 74.9, 79.4, 82.2, 82.3, 83.8, 83.9, 85.2, 85.3, 86.3, 86.4, 86.7, 87.3, 110.8, 112.0, 112.1, 118.6, 137.7, 138.0, 138.3, 152.1, 152.2, 152.3, 166.5, 172.2, 210.4 and 210.5; δ_P([$^2H_4$]methanol) −1.50; *m*/*z* (FAB) 906 ($M^+ + 1$, 30%), 834 (100) and 720 (100).

Palladium hydroxide (20%) on carbon (20 mg) was added to a solution of the unsaturated *C*-linked trinucleotide **37** (30 mg, 0.026 mmol) in methanol (1 cm^3). The reaction mixture was stirred under an atmosphere of hydrogen for 16 h, then filtered through Celite and the retained solids washed with methanol. The filtrate was concentrated under reduced pressure to afford the saturated *C*-linked trinucleotide **38** which was identical (NMR, IR, MS, TLC) to samples prepared from the dimethoxytrityl compound **43**.

(5′-{3-[(3′*R*)-3′-Deoxythymidin-3′-yl]-2-oxopropyl}-5′-deoxythymidin-3′-yl)(thymidin-5′-yl)phosphinate 44

Ammonia was bubbled through a solution of the phosphate triester **38** (20 mg, 0.022 mmol) in methanol (1 cm^3) for 10 min and the flask sealed and stored at room temperature for 16 h. The reaction mixture was then concentrated under reduced pressure. The residue was azeotroped with water–methanol (1 : 1) and then with anhydrous methanol to give the *title compound* **44** (18 mg, 100%) as a colourless glass; ν_{max}/cm^{-1} 3500br,

1692, 1472, 1274, 1228 and 1059; δ_H([$^2H_4$]methanol) 2.07, 2.08 and 2.12 (each 3 H, d, *J* 1, Me), 2.18 (3 H, m, 5‴-H_2 and 2⁗-H), 2.46 (4 H, m, 2′-H_2, 2‴-H and 2⁗-H′), 2.62 (1 H, ddd, *J* 4, 6, 14, 2‴-H′), 2.8 (5 H, m, 1⁗-H_2, 3⁗-H_2 and 3⁗-H), 3.90 (2 H, m, 4⁗-H and 5⁗-H), 4.06 (1 H, dd, *J* 3, 14, 5⁗-H′), 4.24 (4 H, m, 4′-H, 5′-H_2 and 4‴-H), 4.66 (1 H, m, 3′-H), 4.78 (1 H, m, 3‴-H), 6.22 (1 H, dd, *J* 3, 7, 1⁗-H), 6.38 (1 H, dd, *J* 6, 7.5, 1‴-H), 6.53 (1 H, t, *J* 7, 1′-H), 7.58 (1 H, d, *J* 1, 6″-H), 7.96 (1 H, d, *J* 1, 6-H) and 8.17 (1 H, d, *J* 1, 6⁗-H); δ_P([$^2H_4$]methanol) 0.60; *m*/*z* (−ES) 809 (M^-, 55%) and 82 (100).

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