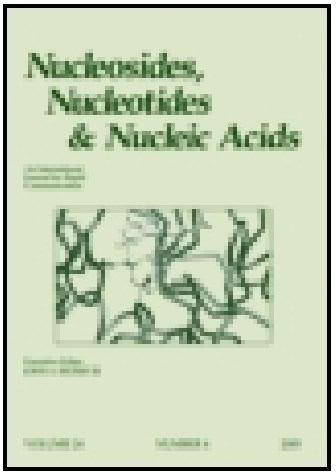


Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

The Preparation of a 3′-(2-Cyanoethyl)phosphoramidite of 5′-O-(3-Thiopropyl)methylphosphorylthymidine

Karen J. Percival [a] & Colin W. G. Fishwick [a]
[a] School of Chemistry, University of Leeds , Leeds, LS2 9JT, United Kingdom
Published online: 24 Sep 2006.

To cite this article: Karen J. Percival & Colin W. G. Fishwick (1995) The Preparation of a 3′-(2-Cyanoethyl)phosphoramidite of 5′-O-(3-Thiopropyl)methylphosphorylthymidine, Nucleosides and Nucleotides, 14:8, 1785-1794, DOI: 10.1080/15257779508009756

To link to this article: http://dx.doi.org/10.1080/15257779508009756

NUCLEOSIDES & NUCLEOTIDES, 14(8), 1785-1794 (1995)

THE PREPARATION OF A 3'-(2-CYANOETHYL)PHOSPHORAMIDITE OF 5'-*O*-(3-THIOPROPYL)METHYLPHOSPHORYLTHYMIDINE

Karen J. Percival and Colin W.G. Fishwick*
School of Chemistry, University of Leeds, Leeds LS2 9JT, United Kingdom

ABSTRACT: A high-yielding three-step reaction sequence to a useful novel phosphoramidite, using 3'-*O*-acetylthymidine as starting material, is reported.

There is at present intense interest in the synthesis and study of nucleic acids containing structurally variant features. In particular the effects of interstrand covalent cross-links in double helical DNA has aroused considerable attention. Methods used to gain access to such cross-linked strands include: nitrous acid-induced cross-linking through deoxyguanosine residues,[1] aziridines introduced on the purine bases[2] and use of 1,3,4,6-tetraazapentalene[3] chemistry affording covalently cross-linked mimics directing parallel strandedness in DNA. Introduction of dinucleoside analogues containing 6-*N*-(2-aminoethyl)-2'-deoxyadenosine has been aimed at producing cross-links in synthetic oligonucleotides.[4] Other cross-linking reagents which have been used include a naphthoquinone derivative,[5] the *p*-azidophenacyl group,[6,7] the proflavin residue,[8] the azidoproflavin residue,[9] a 5-azidosubstituted uridine residue,[10,11] and a 5'-methyl-N^4,N^4-ethanylcytidine residue.[12] The first five approaches require irradiation for cross-linking to occur, which is also the case for psoralen oligonucleotides.[13,14]

Recently, disulphide cross-linkages[15,16] have been introduced between modified thymidine bases to stabilise DNA hairpin structures. Such cross-linked DNA has seen application in a number of important areas. For instance in the inhibition of RNA transcription,[17,18] by prolonging the life-time of the duplex. Molecular recognition of DNA-protein complexes has also been studied using cross-linkers.[19-22] Other areas from which covalent cross-linking can yield interesting information include mutagenesis,[23] drug-DNA interactions[24] and, most importantly in our case, DNA-protein interactions.[22]

Our interest in developing methods for the preparation of such covalently linked structures arose from NMR-based structural studies of short DNA duplexes. Much work has been carried out on such oligonucleotides using modern NMR techniques.[25] However a common problem is difficulty in making a full spectroscopic assignment of such duplexes, owing to "fraying" of the duplex termini – due to the relative weakness of the interstrand hydrogen bonding at the terminal

bases. We planned to address this problem by covalently linking the termini of the duplex, in an attempt to prevent end fraying.

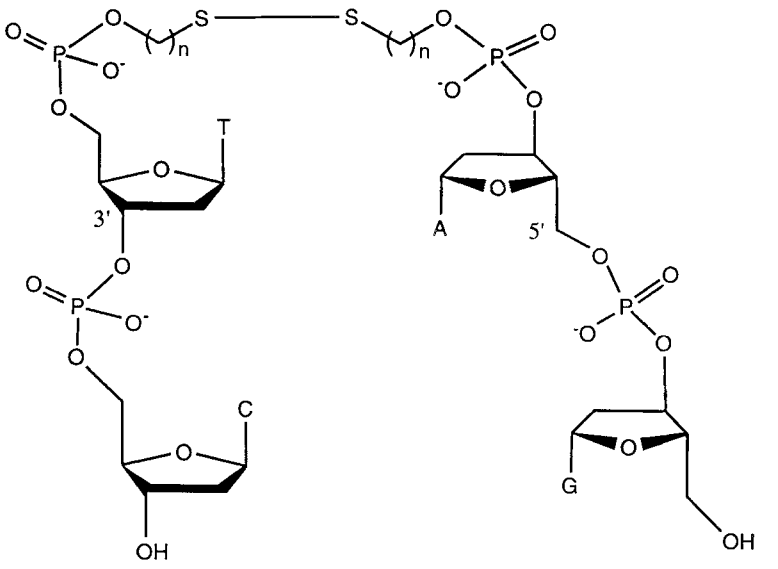
Therefore, our objective was to synthesise a DNA duplex, tied at the termini, using a disulphide bridge (**Figure1a, 1b**). Use of this particular covalent link was an attractive option, due to the ease of formation of disulphides and their facile reduction back to free sulphide[26] – and thus potential access to an untied analogue of the natural duplex, if required. We have used molecular modelling techniques to establish the optimum length of the duplex interstrand link (**Figure 1b**).

These have shown that an interstrand chain length corresponding to n = 3 (**Figure 1a**) is optimal in terms of minimising perturbations of the proximal base pairs. In addition, it was reasoned that utilising ω-thioalkyl substituted 5'- and 3'-terminal phosphoesters as a basis for such a covalent link (**Figure 1a**) would render the synthesis of the oligonucleotide relatively straightforward.

Although disulphide cross-links have been introduced in the central region of a duplex, by Glick[16] and others,[13] these have been centred around the bases – either using mismatched T-T pairs or introducing additional *N*-6-thiopropyl deoxyadenosine disulphide cross-links (which sit in the major groove and do not perturb the duplex, as judged by UV melting point studies). In this case,[15] the disulphide was formed by reduction of aminopropanethiol, using dithiothreitol, to produce free thiols on opposite strands. Aerobic dialysis subsequently produced the required disulphide link. Since we planned to form the linker from 3'- and 5'-substituted thiols on opposite strands in the preformed duplex, we therefore required access to the corresponding thionucleosides. We report here an effective preparation of a previously unknown novel phosphoramidite which is suitable for incorporation as the final 5'-nucleoside in solid-phase phosphoramidite[27] synthesis of end-linked oligonucleotides.

In order to prepare oligonucleotides capable of linking to fluorescent conjugates, Connolly and Rider[28] had previously prepared *S*-trityl-*O*-methoxymorpholino-3-mercaptopropoxyphosphite and incorporated it at the 5'-terminal end of an oligonucleotide. They have shown that these reagents appear to be compatible with the solid phase phosphoramidite approach. However, the coupling efficiency was not reported for this morpholinophosphoramidite. Therefore, we wished to develop an approach to similar species, based around *N,N*-diisopropylaminophosphoramidites – which have been shown to couple much more efficiently[29] than the *N*-morpholino analogues. Furthermore, incorporation of the *N,N*-diisopropylamino group facilitates purification of the monomers and increases their stability in solution.[30] We report here that *N,N*-diisopropylaminophosphoramidites undergo highly efficient coupling to 3'-*O*-acetyl thymidine, to give access to the title compound in essentially quantitative yields.

Initially, we chose to prepare β-cyanoethyl-*N,N*-diisopropylaminephosphite (**1a**),[31] for subsequent manipulation with cyanoethylphosphoramidites (**Scheme 1**). Synthesis of



n = 3 for optimimum linker length

Figure 1a

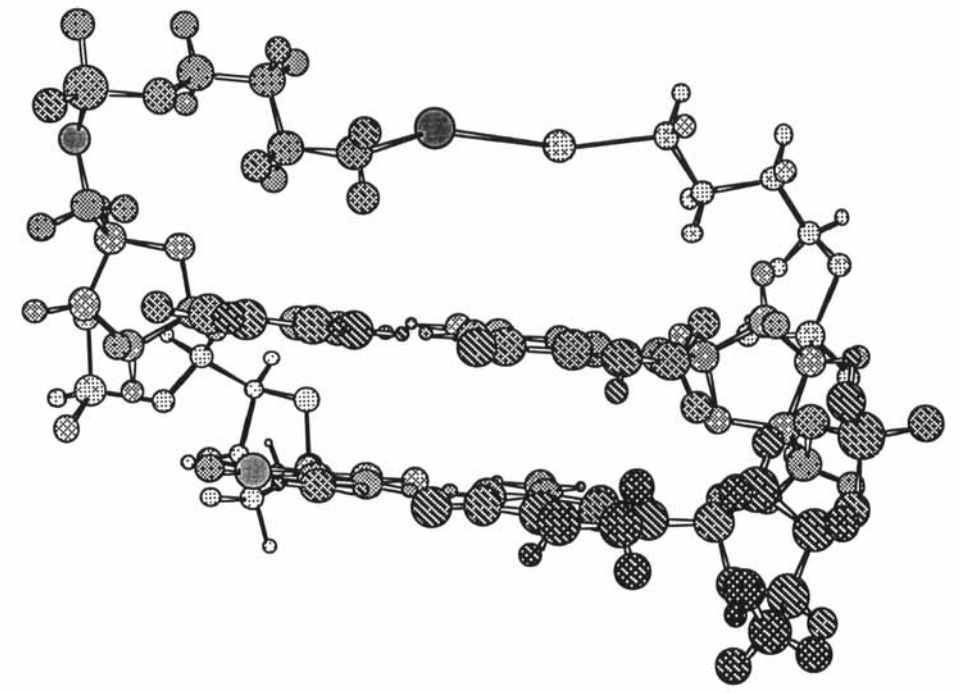
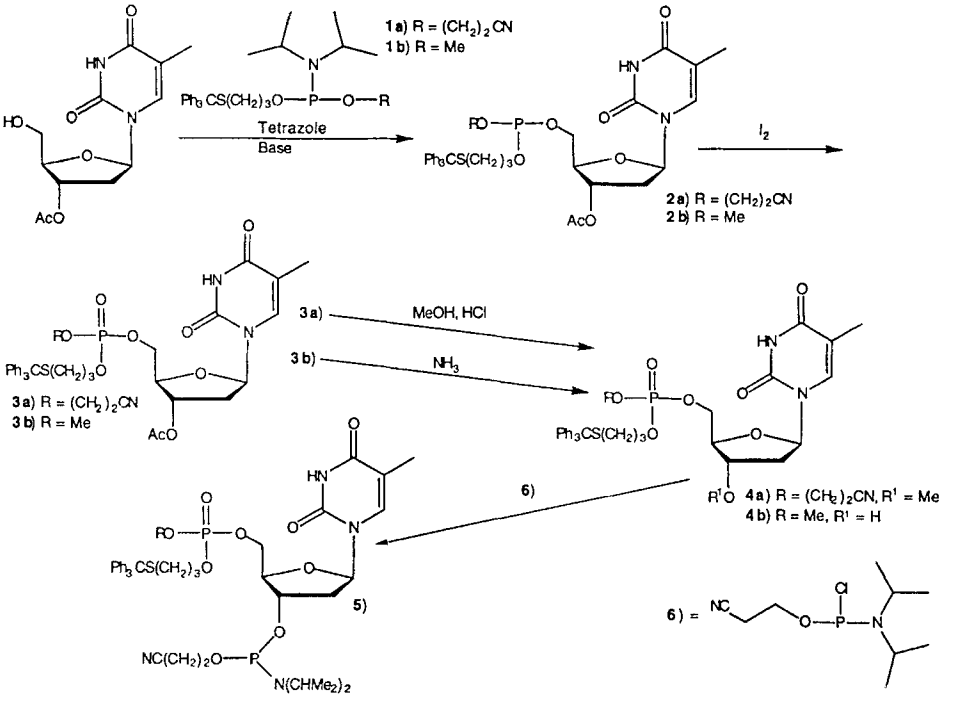


Figure 1b

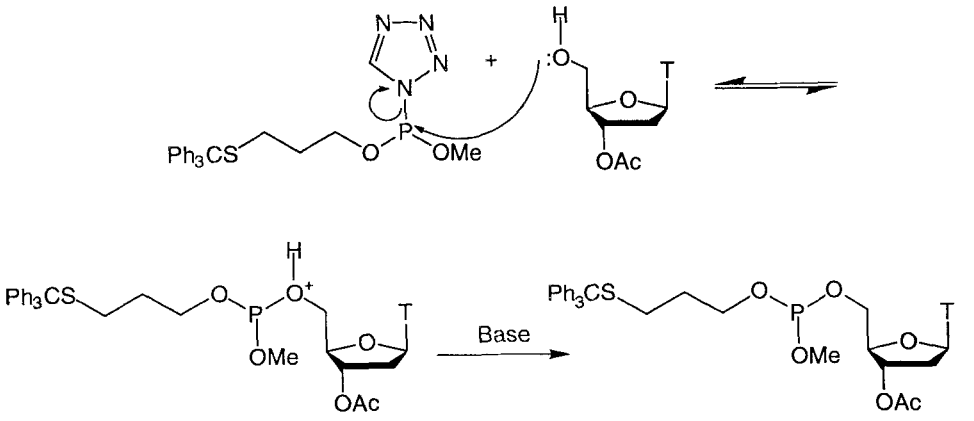


Scheme 1

phosphite (**1a**) was straightforward, but it should be used immediately as we found further purification leads to de-amination and decomposition. Attempts to solidify the glassy oil were unsuccessful, since prolonged exposure to air apparently leads to destructive oxidation.

Connolly and Rider[28] had reported solubility problems associated with the use of morpholinophosphoramidites. However, in the case of the *N,N*-diisopropyl-phosphoramidites we observed no such difficulties and all reagents for subsequent phosphitylation of 3'-*O*-acetylthymidine dissolved smoothly in THF. Addition of 3'-*O*-acetylthymidine to a solution of phosphite (**1a**) and tetrazole was expected rapidly to yield 3'-*O*-acetylthymidine-5'-*O*-(*S*-trityl-3-thiopropyl)(2-cyanoethyl)phosphoramidite (**2a**). However, monitoring by TLC (DCM-MeOH 8:2) showed that the reaction did not proceed in the absence of base.

Most reports[32] indicate spontaneous reaction of phosphite tetrazolides, resulting from nucleophilic displacement of diisopropylamine by tetrazole, with nucleosides at room temperature. Mechanistic studies[33,34] suggest that tetrazole behaves as an acid catalyst, as well as a nucleophile. Furthermore, the steric effect of substituents appears to impede these reactions which can be represented as involving an equilibrium step (**Scheme 2**). In our case, it



Scheme 2

was apparent from TLC that the tetrazolide formed successfully. However, in the absence of *N,N*-diisopropylethylamine, no forward reaction of the tetrazolide was observed. We suggest, therefore, that addition of base displaces the equilibrium (**Scheme 2**), *via* deprotonation, thus promoting formation of the product 3'-*O*-acetylthymidine-5'-*O*-(*S*-trityl-3-thiopropyl)(2-cyanoethyl)phosphoramidite (**2a**). Under these conditions, (**2a**) can be obtained in 70-80% yield. Recent work[35,36] has also shown facilitation of the coupling reaction by dialkylammonium tetrazolides, in high yield, using an equimolar mixture of tetrazole and diisopropylamine.

Phosphite (**2a**) was fully characterised by 2D-NMR and shown to be a mixture of diastereoisomers. Phosphite (**2a**) was oxidised, using conventional methodology,[36] to yield the corresponding phosphate (**3a**). (*S*-Trityl-3-thiopropyl)(2-cyanoethyl)diisopropylamino-phosphoramidite (**1a**) could not be adequately purified for use with a solid-phase synthesiser, due to its apparent instability. Crude phosphoramidite (**1a**) was therefore used in a solution-phase preparation of the more stable phosphoramidite (**5**).

Access to phosphoramidite (**5**) required deacylation of phosphate (**3a**). Since the β-cyanoethyl protecting group is base labile, we chose acid conditions for this procedure with methanol as solvent. Unfortunately, this lead to transmethoxylation of the 3'-hydroxyl group to give the corresponding 3'-methoxythymidine analogue (**4a**).

A change of solvent appeared impractical, on solubility grounds, so we instead opted to modify our synthesis. We chose to change phosphate protecting group, replacing the β-cyanoethyl function by methyl – since the latter is stable under both acid and base conditions, but deprotection can be readily achieved by thiophenol.[37] This approach required preparation of *S*-

trityl-3-thiopropylmethoxydiisopropylaminophosphoramidite[37] (**1b**), which was transformed, by application of the previous methodology and without difficulty, into phosphate (**3b**). Treatment with ammonia afforded hydroxynucleoside (**4b**), in high yield, after 22 hours at room temperature. Purification involved removal of acetamide *in vacuo* and filtration of a dichloromethane-methanol solution of the resultant white solid through a short pad of silica. A significant shift was observed in the position of the 3'-sugar proton resonance in the ^{1}H NMR spectrum, as was the disappearance of the methoxy signal at 2 ppm.

Excellent yields of the desired phosphoramidite (**5**) were achieved using the general procedure of Sinha *et al.*[38] Reaction of (**5**) and tetrazole lead to formation of the corresponding tetrazolide, as observed by TLC, with no apparent precipitation. In order to assess the coupling efficiency of phosphoramidite (**5**), a solution-phase reaction was performed with 3'-*O*-acetylthymidine. Analysis by TLC indicated complete coupling to produce the corresponding dinucleotide within 30 minutes.

This methodology for preparing 5'-*S*-tritylthioalkoxythymidine-3'-phosphoramidites has been shown to give good yields from cheap reagents under easily reproducible conditions. It affords products which are suitable for further reaction using solid support conditions. The stability of these compounds is also advantageous, making any further manipulation straightforward.

Melting points were measured on an Electrothermal apparatus and are uncorrected. ^{1}H NMR spectra were run on a 300 MHz General Electric spectrometer, or, for 2D spectra, on a Jeol 400 MHz spectrometer. $CDCl_3$ was used as solvent, with TMS as internal reference. In the ^{1}H NMR data, * indicates the presence of diastereoisomers. Phosphoric acid was used as an internal reference for ^{31}P NMR. All solvents were freshly distilled from calcium hydride and stored under nitrogen. Dichloromethane was neutralised by filtration through basic alumina and ethyl acetate was shaken with aqueous sodium hydrogen carbonate. The Macromodel molecular modelling package, applying the Amber force field for energy minimisation to a stable B-DNA fragment taken from the Brookhaven database, was used to generate **Figure 1b**.

(*S*-Trityl-3-thiopropyl)(2-cyanoethyl)diisopropylaminophosphoramidite (1a):

To a solution of *S*-trityl-3-thiopropanol[28] (0.5g, 1.49 mmol) dissolved in dry $CHCl_2$ (2 mL) was added *N,N*-diisopropylethylamine (1.04 mL, 5.9 mmol) and the reaction mixture was cooled to -78 ^{0}C. 2-Cyanoethyldiisopropylchlorophosphite (0.42 g, 1.7 mmol) was added slowly, by syringe, over 5 min. The mixture was then allowed to warm to r. t. over a period of 15 h, washed with EtOAc, shaken with aq $NaHCO_3$ (5%) and brine, dried (Na_2SO_4) and evaporated to give a pale yellow oil. Flash chromatography (petroleum ether-Et_3N, 9:1) gave (**1a**) as a colourless, viscose oil (0.75 g, 95%). Further attempts to purify this material lead to extensive decomposition. ^{1}H NMR: δ = 7.6-7.2 (15H, m, Ar), 3.75 (2H, m, CH_2OP), 3.56 (4H, m, $NCCH_2CH_2O$, $CH(CH_3)_2$), 2.56 (2H, t, J = 6 Hz, CH_2CN), 2.25 (2H, t, J = 7 Hz, CH_2S), 1.68 (2H, m, $CH_2CH_2CH_2$), 1.15 (12H, dd, J = 7, 15 Hz, $(CH_3)_2CH$). MS (EI, 70 eV): *m/z* (%) = 535 (M^+, 25). ^{31}P NMR ($CDCl_3$): δ = 147.8.

(*S*-Trityl-3-thiopropyl)methoxydiisopropylaminophosphoramidite (1b):

To a solution of *S*-trityl-3-thiopropanol[28] (0.1 g, 0.29 mmol) in dry CH_2Cl_2 (2 mL) under nitrogen, was added *N,N*-diisopropylethylamine (1.19 mmol, 0.15 g). This solution was cooled to -78 ^{0}C and 2-methoxydiisopropylchlorophosphite (0.35 mmol, 0.07 g) was added over 2-3 min. The reaction mixture was allowed to warm to r. t. over 15 h, when TLC analysis (CH_2Cl_2-MeOH, 8:2) indicated two partially overlapping spots. The mixture was poured into EtOAc, washed with aq $NaHCO_3$ (5-10%) and brine, dried (Na_2SO_4) and evaporated to give (**1b**) as a pale yellow oil (0.129 g, 87%). Due to its apparent instability, this material was used without further purification. ^{1}H NMR: δ = 7.5-7.2 (15H, m, Ar), 3.75 (3H, dd*, CH_3O), 3.5 (4H, m, CH_2O, $CH(CH_3)_2$), 2.3 (2H, t, J = 7 Hz, CH_2S), 1.8 (2H, m, $CH_2CH_2CH_2$), 1.3 (12H, dd, J = 7, 15 Hz, $(CH_3)_2CH$). MS (EI, 70 eV): *m/z* (%) = 496 (M^+, 10).

3'-*O*-Acetylthymidine-5'-*O*-(*S*-trityl-3-thiopropyl)(2-cyanoethyl)phosphoamidite (2a):

To a solution of (**1a**) (0.28 g, 0.5 mmol), which had been co-evaporated twice with dry THF, in dry THF (2 mL), was added tetrazole (0.1 g, 1.2 mmol) in dry THF (5 mL). The reaction was monitored by TLC (CH_2Cl_2-MeOH, 9:1) and was complete after 15 min. 3'-*O*-Acetylthymidine (0.127 g, 0.44 mmol) in dry THF (5 mL) was added, with *N,N*-diisopropylethylamine (0.23 g, 1.7 mmol). After 1 h, TLC analysis indicated completion of the reaction and the mixture was poured into $CHCl_3$, washed with aq $NaHCO_3$ and brine, then dried (Na_2SO_4) and evaporated to give an off-white gum. Flash chromatography over silica gel ($CHCl_3$-MeOH, 9:1) gave (**2a**) as a white foam (0.36 g, 80%). 2D ^{1}H NMR: δ = 8.35 (d, 1H, J = 4.5 Hz, HC=C), 7.4 (d, 6H, J = 3 Hz, Ar), 7.25 (m, 10H, Ar), 6.25 (dd, 1H, J = 3, 9 Hz, H-1'), 5.25 (m, 1H, H-3), 4.25 (m, 5H, $CNCH_2CH_2O$, H-4, H-5', H-5"), 4.2 (dd, 2H, J = 6 Hz, $S(CH_2)_2CH_2O$), 2.75 (m, 2H, CH_2CN), 2.40 (dd, 2H, H-2', H-2"), 2.25 (m, 2H, CH_2S), 2.12 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3C=C), 1.65 (m, 2H, $SCH_2CH_2CH_2O$). ^{31}P NMR: δ = 12.2, 12.56*.

Oxidation of 3'-*O*-acetylthymidine-5'-*O*-(*S*-trityl-3-thiopropyl)(2-cyanoethyl)-phosphoramidite (2b) to phosphoramidate (3b):

To (**2b**) (1.1 g, 1.73 mmol) in dry THF (2 mL) was added iodine (0.44 g, 1.73 mmol) in a mixture of lutidine-THF-water (0.37 g, 3.46 mmol:3.2 mL:0.4 mL). The reaction mixture was stirred at r. t. for 1.5 h, shaken with aq $NaHSO_3$, extracted with $CHCl_3$ and dried (Na_2SO_4). Evaporation gave a viscous yellow oil. Treatment with Et_2O produced a white foam, after decanting solvent and evaporation. Further purification by filtration through a short plug of silica (CH_2Cl_2) removed the last traces of lutidine and evaporation produced (**3b**) as a colourless oil (1.14 g, 95%). 2D ^{1}H NMR: δ = 9.8 (s, 1H, NH), 7.98 (s, 1H, HC=C), 7.5-7.2 (m, 15H, Ar), 6.45 (dd, 1H, H-1'), 5.24 (d, 1H, H-3'), 4.25-4.19 (m, broad, 3H, H-4', H-5', H-5"), 4.05 (m, 2H, CH_2OP), 3.75 (dd, 3H, J = 3 Hz, CH_3OP), 2.4 (m, 2H, H-2', H-2"), 2.25 (dt, 2H, J = 3 Hz, 4 Hz, CH_2S), 2.15 (s, 3H, CH_3CO), 1.98 (3H, s, CH_3C=C), 1.55 (m, 2H, $CH_2CH_2CH_2$).

Deacylation of 5'-*O*-(*S*-trityl-3-thiopropyl)methoxy-3'-*O*-acetylphosphoryl-thymidine phosphate (3b):

To a solution of (**3b**) (0.058 g) in methanol (5 mL) was added an excess of 8M NH_3 and the mixture was stirred at r. t. for 22 h. Initially the phosphate was not soluble in the MeOH-water mixture, but as reaction neared completion solubility improved. Evaporation at high vacuum removed solvent and acetamide, producing a viscous oil. Purification, by filtration through a short plug of silica gel (CH_2Cl_2-MeOH, 9:1), produced (**4b**) as a white foam (0.05g, 95%). 2D ^{1}H NMR: δ = 7.5-7.2 (m, 16H, Ar, HC=C), 6.3 (t, 1H, *J* = 6 Hz, H-1), 4.49 (s, broad, 1H, H-3), 4.4 (m, 2H, H-4, H-5'), 4.2 (m, 3H, CH_2O, H-5"), 3.7 (d*, 3H, CH_3O), 2.31 (m, 1H, H-2'), 2.26 (t, *J* = 7 Hz, 2H, CH_2S), 2.17 (m, 1H, H-2"), 1.9 (s, 3H, CH_3C=C), 1.6 (m, 2H, $CH_2CH_2CH_2$).

Phosphorylation of 5'-*O*-(*S*-trityl-3-thiopropyl)methoxyphosphorylthymidine (4b):

To a solution of (**4b**) (0.014 g, 0.021 mmol) in dry CH_2Cl_2 (2 mL) was added 4 eq of *N,N*-diisopropylethylamine (0.13 mL, 0.077 mmol). The reaction was cooled to -78 ^{0}C and 2-cyanoethyldiisopropylaminophosphine chloride (0.006 g, 0.0025 mmol) was added over a 5 min period. The reaction mixture was allowed to warm to r. t. and stirred for 4 h, poured into EtOAc, washed with aq $NaHCO_3$ and brine, then dried (Na_2SO_4) and evaporated to give a pale yellow oil. This was redissolved in CH_2Cl_2-pentane-Et_2O to give a cloudy white precipitate. After 30 min, an oil separated, the supernatant fluid was decanted and the resulting gum was evaporated to dryness, producing (**5**) as a white foam (0.02 g, 99%). ^{1}H NMR: δ = 8.5 (s, broad, 1H, NH), 7.4 (m, 6H, Ar, HC=C), 7.2 (m, 10H, Ar), 6.4 (t, *J* = 6 Hz, 1H, H-1), 5.2 (s, broad, 1H, H-3), 4.35 (m, 2H, $CNCH_2CH_2O$), 4.2 (m, 3H, H-4, H-5', H-5"), 4.0 (m, 2H, $S(CH_2)_2CH_2O$), 3.7 (d*, 3H, OCH_3), 3.45 (m, 2H, $N[CH(CH_3)_2]_2$), 2.8 (m, 3H, CH_2CN, H-2'), 2.45 (m, 1H, H-2"), 2.2 (t, *J* = 7 Hz, 2H, CH_2S), 1.9 (s, 3H, CH_3C=C), 1.65 (m, 2H, $CH_2CH_2CH_2$), 1.25 (dd, *J* = 7, 15 Hz, 12H, $N[CH(CH_3)_2]_2$).

We would like to thank the Wellcome Trust for the funding of KJP, also Julie Fisher and Mike Gradwell for advice and acquisition of 2D NMR.

REFERENCES

1. Kirchner, J.J., Hopkins, P.B. *J. Am. Chem. Soc.*, **1991**, *113*, 4681.
2. Cowart, M., Benkovic, S.J. *Biochemistry*, **1991**, *30*, 788.
3. Leonard, N.J., Bhat, B., Wilson, S.R., Cruickshank, K.A. *J. Am. Chem. Soc.*, **1991**, *113*, 1398.
4. Cosstick, R., Douglas, M.E. *J. Chem. Soc. Perkin Trans . 1*, **1991**, 1035.
5. Chatterjee, M., Rokita, S.E. *J. Am. Chem. Soc.*, **1990**, *112*, 6397.
6. Praseuth, D., Chassignal, M., Takasugi, M., De Doan, T., Thuong, N.T., Helene, C. *J. Mol. Biol.*, **1987**, *196*, 939.

7. Praseuth, D., Chassignal, M., Takasugi, M., Thuong, N.T., Helene, C. *Proc. Natl. Sci. USA*, **1988**, *85*, 1349.
8. Praseuth, D., Le Dean, T., Chassignol, M., Decout, J.L., Habhoub, N., Lhomme, J., Thuong, N.T., Helene, C. *Biochemistry*, **1988**, *27*, 3031.
9. Le Dean, T., Perrouault, L., Prascuth, D., Habhoub, N., Decout, J.-L., Thuong, N.T., Lhomme, J., Helene, C. *Nuc. Acid . Res.*, **1987**, *15*, 7749.
10. Evans, R.K., Johnson, J.D., Haley, B.E. *Proc. Natl. Acad. Sci . USA*, **1986**, *83*, 5382.
11. Evans, R.K., Haley, B.E. *Biochemistry*, **1987**, *26*, 269.
12. Webb, T.R., Matteucci, M. *Nucleic Acids Res.*, **1986**, *14*, 7661.
13. Lee, B.L., Blake, K.R., Miller, P.S. *Nucleic Acids Res.*, **1988**, *16*, 10681.
14. Lee, B.L., Murakami, A., Blake, K.R., Miller, P.S., Lin, S.-B. *Biochemistry*, **1988**, *27*, 3197.
15. Ferentz, A.E., Verdine, G.L. *J. Am. Chem. Soc.*, **1991**, *113*, 4000. Ferentz, A.E., *et al*, *ibid*, **1993**, *115*, 9006.
16. Glick, G.L. *J. Org. Chem.*, **1991**, *56*, 6746.
17. Knorre, D.G., Vlassa, V.V., Zarytova, V.F. In Oligodeoxynucleotides Antisense Inhibitors of Gene Expression, Macmillan Press, London, 1989; p. 173-196.
18. Vlassov, V.V., Godovikov, A.A., Kobets, N.D., Ryte, A.S., Yurchenko, L.V., Bukrinskaga, A.G. *Adv. Enzyme Regul.*, **1985**, *24*, 301.
19. Brent, T.P., Remack, J.S. *Nucleic Acids Res.*, **1988**, *16*, 6779.
20. Godorikova, T.S., Grachev, M.A., Kutyavin, J.V., Tsarev, J.G., Zarytova, V.F., Zaychikov, E.F. *Eur. J. Biochem.*, **1987**, *611*, 1166.
21. Chu, B.C.F., Orgel, L.E. *Nucleic Acids Res.*, **1988**, *16*, 3671.
22. Steitz, T.A. *Quart. Rev. Biophy.*, **1990**, *23*, 205.
23. Shaw, J.P., Milligan, J.F., Krawczyk, S.H., Matteucci, M. *J. Am. Chem. Soc.*, **1991**, *113*, 7765. Kirchner, J.J., Hopkins, P.B. *J. Am. Chem. Soc.*, **1991**, *113*, 4681.
24. Li, V.S., Kohn, H. *J. Am. Chem. Soc.*, **1991**, *113*, 275.
25. Patel, D.J., Shapiro, L. *Ann. Rev. Biophys. Biophys. Chem.*, **1987**, *16*, 423. Nikonowicz, E.P., Meadows, R.P., Fagen, P., Gorenstein, D.G. *Biochemistry*, **1991**, *30*, 1323.
26. Burns, J.A., Butler, J.C., Moran, J., Whitesides G.M. *J. Org. Chem.*, **1991**, *56*, 2648. Burns, J.A., Butler, J.C., Moran J., Whitesides, G.M. *J. Org. Chem.* **1991**, *56*, 2332.
27. Beaucage, S.L., Caruthers, M.H. *Tetrahedron Letters*, **1981**, *22*, 1859. Gait, M.J., Oligonucleotide Synthesis:- A Practical Approach, I.R.L. Press, Oxford, 1984. Narang, S.A. *Tetrahedron* , **1983**, *39*, 3.
28. Connolly, B.A., Rider, P. *Nucleic Acids Res.*, **1985**, *13*, 4485.
29. Dahl, B.H., Nielson, J., Dahl, O. *Nucleotides and Nucleosides*, **1987**, *6*, 457.
30. McBride, L.J., Caruthers, M.H. *Tetrahedron Letters*, **1983**, *22*, 245.

31. Sinha, N.D., Cook, R.M. *Nucleic Acids Res.*, **1988**, *16*, 2659.
32. Brill, W.K.D., Nielson, J., Caruthers, M.H. *J. Am. Chem. Soc.*, **1991**, *113*, 3972. Stec, W.J., Zon, G. *Tetrahedron Letters*, **1984**, *25*, 5279.
33. Dahl, B.H., Nielson, J., Dahl, O. *Nucleic Acids Res.*, **1987**, *15*, 1729.
34. Berner, S., Muhlegger, K., Selger H. *Nucleic Acids Res.*, **1989**, 853.
35. De Mesmaeker A., Lebreto, J., Waldner, A., Wolf, R.M., Frier, S.M. *Synlett*, **1993**, 733.
36. Fontanal, M.L., Bazin, H., Teoule R. *Nucleic Acids Res.*, **1994**, *22*, 2022.
37. Mori, K., Subasingher, C., Stein, C.A., Cohen, J.S. *Nucleosides and Nucleotides*, **1988**, *8*, 649.
38. Sinha, N.D., Biemat, J., Koster H. *Tetrahedron Letters*, **1983**, *24*, 5843.

Received March 4, 1994
Accepted June 30, 1995