Synthesis and Phosphorus–Sulphur Bond Cleavage of 3′-Thiothymidylyl(3′-5′)thymidine

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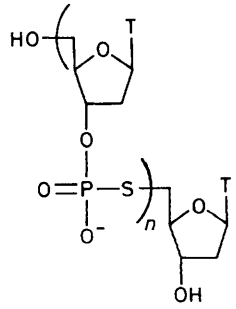
The title compound is readily prepared from 5′-*O*-monomethoxytrityl-3′-thiothymidine (**5**); cleavage of the P–S bond can be accomplished by mild oxidative hydrolysis.

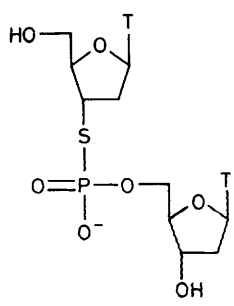
Oligonucleotides containing phosphorothioate linkages have proved useful tools for the study of DNA-processing enzymes and DNA structure.[1] In addition their increased resistance to nuclease activity suggests a potential application in the antisense approach[2] to viral chemotherapy. Although there is a considerable literature relating to these analogues, the isomeric linkages in which a sulphur atom replaces one of the two bridging oxygen atoms in the phosphodiester bond have received comparatively little attention. To our knowledge reports concerning the bridging sulphur analogues have been confined to that in which the 5′-oxygen atom is replaced by sulphur[3] for example the oligo-5′-thiothymidylates (**1**).[3,4] We report here the synthesis, enzymatic cleavage, and chemical hydrolysis of 3′-thiothymidylyl(3′-5′)thymidine (**2**), in which the corresponding 3′-oxygen atom is replaced by sulphur. The position of the sulphur atom makes this dinucleotide analogue a particularly interesting substrate for many nucleases.

The strategy adopted by Nagyvary *et al.*[4] for the synthesis of a variety of oligo-5′-thiothymidylates involved the nucleophilic displacement of a 5′-tosyl group by a nucleoside 3′-phosphorothioate. However, this route gave only mediocre yields and appears to be of limited applicability. In designing the synthesis of (**2**) we were primarily concerned with developing a strategy that would be compatible with the modern solid-phase methods of oligonucleotide synthesis. We therefore chose to prepare 5′-*O*-monomethoxytrityl-3′-thiothymidine (**5**) as an intermediate which could be utilised in phosphite triester protocols (Scheme 1).

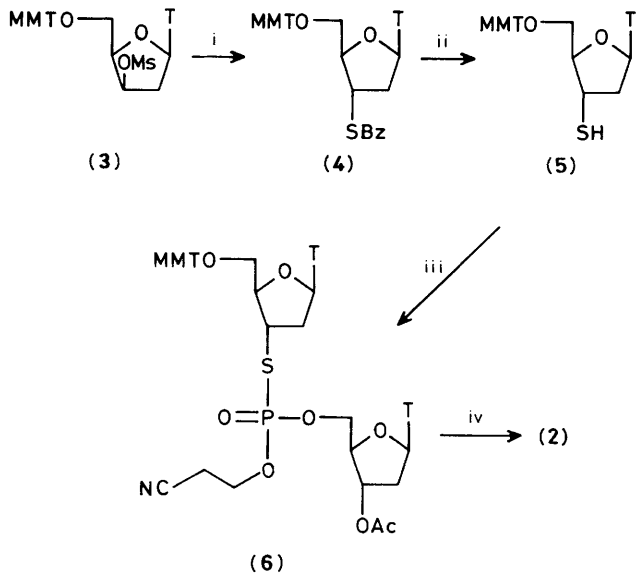
3-(2-Deoxy-5-*O*-monomethoxytrityl-3-*O*-methylsulphonyl-xylosyl) thymine (**3**) was prepared according to the literature[5] and treated with sodium thiobenzoate in dimethylformamide (DMF) to give the thioester (**4**) in 91% yield. Debenzoylation



(**1**)



(**2**)



Scheme 1. *Reagents and conditions:* i, NaSBz, dimethylformamide, 100 °C, 4 h; ii, NH_3, MeOH, room temp., 1 h; iii, (tetrazol-1-yl)$_2$-PO[CH_2]$_2$CN, 4:1 tetrahydrofuran–2,6-lutidine, −78 °C, 15 min; 3′-*O*-acetylthymidine, 20 min; then aqueous work-up; iv, Bu^tNH_2, pyridine, room temp., 1 h; aqueous 80% AcOH, room temp., 5 h; aqueous 35% NH_3, 50 °C, 3 h.

with saturated methanolic ammonia for 1 h gave the free thiol† (**5**) (87%) and a small quantity of the corresponding disulphide; prolonged exposure to these conditions gave the disulphide quantitatively.

Coupling of (**5**) to 3′-*O*-acetylthymidine was performed according the procedure of Fourrey and Shire[6] using 2,6-lutidine instead of pyridine (Scheme 1). The bistetrazolyl derivative of 2-cyanoethyl phosphorodichloridite[7] was prepared *in situ* at −20 °C, cooled to −78 °C, and treated with (**5**). After 15 min 3′-*O*-acetylthymidine was added and 20 min later the solution was allowed to warm to room temperature. ^{31}P N.m.r. analysis at this stage indicated the presence of the two diastereoisomers of the intermediate thiophosphite. Oxidation was accomplished by partitioning the reaction mixture between dichloromethane and sodium hydrogen carbonate and storing at 5 °C overnight. The fully protected dinucleotide (**6**) was isolated in 47% yield after column chromatography. Deprotection under standard conditions (Scheme 1) provided (**2**) in good yield.

As expected, on the basis of previous studies on thymidine 5′-*O*-(*S*-ethyl phosphorothioate),[8] compound (**2**) was cleaved by snake venom phosphodiesterase to give thymidine 5′-phosphate and 3′-thiothymidine. Interestingly this analogue was resistant to hydrolysis by nuclease P1; studies with other nucleases are in progress. The P–S bond can also be cleaved chemically under oxidative conditions:[8] treatment with a solution of iodine in aqueous acetone gave thymidine 5′-phosphate and 3′-thiothymidine disulphide as the sole products. The mild and highly specific nature of this reaction suggests that longer oligonucleotides containing this linkage could be hydrolysed exclusively at the point of modification. The incorporation of dinucleotidic moieties such as (**2**) into oligonucleotide primers and their subsequent chemical cleavage may thus prove to be a useful technique for the 'nicking' and manipulation of DNA.

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† *Selected spectroscopic data.* Compounds (**2**)—(**6**) were characterised by ^{1}H n.m.r., fast atom bombardment (f.a.b.) mass spectrometry and, where appropriate, ^{31}P n.m.r. For (**2**): ^{1}H n.m.r. (D_2O) δ (relative to sodium 3-trimethylsilyl[$^2H_4$]propanoate) 7.84 (1H, s, H-6), 7.79 (1H, s, H-6), 6.31 (1H, t, H-1′ of -pdT), 6.01 (1H, m, H-1′ of dTsp-), 4.57 (1H, m, H-3′ of -pdT), 4.30 (1H, m, H-5′ of -pdT), 4.13 (2H, m, H-5′, H-4′ of -pdT), 3.97 (3H, m, H_2-5′, H-4′ of dTsp-), 3.52 (1H, m, H-3′ of dTsp-), 2.65 (2H, m, H_2-2′ of dTsp-), 2.37 (2H, t, H_2-2′ of -pdT), and 1.84 (6H, s, CH_3); *m/z* (f.a.b.) (*m*-nitrobenzyl alcohol) 561 (M^-); ^{31}P n.m.r. (D_2O) δ 17.40 p.p.m.

For (**6**): *m/z* (f.a.b.) (*m*-nitrobenzyl alcohol) 930 (M + H) and 952 (M + Na); ^{31}P n.m.r. ($CDCl_3$) δ 26.0 and 26.4 p.p.m.

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