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NUCLEOSIDE PHOSPHONODITHIOATES AS INTERMEDIATES IN THE PREPARATION OF DINUCLEOSIDE PHOSPHORODITHIOATES AND PHOSPHOROTHIOATES

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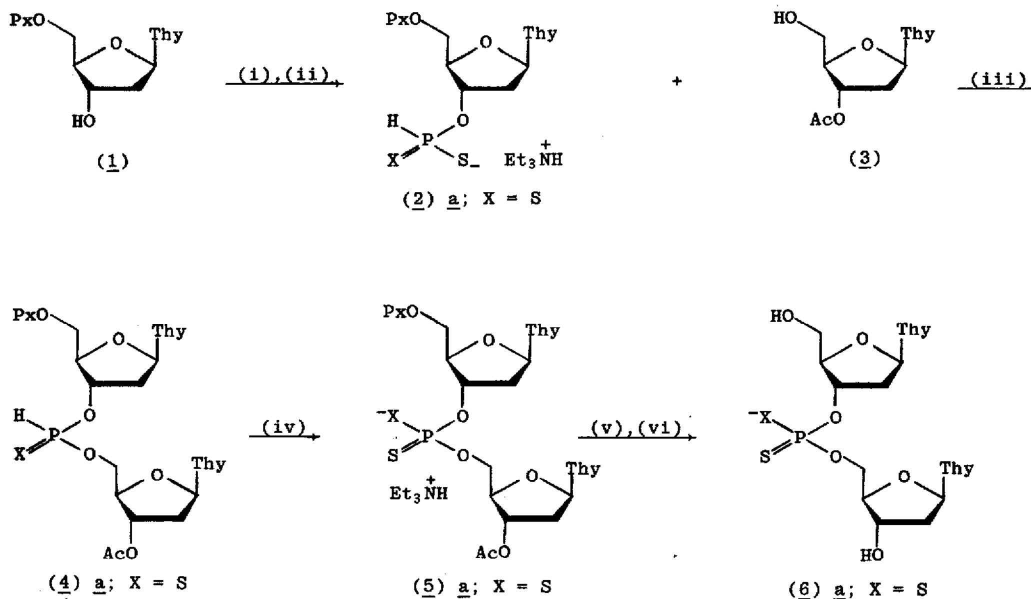
Summary: 5'-*O*-(9-Phenylxanthen-9-yl)thymidine (1) is converted into the triethylammonium salt of its 3'-phosphonodithioate (2a) in good yield; the latter compound is converted into a dinucleoside phosphonothioate (4a) and thence into a dinucleoside phosphorodithioate (6a) in good overall yield.

Phosphorothioate analogues of oligonucleotides have shown potential as antiviral agents[1], and are of value in other studies involving interactions with nucleic acids and proteins[2]. The latter analogues contain chiral internucleotide linkages and therefore suffer from the potential disadvantage that they may consist of complex mixtures of diastereoisomers; they are also to some extent susceptible to phosphodiesterase-promoted digestion. For these reasons, an interest has developed recently[3,4] in the preparation and properties of phosphorodithioate analogues of oligonucleotides. The synthetic methodology used in the work published so far has been based mainly on the phosphoramidite approach[5] to oligonucleotide synthesis. However, a report has just appeared[6] (see below) on the synthesis of dinucleoside phosphonothioates and hence of dinucleoside phosphorodithioates based on the use of protected nucleoside 3'-phosphonothioates [corresponding to (2; X = O)] as starting materials. We now present an alternative and convenient approach to the synthesis of phosphorodithioate analogues which involves the use of protected nucleoside 3'-phosphonodithioates [e.g. (2a)] as starting materials.

The triethylammonium salt of 5'-*O*-(9-phenylxanthen-9-yl)thymidine 3'-phosphonodithioate (2a) was prepared from the corresponding nucleoside derivative (1)[7] by the procedure indicated in the Scheme and, following chromatography on silica gel, was isolated as a stable, colourless precipitated solid in 75% yield; it was found to be t.l.c. homogeneous [R_F 0.65 in Pr^iOH - aq. NH_3 (*d* 0.88) - H_2O (7:1:2 v/v)], and was identified by n.m.r. spectroscopy [δ_P ($CDCl_3$) 85.0 (J_{PH} 546.3 Hz)]. When the latter compound [(2a), 3.0 mol. equiv.] was allowed to react with 3'-*O*-acetylthymidine[8] [(3), 1.0 mol. equiv.] and pivaloyl chloride[9] [2 x 3.0 mol. equiv.] for 20 min at room temperature, the fully-protected dinucleoside phosphonothioate (4a)[10] [R_F 0.44 in $CHCl_3$-MeOH (9:1 v/v); δ_P ($CDCl_3$) 70.8 (J_{PH} 669.8 Hz), 72.2 (J_{PH} 664.0 Hz)] was obtained and isolated in 60% yield [based on (3)]. However, when the crude reaction products were first allowed to react with sulphur [10.0 mol. equiv.] for 15 min at room temperature and then worked-up and

Scheme



Reagents: (i) reagent derived from PCl_3 [4 mol. equiv. with respect to (1)], 1,2,4-triazole [12 mol. equiv.], Et_3N [13 mol. equiv.], tetrahydrofuran, N_2, -35°C, 15 min; (ii)(a) H_2S, Et_3N, tetrahydrofuran, RT, 30 min, (b) N_2, RT, 1 hr; (iii) Me_3CCOCl, C_5H_5N-MeCN (5:1 v/v), RT, 20 min; (iv) S_8, pyridine, RT, 15 min; (v) aqueous NH_3 (*d* 0.88), RT, 2.5 hr; (vi) 0.01M hydrochloric acid (pH 2), RT, 1 hr.

chromatographed on silica gel, the triethylammonium salt of the protected dinucleoside phosphorodithioate (5a) [R_F 0.63 in Pr^iOH - aq. NH_3 (*d* 0.88) - H_2O (7:1:2 v/v); δ_P 113.2] was obtained and isolated as a pure colourless precipitated solid in 72% overall yield, based on (3). Removal of the protecting groups from the terminal 3'- and 5'-hydroxy functions in the usual way [see Scheme] gave the free dinucleoside phosphorodithioate (6a) as an HPLC-homogeneous [R_T 8.8 min, Jones APEX ODS column eluted with 0.1M - aqueous triethylammonium acetate (pH 7.0) - acetonitrile (88:12 v/v)] compound, δ_P (D_2O) 114.1 [lit.[3a], 113.3, for ammonium salt], in virtually quantitative yield. We confirm the recent observation[3a] that (6a) does not undergo digestion in the presence of snake venom phosphodiesterase; indeed no detectable digestion of (6a) was observed in *ca*. 300 times the time required for the complete digestion of thymidylyl-(3'→5')-thymidine under the same conditions. We now report that (6a) also appears to be totally resistant to digestion in the presence of bovine spleen phosphodiesterase and nuclease P_1.

We have also confirmed the reports[3a,6] that treatment of protected dinucleoside phosphonothioates [e.g. (4a)] with iodine in wet pyridine gives the corresponding diastereoisomeric mixtures of protected dinucleoside phosphorothioates [e.g. (5; X = O)]. Thus when (4a) was treated with 1.3 mol. equiv. of iodine in tetrahydrofuran - pyridine - water - 1-methylimidazole (40:5:4:1 v/v) and the protecting groups then removed from the terminal hydroxy functions, a mixture of thymidylyl-(3'→5')-thymidine [TpT, *ca.* 4%] and the corresponding diastereoisomeric phosphorothioates [(6; X = O), *ca.* 96%] was obtained; when 5.0 mol. equiv. of iodine with respect to (4a) was used, the percentage of TpT in the mixture of products obtained increased to 68. It is therefore clearly important to limit the quantity of iodine used in this oxidation reaction.

We have deliberately based our synthesis of protected dinucleoside phosphonothioates [such as (4a)] on phosphonodithioate [e.g. (2a)] rather than on phosphonothioate [e.g. (2; X = O)] building blocks. Nielsen *et al.* reported[3a] that all of their attempts to carry out such a synthesis starting from phosphonothioate building blocks failed and, in any case, the coupling of a phosphonothioate [such as (2; X = O)] with a protected nucleoside derivative [such as (3)] can, in principle, lead to the formation of a dinucleoside phosphonothioate [such as (4a)], a dinucleoside phosphonate [such as (4; X = O)], or a mixture of both. In their recent article, Stawiński *et al.*[6] implied that the pivaloyl chloride - promoted activation of a phosphonothioate building block [corresponding to (2; X = O)] proceeded solely by acylation on oxygen as no dinucleoside phosphonate [corresponding to (4; X = O)] was reported to be present in the products. This is rather surprising in the light of the authors' suggestion[6] that the resulting protected dinucleoside phosphonothioate [corresponding to (4a)] can then readily undergo pivaloyl chloride-promoted activation involving acylation on sulphur. The full clarification of this matter must await further investigation.

In conclusion, we believe that the procedure described in this article is potentially generally applicable to the synthesis of phosphorodithioate analogues of oligodeoxyribo- and oligoribo-nucleotides both in solution and on solid supports.

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[10] The phosphonothioate linkage in (4a) is more stable to alkaline hydrolysis than the phosphonate linkage in (4; X = O) by more than an order of magnitude. When a 0.012M solution of (4a) in tetrahydrofuran - Et_3N - H_2O (8:1:1 v/v) was allowed to stand at room temperature, the substrate underwent *ca.* 50 and 100% degradation after 90 and 720 min, respectively. The corresponding times observed for the degradation of the dinucleoside phosphonate (4; X = O) under the same conditions were 6 and 40 min, respectively. 3'-*O*-Acetylthymidine (3) is completely resistant to hydrolysis under these reaction conditions.

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