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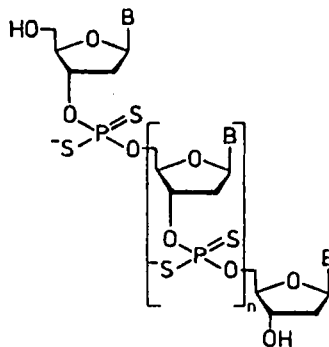
SYNTHETIC APPROACHES TO OLIGODEOXYRIBONUCLEOSIDE PHOSPHORODITHIOATES USING TERVALENT PHOSPHORUS MONOMERS

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Abstract. Two routes to the title compounds using nucleoside phosphorodiamidites or thiophosphoramidites have been explored. A suitably substituted thymidine thiophosphoramidite could be used for tetrazole catalysed solid support syntheses of oligomers (2- to 10-mers).

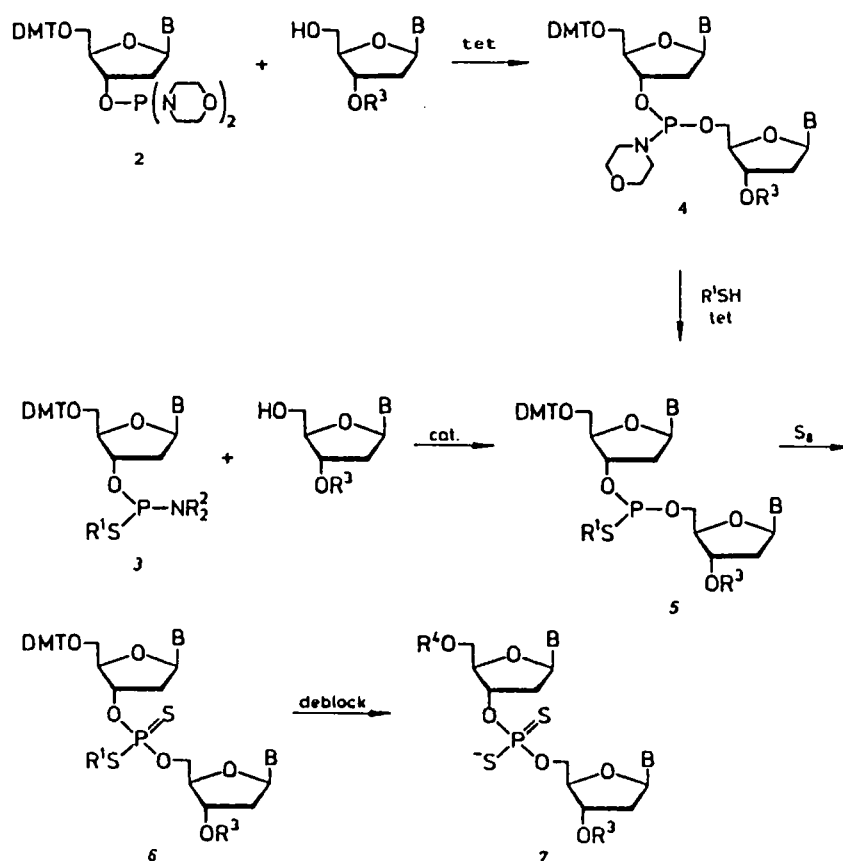
Modified oligodeoxyribonucleotides containing phosphorodithioate groups, 1, are promising alternatives to phosphorothioates as nuclease-resistant antisense probes since they



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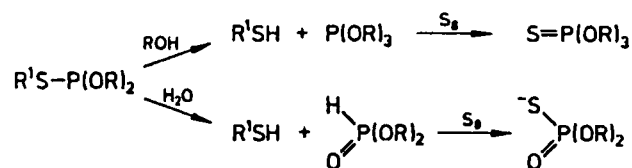
lack chiral phosphorus centers. The first member of this class, a thymidine dimer (1, $n = 0$, $B = T$), has recently been described.¹ We have investigated two potential routes to prepare 1 from the tervalent monomers 2 or 3 (Scheme 1).

In the first route, the known dinucleoside phosphoramidite 4 ($R^3 = \text{Ac}$, $B = T$)² was treated with a thiol ($R^1 = 2\text{-cyanoethyl}$ or $2,4\text{-dichlorobenzyl}$) and tetrazole in acetonitrile. A rapid reaction occurred to give 5 (d_p 191.6-191.8, 2 dia-



Scheme 1

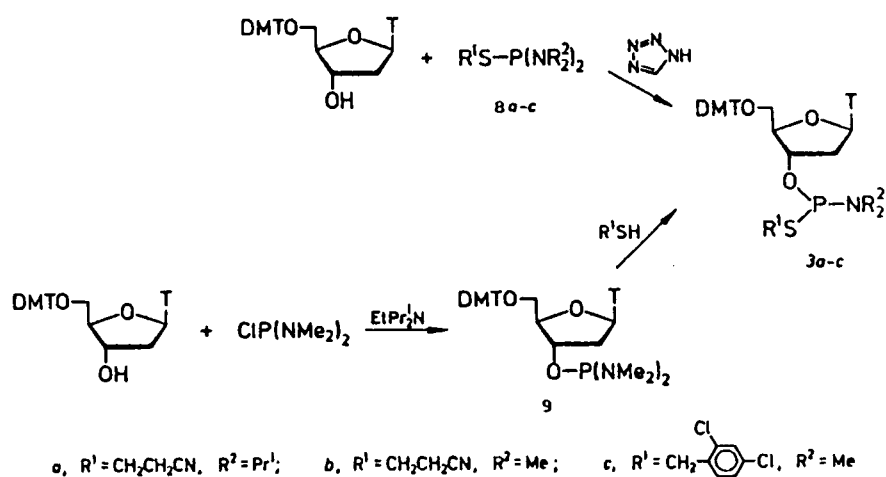
stereomers), and addition of S₈ to give **6** (δ_P 94.3-94.7, 2 diastereomers) and deblocking of the S-alkyl group with aq. NH₃ (R¹ = 2-cyanoethyl) or thiophenolate (R¹ = 2,4-dichlorobenzyl) to give **7** (R⁴ = DMT, R³ = ac, δ_P = 113-114.5, one isomer) proceeded without isolation of intermediates. According to ³¹P NMR the crude yield of **7** was 50-80%, depending of the purity of **4** and the time lapse before S₈ addition. The main impurities were a dinucleoside H-phosphonate, a dinucleoside phosphorothioate, and a trinucleoside phosphorothioate, probably all formed by reaction of **5** with water or 3'-acetylthymidine:



The presence of these impurities indicates that R^1S is a leaving group comparable to R^2_2N in the presence of tetrazole.

Attempts to use the monomer 2 for solid support syntheses of thymidine dimers gave low yields of 7 ($\text{R}^3 = \text{R}^4 = \text{H}$, $\text{B} = \text{T}$, 10–20% (^{31}P NMR) after deblocking). The main impurities were dithymidine phosphorothioate and thymidine H-phosphate, indicating severe hydrolysis of 4 or 5 during coupling.

For the second route the thiophosphoramidites 3a–c were prepared (Scheme 2). DMTdT and 8a gave 3a (70% after column chromatography), which however was very unreactive in coupling experiments with tetrazole as catalyst. The more reactive 3b and 3c were then made from 9 (since 8b and 8c were difficult to purify) and isolated as fairly pure powders (85–90% according to ^{31}P NMR) after aq. NaHCO_3 extraction and precipitation from hexane ($d_{\text{p}}(\text{CDCl}_3)$ 172.6 and 170.9 for



Scheme 2

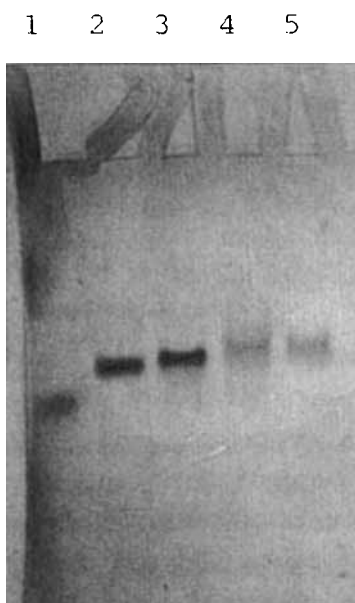


Fig. 1. PAGE (UV-shadowing), lane 1: Bromophenol blue, lane 2: $d(T)_{10}$, lane 3: $d(TpS)_9T$, lane 4 and 5: $d(TpS_2)_9T$.

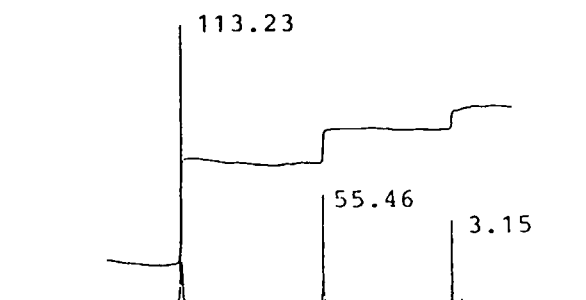


Fig. 2. ^{31}P NMR spectrum (D_2O) of crude $d(TpS_2)_9T$.

3b, 171.8 and 170.1 for 3c). Solution experiments showed that thymidine dimers 5 were formed rapidly with tetrazole catalysis (1-2 min), although subsequent side reactions consumed 5 slowly.

Solid support syntheses with 3b or 3c, using a standard amidite coupling cycle without capping (coupling 3 min, S_8 5 min), gave promising results, e.g. 1 ($n=8$, $B=T$) was synthesised with a mean DMT-efficiency of 99-101%. PAGE showed a main product running close to $d(T)_{10}$ and the phos-

phorothioate analogue (Fig. 1). However ^{31}P NMR showed that ca. 30% of phosphorus in the crude material was phosphorothioates or H-phosphonates (Fig. 2).

Work is in progress to examine the nuclease resistance and hybridisation properties of purified 1, and to extend the method to mixed sequences.

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