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The Synthesis of Di- and Oligo(deoxyribonucleoside phosphorodithioates) by Dithiaphospholane Method

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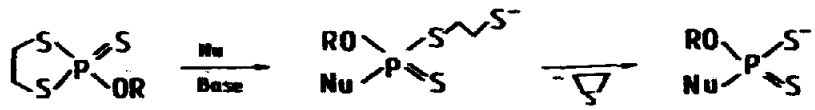
Abstract: Four dinucleoside phosphorodithioates: $T_{ps}T$ (1a), $d(C_{ps}C)$ (1b), $d(A_{ps}A)$ (1c) and $d(G_{ps}G)$ (1d) were synthesized by reacting solid support-bound nucleosides with nucleoside 5'-O-DMT-3'-O-(2-thio-1,3,2-dithiaphospholanes) (2a-d) in the presence of DBU. Pyrimidine pentamers and decamers, free from phosphorothioate contamination, were obtained by this method.

Oligonucleoside phosphorodithioates have proven useful as antisense modulators of gene expression and inhibitors of viral reverse transcriptases.[1-3] Several approaches to the synthesis of these analogues involving the use of phosphorodiamidite[2,4-7], thiophosphoramidite[1-3,7-11], H-phosphonothioate[12-14], H-phosphonodithioate[2,7,15-17] or phosphorodithioate[18,19] monomers have been reported. Recently, we have found, that 2-alkoxy-2-thio-1,3,2-dithiaphospholanes are phosphorodithioate precursors[20] *viz* a route analogous to that discovered by us earlier for the synthesis of oligo(nucleoside phosphoromonothioates).[21]



Thus, 2-n-butoxy-2-thio-1,3,2-dithiaphospholane reacts with n-butanol in the presence of base catalyst, such as t-BuOK or tertiary amines (Et_3N, N-methylimidazol, DMAP, DBU) to give O,O-di-n-butylphosphorodithioate in high yield.[22] In an effort to extend this observation as a possible basis for the formation of internucleoside phosphorodithioate bond, we have synthesized four base protected (except thymidine) 5'-O-DMT-deoxyribonucleoside-3'-O-(2-thio-1,3,2-dithiaphospholanes) (2a-d) by the reaction of the appropriate 3'-OH nucleoside with 2-N,N-diisopropylamino-1,3,2-dithiaphospholane[20] in the presence of tetrazole, followed by oxidation with elemental sulphur. The yields and physicochemical characteristics of 2a-d are listed in Table 1.

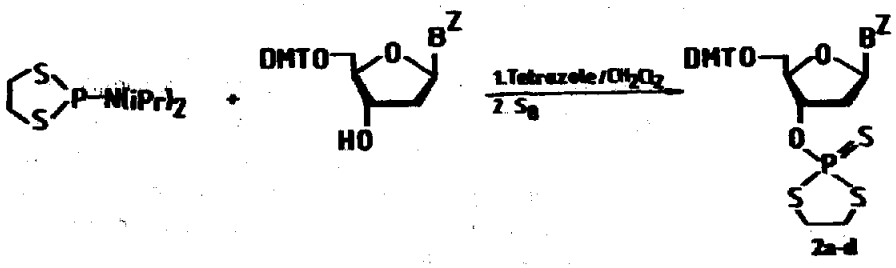


Table 1

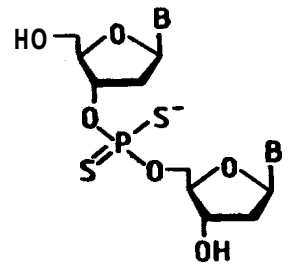
2	B	Z	δ ^{31}P NMR[a]	R_f[b]	Yii (%)
2a	Thy	-	124.74[c]	0.76	88.6
2b	Cyt	Bz	124.m	0.78	80.0
2c	Ade	Bz	124.349	0.79	50.4
2d	Gua	iBu	124.96[c]	0.61	74.4

[a] In CD_3CN, 85% H_3PO_4 as external reference, Bruker AC 200.
[b] TLC in $CHCl_3$:MeOH (9:1) as developing system.
[c] For 2-phenoxy-2-thio-1,3,2-dithiaphospholane δ ^{31}P NMR 120.7 ppm ($CHCl_3$) was reported.[23]

The thymidine derivative (2a) was reacted with a 10% excess of 3'-O-acetylthymidine in CH_3CN solution in the presence of equimolar amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give $T_{PS2}T$ (δ_{31P} = 113.8 ppm, D_2O) in 47% yield after ion exchange chromatography purification (DEAE Sephadex A-25). The following protocol have been proposed for the synthesis of oligo(nucleoside phosphorodithioates) *via* the dithiaphospholane method by using for 1 μmole of LCA CPG bound nucleoside, 30 μmole of 2 and 30 μmole of DBU.

Step (Reagent or Solvent)	Volume	Time (min)
1. Detritylation (3% $Cl_2CHCOOH$ in CH_2Cl_2)	3 ml	3
2. Wash (CH_3CN)	10 ml	3
3. Condensation [0.22M solution of dithiaphospholane 2 in CH_3CN (140μl) + 2M solution of DBU in CH_3CN (15 μl), premixed]		10
4. Wash (CH_3CN)	10 ml	3
5. Capping - a) and b) premixed a) Ac_2O : Lutidine : THF (1:1:8) b) N-methylimidazole : THF (16%)	0.4 ml 0.4 ml	3
6. Wash (CH_3CN)	10 ml	3

The above protocol (except capping) has been checked in the synthesis of dinucleoside phosphorodithioates 1a-d (Table 2). The $T_{PS2}T$ (1a) obtained by this method appeared to be identical with that obtained in solution (HPLC, ^{31}P NMR). The structures of 1a-d were confirmed by Negative LSIMS mass spectroscopy.[24] The identities of 1a-d were additionally confirmed by their conversion to the corresponding dinucleoside phosphates (3a-d) by treatment with butylene 1,2-oxide.[25] The products of oxidation (3a-d) were identified by HPLC co-injection with authentic samples prepared independently.[26]



1a, B=Thy
lb, B=Cyt
1c, B=Ade
1d, B=Gua

Table 2

1	HPLC[a] t_R (min)	Yield (%) (trityl test)	Yield (%) (from HPLC)
1a	16.49	99	97
1b	11.86	95	84
1c	14.80	96	68
Id	11.83	83	56

[a] HPLC:ODS Hypersil (5 μ), column 4.7x30, 1.5 ml/min, 0-20% CH_3CN ill 0.1M TEAB (Iii) in 20 min.

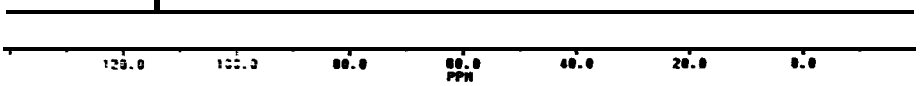


Figure 1. The ^{31}P NMR spectrum of phosphorodithioate pentamer 5 after HPLC Trityl ON purification. The blip at *ca* 57 ppm may indicate *ca* 1% phosphorothioate contamination.

Since the yields of the coupling reaction for the purine derivatives (1a, 1d) are still far from satisfactory, we have used the above protocol to synthesize only longer oligo(nucleoside phosphorodithioates) of the pyrimidine series. Thus, trinucleotide $T_{PS2}T_{PS2}T$ (4) and pentanucleotide $T_{PS2}T_{PS2}T_{PS2}T_{PS2}T$ (5) were obtained in good yields using sarcosinylated[27] LCA CPG. The structure of trinucleotide 4 was confirmed by negative LSIMS mass spectroscopy. ^{31}P NMR examination of the pentamer 5, synthesized on a 3 μmole scale, after purification by Trityl ON HPLC, showed two signals at 113.73 and 113.80 ppm (D_2O), characteristic for phosphorodithioate diesters[11], with almost no phosphate or phosphorothioate contamination (Figure 1). The pentamer 5 shows a PAGE mobility close to that of $T_{PS}T_{PS}T_{PS}T_{PS}T$ (slightly lower).[28] Similarly, tbe decamer $d[(C_{PS2})_9C]$ (6) was synthesized with an average "trityl yield" 96%. The analysis of the product, following its purification by Trityl ON - Trityl OFF HPLC, was performed by PAGE (20%. TBE buffer) showing the main product (ca 80%) to have slightly lower mobility than that of $d[(C_{PS})_9C]$. A minor product (a 20%) of apparent doubled length, as judged from PAGE mobility, was observed. Its detailed structure has not, as yet, been determined. Further experiments to improve coupling efficiency are in progress.

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