Use of 2,5-Dichlorophenyl Phosphorodichloridothioate in the Synthesis of Diastereoisomeric Dinucleoside Phosphorothioates

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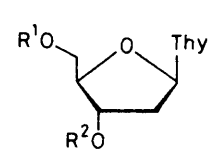
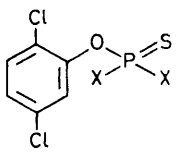
A convenient method for the synthesis, by the phosphotriester approach, of dinucleoside phosphorothioates is illustrated by the preparation and isolation of the pure R_p- and S_p-diastereoisomers of thymidine-3′ thymidine-5′ phosphorothioate (**5**)

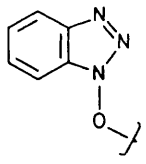
Synthetic dinucleoside phosphorothioates, which contain a chiral centre at phosphorus, have proved to be valuable intermediates in the elucidation of the stereochemistry of phosphorolytic enzymes.[1] It further seems possible that



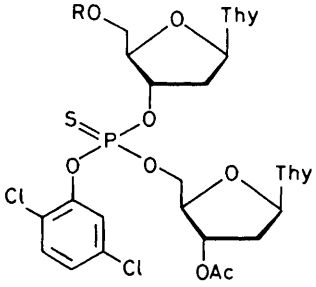
(1) a; R^1 = Px, R^2 = H
b; R^1 = H, R^2 = Ac



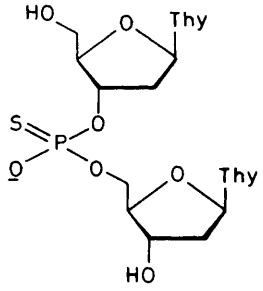
(2) a; X = Cl
b; X = (3)



(3)



(4) a; R = Px
b; R = Ac



(5)

Px = 9-phenylxanthen-9-yl (pixyl)

oligonucleotide analogues containing one or more phosphorothioate internucleotide linkages will prove to be useful in other ways in biological research. For these reasons, we, like other groups of workers,[2-4] have become interested in the development of methods for the synthesis of phosphorothioate analogues of oligonucleotides. In this preliminary communication, we report the preparation of both diastereoisomers of thymidine-3′ thymidine-5′ phosphorothioate [**5**; Tp(S)T] by what we believe to be the most convenient synthetic method so far reported.

The desired phosphorylating agent, 2,5-dichlorophenyl phosphorodichloridothioate[5] (**2a**) [b.p. 100 °C at 0.1 mmHg; $\delta(^{31}P)$ ($CDCl_3$) 53.1 p.p.m.], was readily prepared in 62% yield by heating 2,5-dichlorophenyl phosphorodichloridite,[6] thiophosphoryl chloride, and sulphur in the presence of activated charcoal, following one of Tolkmith's procedures.[5] The phosphorylating agent (**2a**) (1.2 mmol) was first allowed to react with 1-hydroxybenzotriazole[7] (2.6 mmol) and triethylamine (2.4 mmol) in anhydrous tetrahydrofuran (10 ml) at room temperature. After 20 min, 5′-*O*-pixylthymidine[8] (**1a**) (1.0 mmol) and then pyridine (5 ml) were added to the resulting products which were assumed to contain (**2b**).†

After a further period of 75 min, 3′-*O*-acetylthymidine[9] (**1b**) (1.5 mmol) and 1-methylimidazole (4.0 mmol) were added and the ensuing reaction was allowed to proceed for 5.5 h before it was worked up. Short column chromatography[10] of the products afforded the pure higher R_F [0.35, $CHCl_3$–EtOH (95 : 5 v/v)] diastereoisomer of the fully protected dinucleoside phosphorothioate (**4a**) in 36% isolated yield and the pure lower R_F (0.32) diastereoisomer in 17% isolated yield. In addition, a mixture of the two diastereoisomers (richer in the higher R_F component) was isolated in 32% yield. Thus the total isolated yield of (**4a**) was 85%; this material was composed of approximately twice as much of the higher R_F {δ_P[$(CD_3)_2SO$] 61.20 p.p.m.} as of the lower R_F {δ_P[$(CD_3)_2SO$] 61.12 p.p.m.} diastereoisomer.

The higher R_F diastereoisomer of (**4a**) was converted (acidic treatment, followed by acetylation) into the corresponding diacetate (**4b**) in 79% yield. The latter compound (**4b**) was treated first with (*E*)-2-nitrobenzaldehyde oxime[11] (10 mol. equiv.; 0.3 M) and N^1,N^1,N^3,N^3-tetramethylguanidine (9 mol. equiv.) in dioxan–water (9 : 1 v/v) at room temperature to unblock the internucleotide linkage,‡ and then with aqueous ammonia to remove the acetyl groups. The resulting fully unblocked Tp(S)T (**5**) [triethylammonium salt; $\delta_P(D_2O)$ 55.27 p.p.m.], which was virtually the sole nucleotide product obtained, was confirmed by h.p.l.c. (Du Pont Zorbax ODS column) to be diastereoisomerically pure. In the presence of *Crotalus adamanteus* snake venom phosphodiesterase, the latter compound underwent complete digestion, but appreciably more slowly than did thymidylyl-(3′ → 5′)-thymidine; it was thereby identified as the R_P-diastereoisomer[3] of thymidine-3′ thymidine-5′ phosphorothioate (**5**). In the same way the pure S_P-diastereoisomer of (**5**) [triethylammonium salt; δ_P (D_2O) 55.02 p.p.m.] was obtained from the lower R_F diastereoisomer of (**4a**); this compound remained completely unchanged after it had been treated with snake venom phosphodiesterase for more than the time required to effect the total digestion of the R_P-diastereoisomer.

Apart from one of Eckstein's procedures[3] in which unblocking of the internucleotide linkage apparently leads to the formation of some of the corresponding dinucleoside phosphate in addition to the desired dinucleoside phosphorothioate, the other successful synthetic methods reported previously[2,4] all involve the use of the phosphite triester approach. The present phosphotriester synthesis leads to a high yield of fully protected dinucleoside phosphorothioate in only two steps. Unblocking of each diastereoisomer of (**4a**) proceeds stereospecifically and the fully unblocked diastereoisomers (**5**) are obtained as virtually the sole nucleotide products. Finally if, as would be expected, hydroxide ion-promoted unblocking of a suitably protected dinucleoside phosphorothioate 2,5-dichlorophenyl ester were also to proceed stereospecifically, it should be easy to obtain specifically ^{17}O- or ^{18}O-labelled dinucleoside phosphorothioates by this approach. The latter would be valuable substrates for the determination of the stereochemistry of phosphorolytic enzymes.

† The intermediate (**2b**) is a much faster and more effective phosphorylating agent than the corresponding di-(1,2,4-triazolide), obtained by treating (**2a**) with *ca.* 2 mol. equiv. each of 1,2,4-triazole and triethylamine. In the preparation of (**4a**) in which the di-(1,2,4-triazolide) was used as the phosphorylating agent, the reaction times required for the first and second phosphorylation steps were 4 and 50 h, respectively. Furthermore, the total isolated yield of (**4a**) was only *ca.* 60%. It is noteworthy that the (**4a**) prepared in this way was richer in the *lower* R_F diastereoisomer.

‡ Under the conditions indicated, the half-time for 2-nitrobenzaldehyde oximate ion-promoted unblocking of this diastereoisomer of (**4b**) is *ca.* 10 min, and the time required for complete removal of the 2,5-dichlorophenyl group from the internucleotide linkage of (**4b**) is *ca.* 1 h. This may be compared with the time of 30 min required[11] to remove the 2-chlorophenyl protecting group from a fully protected derivative of thymidylyl-(3′ → 5′)-thymidine under the same reaction conditions. Preliminary experiments suggest that the 2-nitrobenzaldehyde oximate ion-promoted removal of the 2-chlorophenyl group from a fully protected dithymidine phosphorothioate occurs some 30 times more slowly than the removal of the 2,5-dichlorophenyl group.

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