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THE STEREOSPECIFIC CONVERSION OF P-CHIRAL DIALKYL PHOSPHOROTHIOATES INTO [^{18}O]-PHOSPHATES

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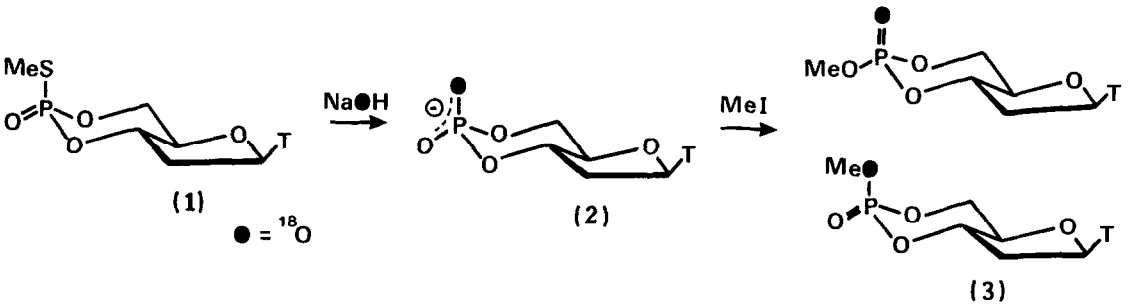
Abstract: S-Methyl thymidine (S_p)-3',5'-cyclic phosphorothioate and S-methyl 5'-O-thymidyl 3'-thymidyl (R_p)-phosphorothioate are readily converted by means of sodium [^{18}O]-hydroxide into the corresponding [^{18}O]-phosphate diesters stereospecifically with retention of configuration at phosphorus.

The development of syntheses of P-chiral isotopically labelled phosphate and thiophosphate esters makes possible the determination of the stereochemical course of enzymatic reactions at phosphorus,[1] e.g. kinases, nucleotidyl transferases and phosphatases. Although methods exist for the synthesis of chiral isotopically labelled phosphate monoesters of defined stereochemistry[2,3] few methods of sufficient generality have been reported that allow the specific incorporation of isotope into phosphate diesters.

In view of the facile synthesis of P-chiral phosphorothioate O,O-diesters we and others have been seeking conditions that will allow their stereospecific conversion into the corresponding [^{17}O] or [^{18}O]-phosphates. Methods so far reported exploit the oxidative displacement of sulphur from the phosphorothioate diester predominantly with inversion of configuration using reagents such as bromine,[4] N-bromosuccinimide,[5] cyanogen bromide[6] or dimethyl sulphoxide[7] in the presence of [^{17}O] or [^{18}O]-water, but these methods appear not to be completely stereospecific especially when the phosphorus is constrained in a 6-membered ring. We report here an extremely easy method for stereospecific conversion of *P-S to P-^{18}O* involving the displacement of a thioalkyl group by hydroxide.

The separate diastereoisomers of thymidine 3',5'-cyclic phosphorothioate can be prepared and their respective configurations at phosphorus have been firmly established.[8] These can be readily converted to S-methyl thymidine 3',5'-cyclic phosphorothioate by treatment with methyl iodide. When S-methyl thymidine (S_p) 3',5'-cyclic phosphorothioate(1) (cTMPS) (δ ^{31}P 24.21) was treated with a slight excess of sodium [^{18}O]-hydroxide in [^{18}O]-water (ca. 50% ^{18}O), thymidine [^{18}O] 3',5'-cyclic phosphate(2), [^{18}O] cTMP, (δ ^{31}P -2.87) was isolated by ion exchange chromatography in essentially quantitative yield, Scheme 1. The distribution of ^{18}O in the exocyclic positions was determined directly by ^{31}P n.m.r. after methylation to the triesters (3),[9] Figure 1. Clearly ^{18}O is found only in the axial position indicating that the reaction proceeded stereospecifically with retention of configuration, Scheme 1. A mixture of (R_p)- and (S_p)-

Scheme 1



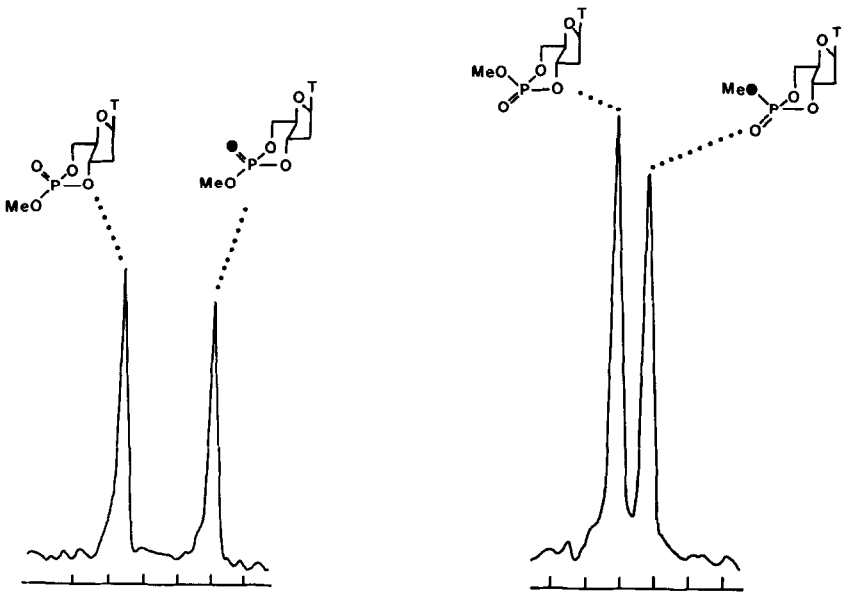


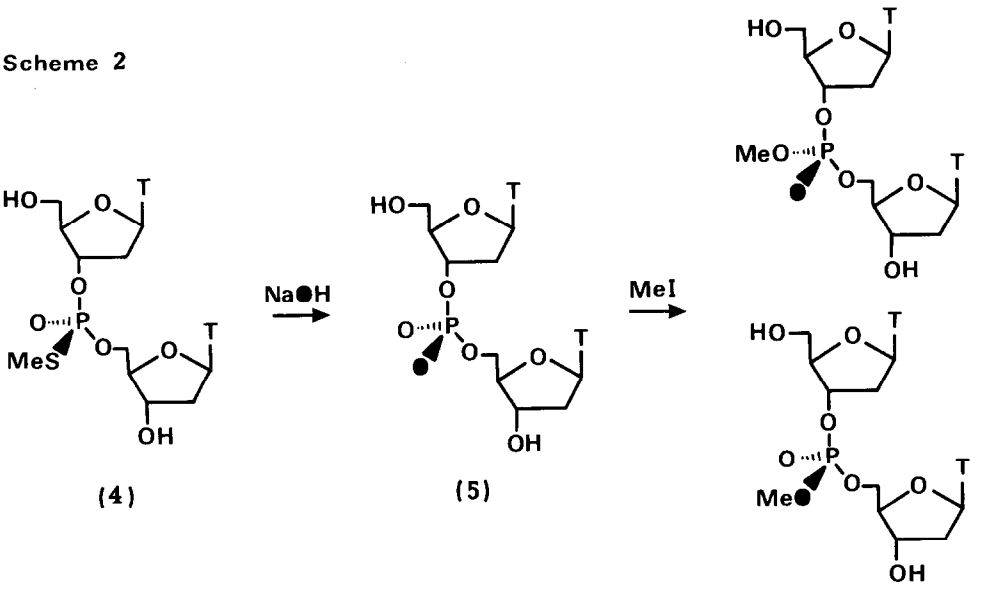
FIGURE 1

^{31}P n.m.r. (162 MHz) of [^{18}O] cTMP triester (3) derived from reaction of [^{18}O]-hydroxide with (S_p) S-methyl cTMPS (1). [1 Hz per division]

diastereoisomers of cTMPS (R_p:S_p, 3:1) when subjected to the same reaction conditions gave [^{18}O]-cTMP(2) with ^{18}O distributed between the equatorial and axial positions in the same ratio, both diastereoisomers would therefore appear to react stereospecifically with retention of configuration.

In order to establish that this method also succeeds with acyclic phosphorothioates, 5'-O-thymidyl 3'-O-thymidyl phosphorothioate, (Tp(S)T),[10] was reacted in exactly the same way. A sample containing 85% (R_p) Tp(S)T and 15% (S_p) Tp(S)T ($\delta_{^{31}P}$ 55.30 and 55.00 respectively) was methylated to give S-methyl Tp(S)T (4) which was not isolated but was directly treated with sodium [^{18}O]-hydroxide in [^{18}O]-water (ca. 50% ^{18}O) to give 5'-O-thymidyl 3'-O- thymidyl[^{18}O]-phosphate (5) ($\delta_{^{31}P}$ -0.618 and -0.648) in excellent yield.

Scheme 2



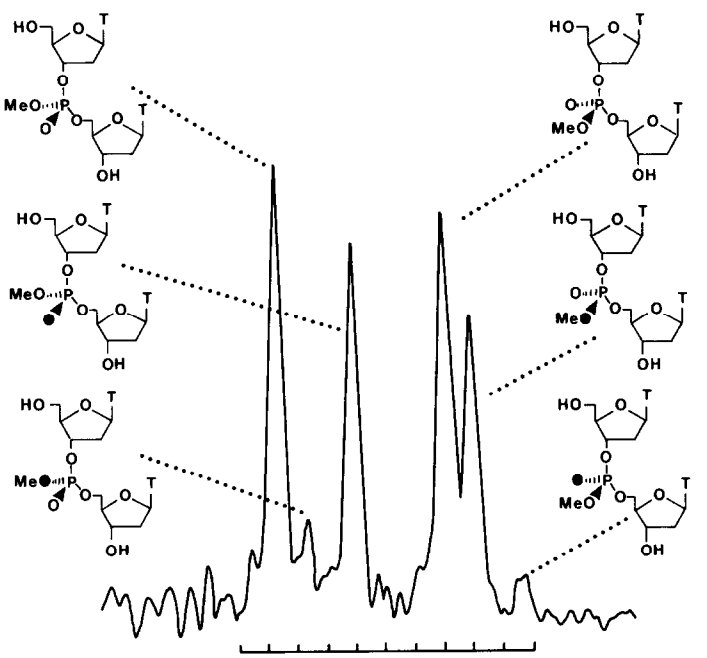


FIGURE 2

^{31}P n.m.r. (162 MHz) of T[^{18}O]pT triester (6) derived from reaction of [^{18}O]-hydroxide with (R_p) S-methyl Tp(S)T (4). [1 Hz per division]

The absolute configurations of the diastereomeric O-methyl TpT triesters have recently been assigned[11] hence the stereochemical course of the above sulphur displacement step can be determined directly from the ^{31}P n.m.r. of the dinucleotide following methylation to the triester (6), Figure 2. The isotopomers of methyl T[^{18}O]pT (6) are in the ratio of 85:15, Figure 2, corresponding to the original ratio of (R_p) : (S_p) Tp(S)T, which strongly implies that the sulphur displacement reaction is stereospecific. Since the major isotopomer contains ^{18}O in the *pro R* position of the diester (5) the reaction has occurred with retention of configuration.

A displacement reaction at phosphorus proceeding with retention of configuration is most readily explained in terms of a single pseudorotation of a pentacoordinate intermediate.

This approach to the synthesis of P-chiral [^{17}O] or [^{18}O] phosphate diesters is simple, generally applicable and introduces isotope directly from its most convenient source, namely water. The only instance where the method cannot be applied is in the case of thiopyrophosphate esters such as adenosine 5'(α-thio)diphosphate where the intermediate P^1P^1-pyrophosphate diester decomposes. However, our oxidative method previously reported[4] appears to be essentially stereospecific in this particular case.

Having completed this work, an alternative method using oxiranes to convert PS to PO has recently been reported[12]. However the application of this to the synthesis of [^{18}O]-phosphate diesters requires the prior synthesis of [^{18}O]-oxiranes, which in view of this report is unnecessary.

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