Tetrahedron Letters,Vol.25,No.27,pp 2897-2900,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

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

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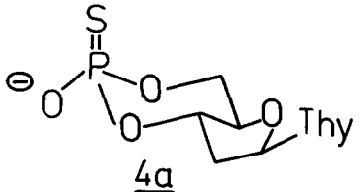
Abstract: P-chiral phosphorothioate analogs of thymidine and adenosine nucleotides are transformed in high yield with retention of configuration by [^{18}O]chloral and [^{18}O]styrene oxide into corresponding nucleoside [^{18}O]phosphates.

It has been shown in our recent reports that ^{18}O-labelled reagents such as dimethyl sulphoxide (1) [1], styrene oxide (2) [2] and chloral (3)[3] may be successfully applied for a stereospecific conversion of P-chiral cyclic dialkyl phosphorothioates into corresponding [^{18}O]phosphates [4]. The exchange of sulphur by oxygen proceeds either with inversion of configuration at phosphorus (dimethyl sulphoxide) or with retention (styrene oxide, chloral) with the stereoselectivity 92-95%. These results prompted us to investigate the applicability of our reagents for the stereospecific synthesis of P-chiral nucleoside [^{18}O]phosphates. For these studies Sp (4a) and Rp (4b) diastereoisomers of thymidine cyclic 3',5'-phosphorothioate, Sp adenosine cyclic 3',5'-phosphorothioate (5), Sp adenosine-5'-O-(1-thiotriphosphate)(6) and Rp thymidine-3'-O-(4-nitrophenyl)phosphorothioate (7) were chosen as model compounds.



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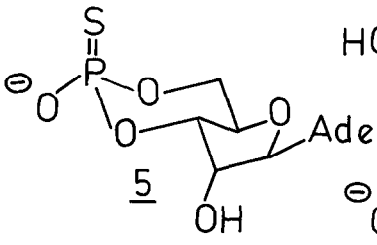

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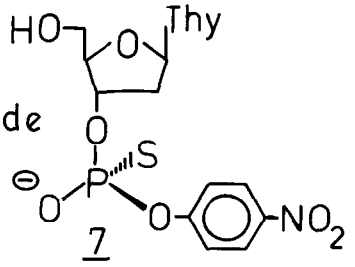

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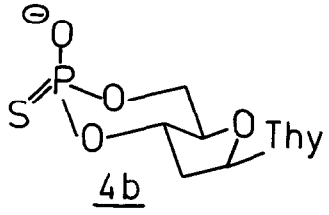

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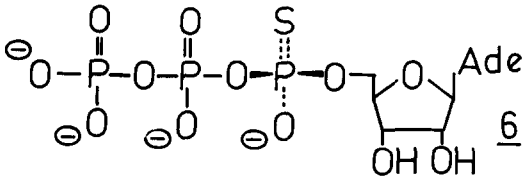

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The nucleoside phosphorothioates 4-7 were prepared according to literature reports[5-8] and the oxygen-18 labelled reagents 1-3 were obtained according to our previously described procedures [1-3,9] with 70% isotope enrichment [10]. The experiments of $PS \rightarrow P^{18}O$ exchange were performed on a 20-40 μmole scale and were monitored by HPLC [11]. The oxo-products were purified by ion-exchange chromatography on DEAE Sephadex A-25. Their identity and purity were checked by comparison of chromatographic (HPLC) and spectroscopic (^{31}P NMR [12], UV [13]) properties with those of authentic samples. The resulting [^{18}O]nucleotides were transformed into cyclic derivatives appropriate for determining of configuration at phosphorus atom by ^{31}P NMR as described by Gerlt [14,15] and Lowe [16]. Potassium salts of [^{18}O]cTMP and [^{18}O]cAMP (obtained from 4 and 5, respectively) treated with methyl iodide in DMSO in the presence of 18-Crown-6 [17] gave mixtures of axial and equatorial methyl esters which were analysed by ^{31}P NMR. Thymidine-3'--O-(4-nitrophenyl) [^{18}O]phosphate obtained from 7 was stereospecifically cyclized with tBuOK in DMF (inversion [7]) into [^{18}O]cTMP and then methylated as above. [α-^{18}O]ATP derived from 6 was transformed into [α-^{18}O]ADP with yeast hexokinase and analysed by ^{31}P NMR as the [α-^{18}O]$ADPCo(NH_3)_4$ complex [15].

The results are listed in the Table. An inspection of the Table reveals that with few exceptions in the series of thymidine and adenosine phosphorothioates the exchange of sulphur by oxygen with reagents such as chloral and styrene oxide proceeds in good to excellent yields. In the case of compounds possessing reactive OH and NH groups silylation of substrate markedly increases yields of reactions with chloral (exp. 4 and 7). The comparison of P-chirality of products with that of substrates shows that for both cyclic and acyclic nucleoside phosphorothioates the $S \rightarrow {}^{18}O$ exchange with [^{18}O]chloral and [^{18}O]styrene oxide proceeds with retention of configuration at phosphorus whereas with [^{18}O]dimethyl sulphoxide predominant inversion is observed. The reactions with styrene oxide are fully stereospecific (exp. 8-12).

In the case of chloral the stereoselectivity of exchange depends upon the structure of phosphorothioate substrate: with acyclic 7 complete retention was observed (exp. 6, 7) while with cyclic 4 and 5 the stereoselectivity of the exchange reaction drops to ca 92% (exp. 1-4). Similar effect of lower stereoselectivity of $PS \rightarrow P^{18}O$ exchange in cyclic nucleoside phosphorothioate series was observed by Lowe [18] and Eckstein [19] for bromination-hydrolysis procedure. Unfortunately, the reaction of 7 with styrene oxide was accompanied by a removal of p-nitrophenyl group making impossible its stereochemical analysis. In the case of [^{18}O]dimethyl sulphoxide (exp. 15,16) in addition to low yield of reaction with 4b much lower stereoselectivity was observed. It is worth mentioning, that the exchange of sulphur by oxygen in ATPαS proceeds with both 2 and 3 in a fully regioselective manner giving [^{18}O]ATP labelled exclusively in α-position. In this case bromination-hydrolysis procedure results in "scrambling" of oxygen label between α and γ positions [18-20]. The recently reported hydrolysis of nucleoside S-methyl phosphorothioates with [^{18}O]sodium hydroxide when applied to ADPαS analog gives decomposition products [21]. Further experiments on the application of chloral and styrene oxide for the exchange of sulphur by oxygen in other nucleoside phosphorothioate systems are underway.

Table

Exp. no.	Substrate [a]	Reagent	Reaction conditions	Yield (%)	Stereochemistry
1	4a	3 [d]	neat [g], 60°, 1.5 h	81	retention (82%)
2	4b	3 [d]	neat [g], 60°, 1.5 h	78	retention (92%)
3	5	3 [d]	neat, 60°, 1.5 h	62	retention (92%)
4	5-silylated [b]	3 [d]	neat, 60°, 2h	84	retention (92%)
5	6	3 [d]	neat, 60°, 1h	34	retention [h]
6	7	3 [d]	neat, 60°, 1.5 h	77	retention (100%)
7	7-silylated [b]	3 [d]	neat, 60°, 2h	83	retention (100%)
8	4a	2 [e]	DMF/H_2O, 60°, 4h	48	retention (100%)
9	4b	2 [e]	DMF/H_2O, 60°, 4h	88	retention (100%)
10	4a	2 [e]	EtOH, 60°, 3h	83	retention (100%)
11	4b	2 [e]	EtOH, 60°, 2h	90	retention (100%)
12	5	2 [e]	DMF/H_2O, 60°, 4h	38	retention (100%)
13	6	2 [e]	DMF/H_2O, 60°, 1.5 h	68	retention [h]
14	6	2 [e]	EtOH, 60°, 1h	76	retention [h]
15	4b-acid [c]	1 [f]	neat, 40°, 0.5 h	21	inversion (76%)
16	7-acid [c]	1 [f]	neat, 60°, 0.5 h	90	inversion (80%)

[a] Triethylammonium salts were used unless otherwise stated.

[b] The sample was dissolved in pyridine, treated with an excess of Me_3SiCl for 1h and evaporated. After exchange reaction silyl protecting groups were removed with 0.2 M triethylammonium bicarbonate.

[c] The sample was evaporated with an excess of 1N HCl prior to exchange reaction.

[d] 30-fold excess of 3 was used. After exchange reaction ca 70% of reagent was recovered by vacuum line technique.

[e] 10-fold excess of 2 was used with 20 µl of solvent per 1 µmole of substrate.

[f] 10-fold excess of 1 was used.

[g] When DMF was used as a solvent the stereoselectivity dropped to 82% (retention) probably due to the exchange of oxygen between 3 and the solvent.

[h] Due to considerable ^{31}P NMR line broadening in the spectrum of $[\alpha\text{-}^{18}O]ADPCo(NH_3)_4$ the stereoselectivity of the reaction could not to be calculated with sufficient accuracy.

Acknowledgements: A major part of this work was performed during a two-months visit of one of us (A.O.) to the laboratory of Prof. F.Eckstein in Göttingen; Max-Planck-Gesellschafft is thanked for sponsoring of this stay. The authors are indebted to Prof. W.J.Stec for his interest in this work and helpful discussion.

References and Notes

1. A.Okruszek, W.J.Stec, Tetrahedron Lett., 23, 5203 (1982)
2. P.Guga, W.J.Stec, Tetrahedron Lett., 24, 3899 (1983)
3. A.Okruszek, W.J.Stec, J.Chem.Soc., Chem.Commun., 1984, 117
4. For a recent review of other procedures leading to a $PS \rightarrow P^{18}O$ exchange see F.Eckstein Angew.Chem.Int.Ed., 22, 423 (1983).
5. W.S.Zieliński, W.J.Stec, J.Am.Chem.Soc., 99, 8365 (1977); Z.J.Lešnikowski, W.Niewiarowski, W.S.Zieliński, W.J.Stec, Tetrahedron, 40, 15 (1984)
6. W.Niewiarowski, W.J.Stec, W.S.Zieliński, J.Chem.Soc., Chem.Commun., 1980, 524
7. J.A.Gerlt, S.Mehdi, J.A.Coderre, W.O.Rogers, Tetrahedron Lett., 21, 2385 (1980)
8. The adenosine derivatives 5 and 6 were kind gifts from Prof. F.Eckstein and Dr.B.Connolly.
9. A.Okruszek, J.Label.Comp. and Radiopharmaceuticals, 20, 741 (1983)
10. The $[^{18}O]$enrichment was measured from the intensity of molecular ion peaks in Mass Spectra recorded with a LKB-2091 spectrometer at 70 eV ionizing energy.
11. High-performance liquid chromatographic analyses were made on Waters Associates chromatograph by using anion-exchange column (Nucleosil 10SB) with a pH 4.5 buffer 200 mM in KH_2PO_4 and 300 mM in CH_3COOK as eluent.
12. ^{31}P NMR spectra were recorded on a Bruker WP200SY spectrometer operating at 81.01 MHz with ^{1}H broad band decoupling.
13. UV spectra were recorded on a Shimidzu UV-200 spectrophotometer.
14. J.A.Gerlt, J.A.Coderre, J.Am.Chem.Soc., 102, 4531 (1980)
15. J.A.Coderre, J.A.Gerlt, J.Am.Chem.Soc., 102, 6594 (1980)
16. R.L.Jarvest, G.Lowe, B.V.L.Potter, J.Chem.Soc., Perkin I, 1981, 3186
17. B.V.L.Potter, B.A.Connolly, F.Eckstein, Biochemistry, 22, 1369 (1983)
18. G.Lowe, G.Tansley, P.M.Cullis, J.Chem.Soc., Chem.Commun., 1982, 595
19. B.A.Connolly, H.H.Füldner, F.Eckstein, J.Biol.Chem., 257, 3382 (1982)
20. R.D.Sammons, P.A.Frey, J.Biol.Chem., 257, 1138 (1982)
21. P.M.Cullis, Tetrahedron Lett., 24, 5677 (1983)

(Received in UK 29 March 1984)