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STEREORETENTIVE CONVERSION OF DIALKYL PHOSPHOROTHIOATES INTO $[^{18}O]$-PHOSPHATES

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Abstract Dicyclohexylammonium salts of *cis*- and *trans*-2-hydroxy-2-thiono-4-methyl-1,3,2-dioxaphosphorinane are quantitatively converted by means of styrene $[^{18}O]$-oxide into the corresponding 2-$[^{18}O]$-oxo-derivatives with retention of configuration at the phosphorus atom.

Among the numerous applications of the nucleoside phosphorothioates as tools for elucidation of the mode of action of several classes of enzymes [1], it became apparent only recently that they can be successfully transformed into nucleoside $[^{18}O]$-phosphates. Methods so far developed for the stereospecific conversion of P-chiral phosphorothioates into $[^{18}O]$-phosphates involve an oxidative bromination in the presence of $[^{18}O]$-H_2O [2-4]. However, these methods are not fully stereospecific and in some cases the formation of undesired by-products occurs.

In the light of our earlier interest in the stereospecific synthesis of nucleoside cyclic 3',5'-phosphorothioates [5] and $[^{18}O]$-phosphates [6] and recent studies on the stereochemistry of PS → PO conversion [7,8] we undertook independent efforts in order to elaborate the method for stereospecific conversion of dialkyl phosphorothioates into dialkyl phosphates From the work of Hamer et al.[9] it is known that aqueous solution of sodium methyl-N-cyclohexyl phosphoroamidothioate upon treatment with an excess of ethylene oxide produces ethylene sulphide and corresponding phosphoramidate The substitution of oxygen for sulphur was explained in terms of pentaco-ordinated intermediate formation, its pseudorotation and ethylene sulphide expulsion. Assuming that polytopal rearrangement of the phosphorane is limited to a minimal number of necessary elementary steps we could expect that the PS → PO conversion by means of ethylene oxide would proceed in the highly stereoselective manner. To prove this hypothesis the following experiments were performed

A. *Trans*-2-phenylamino-4-methyl-2-$[^{18}O]$-oxo-1,3,2-dioxaphosphorinane [<u>1a</u>, R_f 0 29 (chloroform-acetone, 10 3), δ_{31P} -1.0 ppm ($CHCl_3$)[x/], $[^{18}O]$-enrichment 60.0%] was converted by means of NaH/CS_2 approach [10] into sodium *cis*-2-thio-4-methyl-2-$[^{18}O]$-oxo-1,3,2-dioxaphosphorinane

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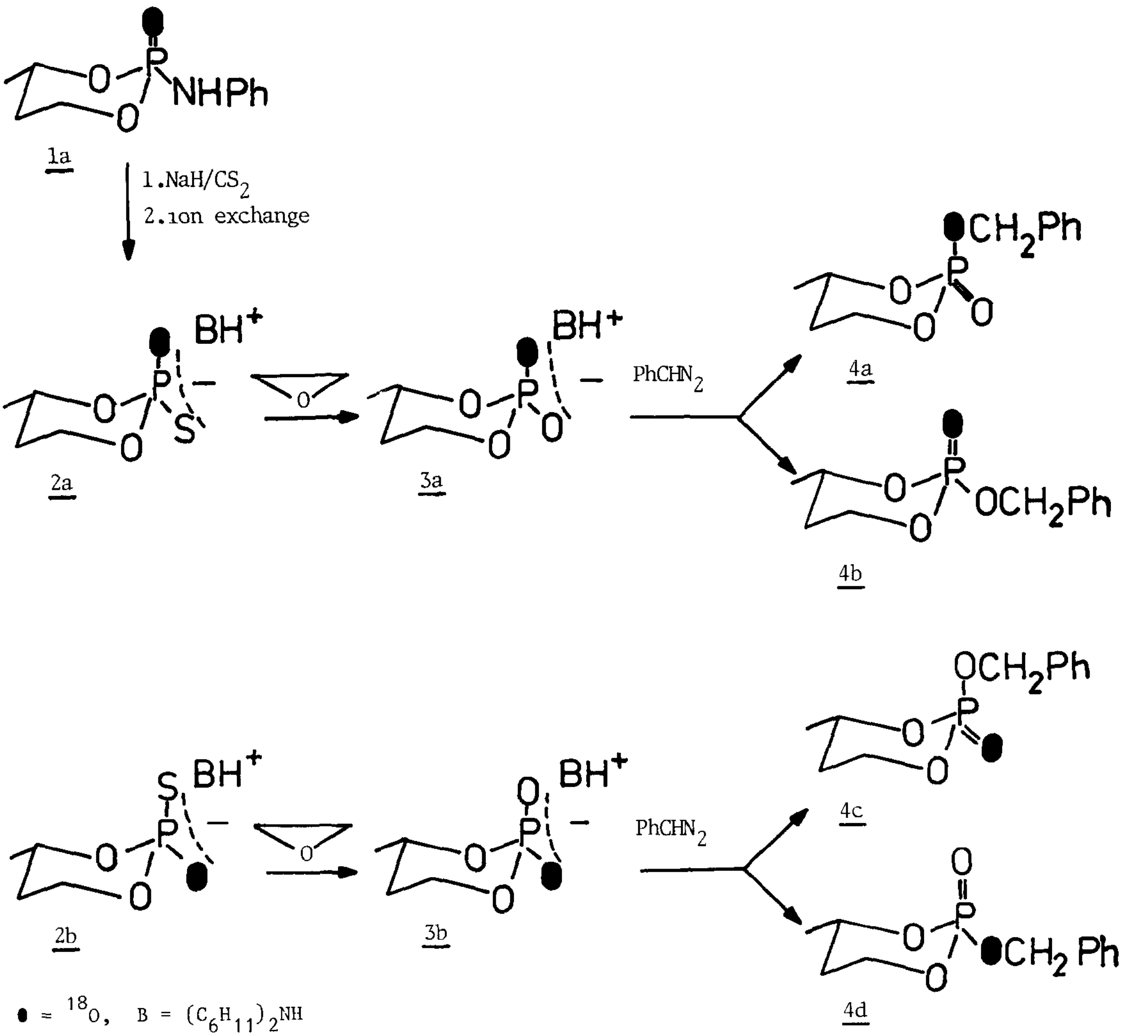
[x/] Negative chemical shifts for compounds absorbing at higher field than 85% H_3PO_4

and then transformed into crystalline dicyclohexylammonium salt [2a, $\delta_{^{31}P}$ 53.3 ppm (H_2O), [^{18}O]-enrichment 60.0%]. The sample of 2a (50 mg) was dissolved in water (2 mL) and gaseous ethylene oxide (1 g) was passed through the solution at room temperature. ^{31}P-NMR spectrum recorded after 2 hours showed only one signal at −3.5 ppm, characteristic for phosphate 3a. The conversion of 3a into the free acid (ion-exchange resin) was followed by treatment of its THF solution (2 mL) with phenyldiazomethane. ^{31}P-NMR spectrum showed the presence of two signals corresponding to diastereoisomeric triesters 4 [4a, $\delta_{^{31}P}$ −8.6 ppm (THF), 58% and 4b, $\delta_{^{31}P}$ −5.7 ppm (THF), 42%]. The diastereoisomers were separated by means of preparative TLC [11].

B. The same procedure repeated with the sample containing 18% *cis*-salt (2a) and 82% *trans*-salt [2b, $\delta_{^{31}P}$ 49.5 ppm (H_2O), [^{18}O]-enrichment 60.0%] gave the corresponding triesters 4 as the mixture 4a + 4c and 4b + 4d.

SCHEME 1.



● = ^{18}O, B = $(C_6H_{11})_2NH$

Mass spectrometry data for triesters 4 isolated from both experiments are collected in the Table.

Substrate	Triester(s)	[^{18}O]-enrichment of 4 $^{a/}$ %	Relative intensities of ions				Stereoretention %
			M-PhCHO m/z 138		M-PhCH^{18}O m/z 136		
			calc.$^{b/}$	measd	calc.$^{b/}$	measd	
2a	4a	59.5	0	4.5	100	95.5	92.5
	4b	59.5	59.5	56 8	40.5	43.2	95.5
2a + 2b$^{c/}$	4a + 4c	60.0	49 2	45.9	50.8	54.1	94.5
	4b + 4d	60.0	10.8	13.0	89.2	87.0	96.0

$^{a/}$ Measured on the molecular ions m/z = 244 and m/z = 242

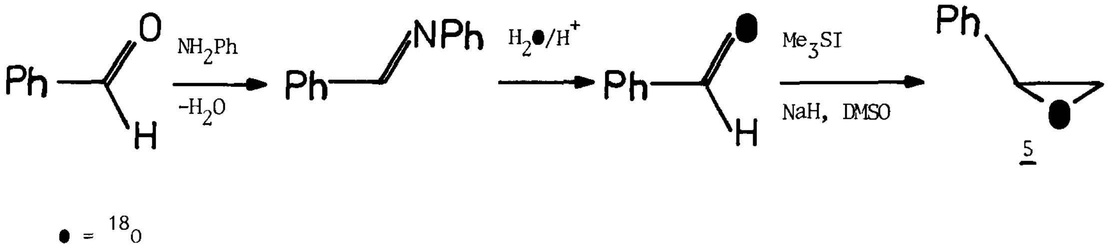
$^{b/}$ The retention of configuration at phosphorus was assumed in calculation

$^{c/}$ This substrate consisted of 82% of 2b and 18% of 2a (^{31}P-NMR assay)

The results clearly show that conversion 2 → 3 proceeds with retention of configuration at phosphorus and its stereoselectivity is not lower than 92.5%.

In the experiments presented above the labelled [^{18}O]-phosphorothioates and non-labelled ethylene oxide were used, but for the possible practical applications of this method labelled [^{18}O]-epoxide is necessary. Since the synthesis of ethylene [^{18}O]-oxide seemed to be rather difficult and its application as well as storage conditions are not convenient, we turned our attention to the substituted oxirane - styrene [^{18}O]-oxide 5, which was synthesized according to Corey and Chaykowsky [12] from the [^{18}O]-benzaldehyde.

SCHEME 2.



The epoxide 5 was successfully used for the substitution of sulphur by [^{18}O]-oxygen in non-labelled phosphorothioates 2a and 2b Owing to the poor solubility of styrene oxide in water, the mixture DMF-H_2O (3 1) was used as a solvent [13]. The stereoretentive course of reaction was confirmed and the identical range of stereoselectivity was observed

The work on the application of [^{18}O]-oxiranes for the oxidation of nucleoside phosphorothioates is in progress.

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11. The silica gel 60 F-254 S plates (Merck) were developed in benzene and then twice in ethyl ether R_f(4a) 0 31, R_f(4b) 0.13
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13. The procedure involved the heating of the solution of salt 2 (50-70 mg) and 3-fold molar excess of epoxide in 2 mL of solvent at 40-60°C for 4 hours.

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