Synthesis and Stereochemical Analysis of Chiral Inorganic [^{16}O,^{17}O,^{18}O]Thiophosphate

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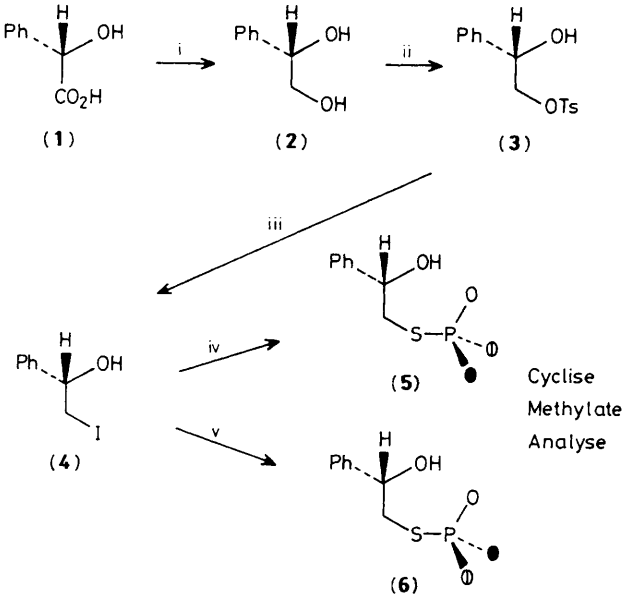
The enantiomers of inorganic [^{16}O,^{17}O,^{18}O]thiophosphate have been prepared and a procedure has been developed which allows them to be distinguished by ^{31}P n.m.r. spectroscopy.

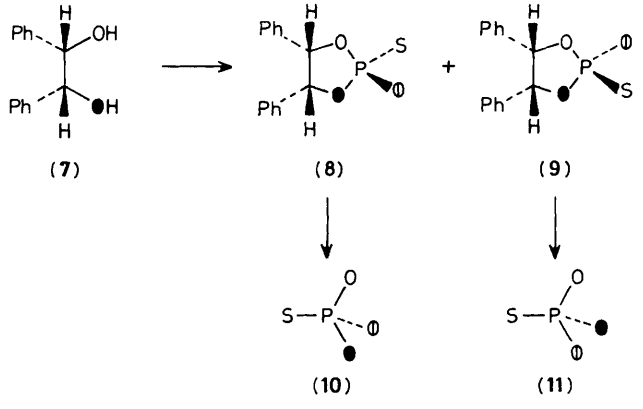
Stereochemical analysis has proved to be a powerful mechanistic probe for a large number of enzyme-catalysed phosphoryl-transfer reactions, but for many reactions of this type the phosphoryl group transferred is released either directly or indirectly as inorganic phosphate. Now inorganic phosphate has a *pro-pro-pro*-chiral centre and since there are only three stable isotopes of oxygen, namely ^{16}O, ^{17}O, and ^{18}O, the stereochemical course of such reactions cannot be followed by isotopic substitution alone. The stereochemical problem, however, can be solved if the fourth oxygen atom is replaced by sulphur; indeed, a method for the stereochemical analysis of chiral inorganic [^{16}O,^{17}O,^{18}O]thiophosphate has been reported.[1] Unfortunately this method depends on the enzymic incorporation of inorganic [^{16}O,^{17}O,^{18}O]thiophosphate into adenosine 5′-*O*-[(2*S*)-thiotriphosphate] (S_p-ATPβS) which leads to a substantial loss of isotope and some racemisation, most probably as a result of the chemical instability of glycerate 1-[^{16}O,^{17}O,^{18}O]phosphorothioate 3-phosphate, an intermediate in the reaction.[1,2] We now report a chemical method for the stereochemical analysis of chiral [^{16}O,^{17}O,^{18}O]thiophosphates which does not suffer from this defect.

The key reagent for this analysis is (*S*)-2-iodo-1-phenylethanol (**4**) which was prepared as outlined in Scheme 1. (*S*)-(+)-Mandelic acid (**1**) was converted by way of (*S*)-(+)-1-phenylethane-1,2-diol (**2**),[3] into the tosyl derivative (**3**).[4] Displacement of the tosylate with iodide ion using tetrabutylammonium iodide in refluxing benzene solution (5 h) gave the iodo-alcohol (**4**), m.p. 30 °C, $[\alpha]_D^{20}$ + 43.6° ($CHCl_3$, *c* 1.58, *l* 1). The iodo-compound (**4**) reacts with the bis-tri-n-octylammonium salts of (R_p)- and (S_p)-[^{16}O,^{17}O,^{18}O]thiophosphate in dimethylformamide at 20 °C to give the [^{16}O,^{17}O,^{18}O]thiophosphate *S*-esters (**5**) and (**6**) respectively.

The enantiomeric inorganic [^{16}O,^{17}O,^{18}O]thiophosphates were prepared as outlined in Scheme 2. (1*R*,2*S*)-1,2-[1-^{18}O]-Dihydroxy-1,2-diphenylethane (**7**)[5] was treated with thiophosphoryl chloride (1 mol. equiv.) in pyridine to give a mixture of diastereoisomeric cyclic thiophosphorochloridates, which were hydrolysed with [^{17}O]water to give the cyclic [^{17}O,^{18}O]phosphorothioates (**8**) and (**9**) in the ratio 3.5 : 1.



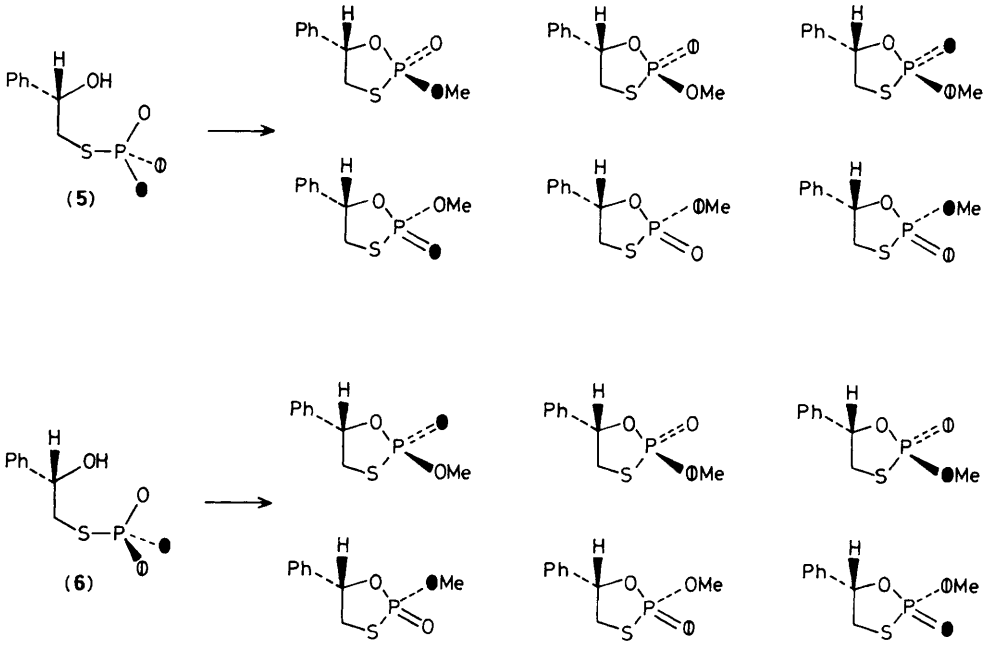
Scheme 1. *Reagents*: i, $LiAlH_4$; ii, *p*-$MeC_6H_4SO_2Cl$ (TsCl), C_5H_5N; iii, $Bu_4N^+I^-$, C_6H_6; iv, (*R*)-[^{16}O,^{17}O,^{18}O]thiophosphate; v, (*S*)-[^{16}O,^{17}O,^{18}O]thiophosphate.



Scheme 2

Table 1. The observed relative peak intensities of the ^{31}P resonances from Figure 1 are compared with the values expected for cyclisation with retention and inversion of configuration at phosphorus. The stereoselectivity of the cyclisation is determined by the ratio of the intensities of the two mono-^{18}O *trans*- and the two mono-^{18}O *cis*-esters, compared with the calculated values. ● = ^{18}O.

	trans-Ester			*cis*-Ester		
	Obs.	Calc.		Obs.	Calc.	
		Retention	Inversion		Retention	Inversion
MeO–P=O	0.47	0.51	0.51	0.50	0.51	0.51
Me●–P=O	1.00	0.66	1.00	0.61	1.00	0.66
MeO–P=●	0.65	1.00	0.66	1.00	0.66	1.00
Me●–P=●	0.38	0.36	0.36	0.36	0.36	0.36



Scheme 3

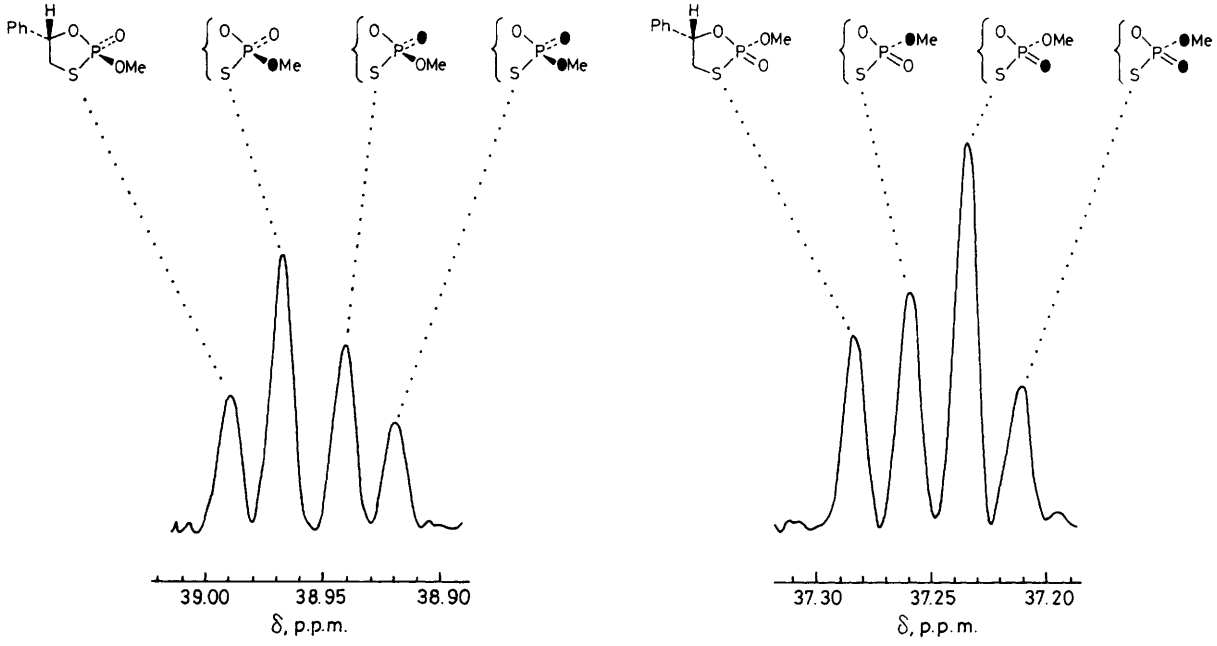


Figure 1. The ^{31}P n.m.r. spectrum of the *trans*- and *cis*-methyl esters derived by cyclisation followed by methylation of the (R_p)-[^{16}O,^{17}O,^{18}O]phosphorothioate (**5**) in [$^2H_3$]acetonitrile–dimethylformamide (v/v, 3 : 1). The ^{31}P n.m.r. spectrum was recorded at 202.47 Hz with a sweep width of 3000 Hz, pulse width of 3 μs, acquisition time 5.4 s, and with broad-band proton decoupling. The chemical shifts of the unlabelled *trans*- and *cis*-methyl esters are about δ_P +39.0 and +37.3 p.p.m. respectively downfield from trimethyl phosphate in ^2H_2O.

The unlabelled diastereoisomers have been well characterised,[6] and the structure of the *cis*-isomer was confirmed by *X*-ray crystallography.[7] After separation by ion exchange chromatography, reductive cleavage of the benzylic oxygen bonds with sodium in liquid ammonia gave the (R_p)- and (S_p)-[^{16}O,^{17}O,^{18}O]thiophosphates (**10**) and (**11**) from the *cis*- and *trans*-[^{17}O,^{18}O]phosphorothioates (**8**) and (**9**) respectively.

The (R_p)- and (S_p)-[^{16}O,^{17}O,^{18}O]phosphorothioate *S*-esters (**5**) and (**6**) were now used to establish a method for the configurational analysis of chiral inorganic [^{16}O,^{17}O,^{18}O]thiophosphate based on ^{31}P n.m.r. spectroscopy in a manner analogous to that developed for chiral [^{16}O,^{17}O,^{18}O]phosphate monoesters.[8] Cyclisation (as their tri-n-butylammonium salts) was achieved with diphenylphosphoryl chloride in dimethylformamide (in about 80% yield) and the products

were esterified with diazomethane (Scheme 3). The ^{31}P n.m.r. spectrum of the isotopomeric mixture of *trans*- and *cis*-methyl esters derived from (R_p)-[^{16}O,^{17}O,^{18}O]phosphorothioate *S*-ester (**5**) is shown in Figure 1.† The spectrum reveals only those isotopomers containing ^{16}O and ^{18}O, since ^{17}O directly bonded to phosphorus causes broadening of the ^{31}P resonance.[9] The three lowest intensity resonances in each quartet arise because the '^{17}O' is not fully enriched; it has 9.4 atom % ^{16}O, 52 atom % ^{17}O, and 38.6 atom % ^{18}O. Since the predominant isotopomer has ^{18}O in the P–OMe bond for the *trans*-methyl ester and in the P=O bond for the *cis*-methyl ester, the cyclisation must have proceeded with inversion of configuration at phosphorus. By comparing the ratio of the peak intensities with those calculated from the known isotopic composition of the (R_p)-[^{16}O,^{17}O,^{18}O]thiophosphate and allowing for an estimated 3% loss of isotope during the cyclisation (Table 1) the cyclisation is seen to occur stereospecifically within experimental error. In a similar experiment with (S_p)-[^{16}O,^{17}O,^{18}O]phosphorothioate *S*-ester (**6**) the cyclisation was also shown to proceed with inversion and with similar stereoselectivity. This analytical procedure can now be applied to the determination of the absolute configuration of inorganic [^{16}O,^{17}O,^{18}O]thiophosphate of unknown chirality.

† The upfield (+37.3 p.p.m.) ^{31}P resonance was assigned to the *cis*-isomer and the downfield (+39.0 p.p.m.) ^{31}P resonance to the *trans*-isomer from the $^3J_{HP}$ to the ring protons.

We gratefully acknowledge support for this work from the S.E.R.C.

Received, 14th February 1986; Com. 205

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