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

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(S)-2-Iodo-1-phenylethanol has been synthesized and shown to be a reagent for the stereochemical analysis of chiral inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate. *R* and *S* Inorganic ^{16}O, ^{17}O, ^{18}O-labeled thiophosphates were synthesized and treated with the iodo compound to give chirally labeled 1(S)-phenylethanol-2-S-thiophosphate, which after cyclization with diphenylphosphoryl chloride followed by methylation with diazomethane could be distinguished by ^{31}P NMR spectroscopy. The cyclization step was shown to proceed with inversion of configuration at phosphorus. © 1987 Academic Press, Inc.

INTRODUCTION

To study the stereochemical course of chemical and enzyme-catalyzed phosphoryl transfer reactions which lead either directly or indirectly to inorganic phosphate, it is necessary for this *pro–pro–pro*-chiral species to be released in a chiral form. Since there are only three stable isotopes of oxygen, namely, ^{16}O, ^{17}O, and ^{18}O, sulfur has been used in place of the fourth oxygen atom. A method for the stereochemical analysis of chiral [^{16}O, ^{17}O, ^{18}O]thiophosphate was developed by Webb and Trentham (*1*) and by Tsai (*2*) in which it was incorporated enzymatically into the S_p-diastereoisomer of ATPβS which was then examined by ^{31}P NMR spectroscopy. The method, however, suffers from two defects which make it unsuitable for quantitative stereochemical analysis. First, there is considerable loss of label during the enzymatic conversion. This is attributable to the intermediacy of glycerate 1-O-[^{16}O, ^{17}O, ^{18}O]phosphorothioate 3-phosphate which is prone to spontaneous hydrolysis by cleavage of the P–O bond releasing inorganic thiophosphate. Thus the inorganic thiophosphate derived from the isotopomers containing ^{17}O or ^{18}O in the bridge will have lost these isotopes, and that derived from the isotopomer containing ^{16}O in the bridge would be expected to give inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate which is inverted or racemized; all these species can be recycled and incorporated into ATPβS. Although this problem can to a large extent be overcome by using very high concentrations of phosphoglycerate kinase to rapidly capture and convert the labile intermediate into (S_p)-ATPβS, the precision of the method is unreliable. Second, the phosphorus–phosphorus coupling constants $^2J_{P_\alpha OP_\beta}$ and $^2J_{P_\beta OP_\gamma}$ of (S_p)-ATPβS are similar but not identical and vary with the ionic strength and pH of the buffer. The lines of P_β therefore appear as a triplet but with a broadened central line of variable width,

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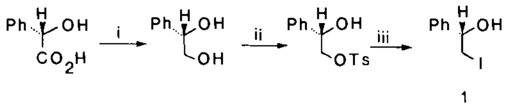


SCHEME 1. Reagents: (i) $LiAlH_4$; (ii) p-$CH_3 \cdot C_6H_4 \cdot SO_2Cl$, C_5H_5N; (iii) $Bu_4N^+I^-$.

the outer lines being sharp. This makes optimization of the resolution enhancement difficult to achieve and again imposes limitations on the precision of the method. Since it is highly desirable to improve the precision with which the chirality of [^{16}O, ^{17}O, ^{18}O]thiophosphate can be measured, we have developed a new method based on a purely chemical protocol. A preliminary account of this work has been reported (*3*).

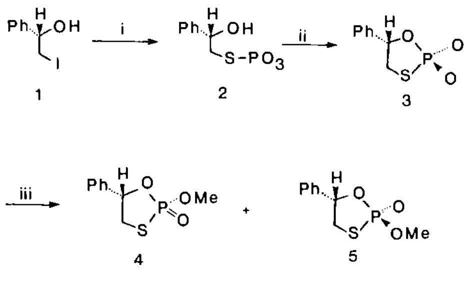
RESULTS AND DISCUSSION

Synthesis of the Key Reagent: (S)-2-Iodo-1-phenylethanol

(*S*)-2-Iodo-1-phenylethanol **1** was selected as the reagent for the configurational analysis of chiral inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate. It was synthesized from (*S*)-(+)-mandelic acid as outlined in Scheme 1, and shown by ^{1}H NMR spectroscopy to be chirally pure using the chiral shift reagent tris[3-heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III).

Strategy for the Stereochemical Analysis of Chiral Inorganic [^{16}O, ^{17}O, ^{18}O] Thiophosphate

The chemistry envisaged for the stereochemical analysis of chiral inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate is outlined in Scheme 2. (*S*)-2-Iodo-1-phenylethanol **1** was found to react completely with inorganic thiophosphate as its bis-trioctylammonium salt in dimethylformamide in 20 h at room temperature (ca. 18°C). The nature of the ammonium salt significantly affects the rate of the reaction; thus, the bis-tri-*n*-butylammonim and bis-triethylammonium salts take 4 and 8 days, respectively, before reaction is complete. Other solvents were also investigated but dimethylformamide proved to be the solvent of choice. The 2-*p*-toluenesulfonyl



SCHEME 2. Reagents: (i) $HOPO_2S^{2-}$ $[HN^+(n\text{-octyl})_3]_2$, DMF; (ii) $(PhO)_2POCl$, NBu_3^n; (iii) CH_2N_2.

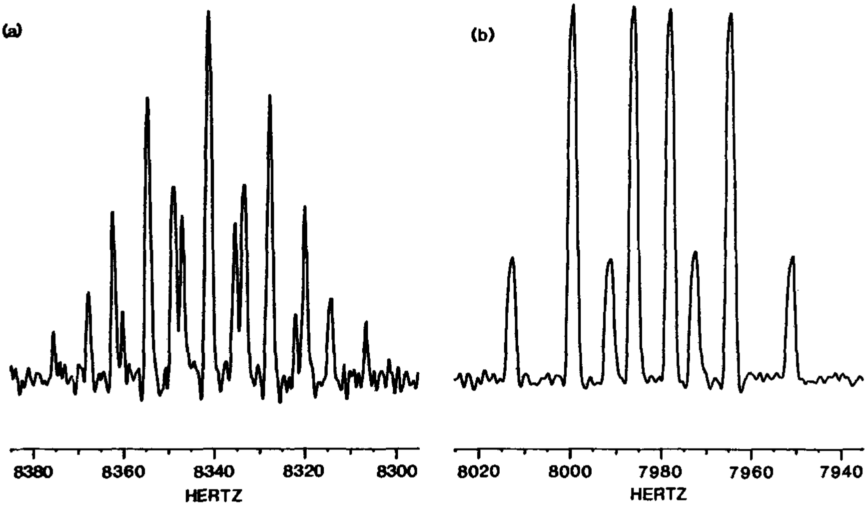


FIG. 1. The observed proton-coupled ^{31}P NMR spectra of the *trans*- and *cis*-4-(*S*)-phenyl-2-methoxy-2-oxo-1,3,2-thiaoxaphospholanes. (a) *trans* isomer centered at +39.0 ppm. (b) *cis* isomer centered at +37.2 ppm downfield from trimethyl phosphate.

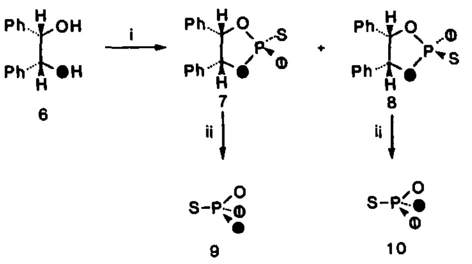
derivative from which (*S*)-2-iodo-phenylethanol **1** is derived (Scheme 1) is much less reactive toward inorganic thiophosphate.

Cyclization of (*S*)-1-phenylethanol-2-*S*-thiophosphate **2** was achieved with diphenylphosphorochloridate in about 5 min (the progress was followed by ^{31}P NMR spectroscopy), the cyclic thiophosphate **3** being isolated in 80% yield after purification on DEAE-Sephadex. Methylation of its tri-*n*-butylammonium or pyridinium salt with diazomethane gave quantitatively a mixture of the *cis* and *trans*-methyl ester **4** and **5** (the choice of counterion influences the ratio of the *cis* and *trans*- esters formed; thus the tri-*n*-*o*-octylammonium salt gives overwhelmingly the *cis*-methyl ester). The ^{31}P NMR spectrum showed two well-separated signals at about +37.2 and +39.0 ppm. These triesters in common with other cyclic five-membered phosphate triesters are sensitive to nucleophiles, including moisture, and must be manipulated carefully under rigorously anhydrous conditions.

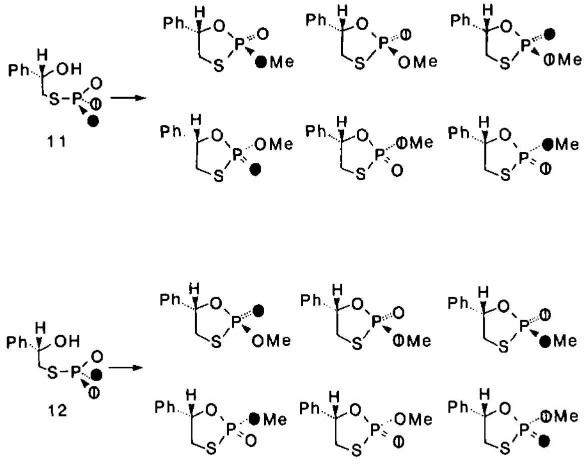
The assignment of the ^{31}P NMR signals to the methyl esters was made from the proton-coupled ^{31}P NMR spectra (Fig. 1). The diastereoisomer resonating at higher field (δ_P +37.2 ppm) is a double quartet, $^3J_{POCH_3}$ = 13.4 Hz and $^3J_{PSCH}$ = 21.5 Hz. Selective proton decoupling showed that the 21.5-Hz coupling was due to one of the methylene protons. The diastereoisomer resonating at lower field is more complex owing to coupling of phosphorus to all three ring protons in addition to the methyl group. The multiplet was analyzed by computer simulation and the coupling constants $^3J_{POCH_3}$ = 13.4 Hz and $^3J_{PH}$ = 12.9, 8, and 7.7 Hz were obtained for the three ring protons. Selective proton decoupling of the methyne proton showed this to be responsible for J = 7.7 Hz. The dihedral angle dependences of both $^3J_{POCH}$ and $^3J_{PSCH}$ in phosphates (*4*) and thiophosphates (*5*) have been shown to obey a Karplus-type relationship. The high-field resonance is assigned to the *cis*-methyl ester **4** since this should adopt the half-chair conformation (the phenyl and methoxy groups will be in pseudoequatorial positions) with two of the ring protons (the methine and one methylene proton) having dihedral angles to phosphorus close to 90° and hence coupling constants close to or equal to zero. No other ring conformation can give this result. The *trans*-methyl ester **5** should adopt the twisted half-chair conformation since one substituent will be in a pseudo axial position. In this conformation all three ring protons would couple to phosphorus as observed for the low-field ^{31}P NMR signal.

To establish the method of analysis with authentic material and determine the stereochemical course of cyclization, the enantiomeric inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphates were required. The route envisaged for their preparation is outlined in Scheme 3. The unlabeled *cis*- and *trans*-4,5-diphenyl-2-oxo-2-thio-1,3,2-dioxaphospholanes had been prepared previously and fully characterized (*6*), one of them by X-ray crystallography (*7*). However, since ^{17}O had to be introduced as $H_2{}^{17}O$ a somewhat modified synthetic route was adopted, which was initially investigated with unlabeled material. The reaction between mesohydrobenzoin and thiophosphoryl chloride gives a mixture of diastereoisomeric cyclic thiophosphorochloridates the ratio of which is solvent dependent. Thermodynamic control appears to be favored by using pyridine as solvent and under these conditions the overall yield is optimal. The reaction of the mixture of cyclic thiophosphorochloridates with water is facile and quantitative, the *cis* and *trans* cyclic thiophosphates being obtained in a ratio of 3.5 : 1 and overall yield of 60% from mesohydrobenzoin. The separation of the stereoisomers was achieved on DEAE-Sephadex with triethylammonium bicarbonate buffer at pH 10. The synthesis of the enantiomeric inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphates was now achieved starting with (1*R*, 2*S*)-[1-^{18}O]dihydroxy-1,2-diphenylethane **6** (*8*), as outlined in Scheme 3. Since the reductive cleavage of the cyclic thiophosphates **7** and **8** with sodium in liquid ammonia occurs at the benzylic oxygen bond, the stereochemistry at phosphorus is unperturbed. Thus the *cis* isomer gives the R_p inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **9** and the *trans* isomer, the S_p inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **10**.

To investigate whether any loss of isotope might occur during the cyclization of chirally labeled thiophosphate ester **11** or **12** (*S*)-1-phenylethanol-2-*S*-[$^{18}O_3$]thiophosphate was made and cyclized with one and with two equivalents of diphenylphosphoryl chloride. The cyclic ester was esterified with diazomethane and the diastereoisomeric esters were investigated by ^{31}P NMR spectroscopy.



SCHEME 3. Reagents: (ia) $PSCl_3$, C_5H_5N; (ib) $H_2{}^{17}O$; (ii) Na, liquid NH_3. ⦶ = ^{17}O, ● = ^{18}O.



SCHEME 4

When one equivalent of diphenylphosphoryl chloride was used the loss of label from the cyclic thiophosphate diester was negligible (<3%), whereas when two equivalents were used the loss was substantial (about 50% from one site). It is important therefore to ensure that only one equivalent of reagent is used for the cyclization step.

The strategy for the stereochemical analysis is outlined in Scheme 4. Cyclization of (*S*)-1-phenylethanol-2-S-[(*R*)-^{16}O, ^{17}O, ^{18}O]thiophosphate **11** (derived from **1** and **9**) with diphenylphosphorochloridate should give a mixture of isotopomeric cyclic phosphates by loss of one of the three peripheral oxygen isotopes with equal probability (the kinetic isotope effect being negligible). The isotopomeric mixture obtained was methylated with diazomethane. The high-resolution ^{31}P NMR spectrum of the product is shown in Fig. 2. ^{17}O is quadrupolar nucleus ($I = \frac{5}{2}$) and causes scalar relaxation of a ^{31}P signal when directly bonded to phosphorus, which broadens the resonance to such an extent that it merges with the noise in the baseline (*9, 10*). ^{18}O, on the other hand, has a nuclear spin quantum number $I = 0$, as does ^{16}O, but it does cause an isotope shift to higher field (*11*), the magnitude of which is related to bond order (*9*). If the ^{17}O and ^{18}O sites were fully enriched only the one isotopomer, namely, that containing ^{16}O and ^{18}O, for each diastereoisomer, would appear in the ^{31}P NMR spectrum. The predominant signal in the ^{31}P NMR spectrum in fact arises from this species and establishes that the stereochemical course of the cyclization occurs with inversion of configuration at phosphorus. The ^{16}O, ^{16}O-labeled, the minor ^{18}O, ^{16}O-labeled, and the ^{18}O, ^{18}O-labeled isotopomers arise because the ^{17}O site is not fully enriched, the isotopic abundance being 9.4 atom% ^{16}O, 52 atom% ^{17}O and 38.6 atom% ^{18}O. From this isotopic composition, as well as that for ^{18}O at 98 atom%, the relative peak heights can be calculated and compared with the observed values.

A computer program was written which allows loss of label and racemization to be incorporated into the calculation. The program steps through values for label loss and racemization, producing ratios which may be compared with the ob-

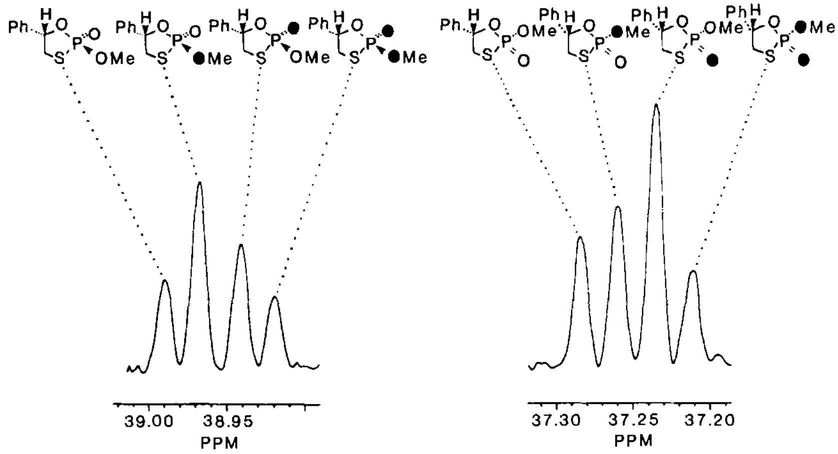


FIG. 2. The ^{31}P NMR spectrum at 202.46 MHz of the labeled 4(*S*)-phenyl-2-methoxy-2-oxo-1,3,2-thiaoxaphospholanes derived from *R* inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **9**. Spectral parameters: sweep width, 3030 Hz; pulse width, 3 μs (49°); acquisition time, 5.4 s; broadband proton decoupling. Gaussian multiplication (line broadening, −1.2 Hz, Gaussian broadening, 0.075) in 32K, Fourier transform in 64K.

served values. Table 1 shows a comparison of the observed and calculated intensities where the calculation includes a 3–5% loss of label but no racemization for the best fit.

(*S*)-1-Phenylethanol-2-*S*-[(*S*)-^{16}O, ^{17}O, ^{18}O]thiophosphate (derived from **1** and **10**) was treated in a similar way and the relative intensities from the ^{31}P NMR spectrum were compared with the calculated values in Table 2. It should be noted that the sample of inorganic [(*S*)-^{16}O, ^{17}O, ^{18}O]thiophosphate used in this experiment was obtained from a preparation different from the inorganic [(*R*)-^{16}O, ^{17}O, ^{18}O]thiophosphate and the ^{17}O site has an isotopic composition of 29 atom% ^{16}O, 42 atom% ^{17}O, and 29 atom% ^{18}O. The ^{18}O site was 98 atom% ^{18}O. The calculated

TABLE 1

OBSERVED RELATIVE PEAK INTENSITIES OF THE ^{31}P RESONANCES FROM FIG. 2 COMPARED WITH THE VALUES CALCULATED FOR CYCLIZATION OF (*S*)-1-PHENYLETHANOL-2-*S*-[(*R*)-^{16}O, ^{17}O, ^{18}O]THIOPHOSPHATE **11** WITH RETENTION AND INVERSION OF CONFIGURATION AT PHOSPHORUS FOLLOWED BY METHYLATION

	trans ester			*cis* ester		
		Calculated			Calculated	
	Observed	Retention	Inversion	Observed	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

TABLE 2

OBSERVED RELATIVE PEAK INTENSITIES OF THE ^{31}P RESONANCES COMPARED WITH THE VALUES CALCULATED FOR CYCLIZATION OF (*S*)-1-PHENYLETHANOL-2-*S*-[(*S*-1-^{16}O, ^{17}O, ^{18}O]THIOPHOSPHATE **12** WITH RETENTION AND INVERSION CONFIGURATION AT PHOSPHORUS FOLLOWED BY METHYLATION

	trans ester			*cis* ester		
		Calculated			Calculated	
	Observed	Retention	Inversion	Observed	Retention	Inversion
MeO—P=O	0.79	0.73	0.73	0.72	0.73	0.73
Me●—P=O	0.75	1.00	0.73	1.00	0.73	1.00
MeO—P=●	1.00	0.73	1.00	0.71	1.00	0.73
Me●—P=●	0.27	0.23	0.23	0.28	0.23	0.23

intensities incorporate a 6% loss of label during the cyclization but no racemization to obtain the best fit.

These results suggest that the cyclization is stereospecific within the experimental and instrumental limits. The low label loss and simple ^{31}P NMR spectra make the method suitable for the quantitative analysis of chiral inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate. The method is similar to those developed for the stereochemical analysis of chiral [^{16}O, ^{17}O, ^{18}O]phosphate monoesters (*12, 13*).

EXPERIMENTAL PROCEDURES

All solvents were purified and dried by conventional procedures. Deionized water was obtained by distillation (Fisons Fi-streem still) followed by passage through a Milli Q3 purification system (Millipore Ltd., London). Diphenylphosphorochloridate was distilled, bp 175°C/1 mm Hg. Thiophosphoryl chloride was distilled at atmospheric pressure under dry nitrogen, bp 125°C. Tributylamine was distilled from calcium hydride, bp 130°C/1 mm Hg.

Melting points were determined using a Kofler block and are uncorrected. Optical rotations were determined using a Perkin–Elmer 241 polarimeter. The pH of aqueous solutions was measured with a Radiometer PHM84 pH meter with a combination electrode. Optical densities and UV–visible spectra were obtained using a Pye-Unicam SP8-100 spectrophotometer. Mass spectra were recorded on a VG 16F mass spectrometer.

Proton magnetic spectra were recorded on a Bruker AM500 Fourier transform spectrometer at 500.13 MHz. ^{13}C NMR spectra were recorded on a Bruker AM500 Fourier transform spectrometer at 125.77 MHz using a ^{13}C-dedicated probe. Broadband proton decoupling was used, with subsequent off-resonance decoupling to show signal splitting by directly bonded protons. ^{17}O NMR spectra were recorded on a Bruker AM250 Fourier transform spectrometer at 33.91 MHz. ^{31}P NMR spectra were recorded on the following Bruker Fourier transform instruments: AM250 (at 101.26 MHz) and AM500 (at 202.46 MHz). The latter instrument was used for most of the isotopic work, with a ^{31}P-dedicated probe. Spectra were recorded with broadband proton decoupling and, subsequently, proton coupling when quoted. All spectra were recorded at room temperature.

All chemical shifts are reported as positive for resonances downfield of the relevant reference signal. The reference for ^{1}H and ^{13}C NMR spectra was external tetramethylsilane. For ^{17}O NMR spectra the reference was internal or external D_2O. For ^{31}P NMR spectra the reference was external trimethyl phosphate in D_2O.

Anion-exchange chromatography was carried out using DEAE-Sephadex A-25 resin (Pharmacia Fine Chemicals, Uppsala) using stated linear concentration gradients of triethylammonium bicarbonate at 4°C. Buffer was removed from product fractions by addition and evaporation (under reduced pressure) of three or four aliquots of methanol.

The presence of sulfur in chromatographic fractions was indicated by a palladium spray test: $PdCl_2$ (0.5 g) in hydrochloric acid (0.1 M, 100 ml). Sulfur-containg fractions gave rise to a yellow or brown color.

(S)-1-Phenylethanol-2-tosylate. (*S*)-(+)-Mandelic acid was reduced with lithium aluminum hydride to give (*S*)-phenylethane-1,2-diol mp 67°C, $[\alpha]_D^{20}$ + 60.7° (ether, $c = 0.97$, $l = 1$) (*14*). The diol (2.2 g, 15.8 mmol) was dissolved in pyridine (5 ml) and cooled to 0°C. *p*-Toluenesulfonyl chloride (3 g, 15.8 mmol) was added and the mixture stirred at 4°C for 3 days, poured into ice water (50 ml), and extracted with ether (3 × 50 ml). The combined ethereal extracts were washed with 1 M hydrochloric acid (2 × 50 ml) and water (50 ml) and dried (Na_2SO_4/K_2CO_3), and the solvent was removed to give the product as a white solid (3.54 g, 86%). The title compound was purified by flash silica chromatography (eluting with 5% ethyl acetate in dichloromethane). Recrystallization from benzene/hexane gave white needles, mp 72°C, $[\alpha]_D^{20}$ +53.6° (chloroform, $c = 2.48$, $l = 1$) and +29.7° (methanol, $c = 3.35$, $l = 1$). Literature (*15*) mp 72–73°C, $[\alpha]_D^{20}$ +30.3° (methanol, $c = 3.35$, $l = 1$).

(S)-2-Iodo-1-phenylethanol 1. (*S*)-1-Phenylethanol-2-tosylate (782 mg, 3 mmol) and tetrabutylammonium iodide (2.22 g, 6 mmol) in benzene (15 ml) were heated under reflux for 5 h. After cooling, the solution was washed with water (50 ml), 5% sodium bisulfite solution (2 × 50 ml), saturated sodium bicarbonate solution (50 ml), and finally water (50 ml). The solution was dried ($MgSO_4$) and the solvent removed to give the crude product as a white gum (630 mg, 86%). The product was purified by flash silica chromatography (eluting with dichloromethane:*n*-pentane, 2:1 v/v). After recrystallization twice from petroleum ether (bp 40–60°C) white needles, mp 30°C, $[\alpha]_D^{20}$ +43.6° (chloroform, $c = 1.58$, $l = 1$) were obtained. (Found: C, 38.6%; H, 3.6%; I, 51.1%. C_8H_9OI requires C, 38.7%; H, 3.6%; I, 51.1%.) δ_H ($CDCl_3$): 2.80 (1H, bs, -O*H*), 3.38 (1H, dd, J = 10.3 and 8.6 Hz, H_2), 3.47 (1H, dd, J = 3.8 and 10.3 Hz, $H_{2'}$), 4.81 (1H, dd, J = 3.8 and 8.6 Hz, H_1), 7.34 ppm (5H, m, Ar*H*). δ_C($CDCl_3$): +16 (t, C_2), +74 (d, C_1), +126 (d, *o*- or *m*-Ar), +128.5 (d, *p*-Ar), +129 (d, *o*- or *m*-Ar), +141 ppm (s, quaternary Ar). *m/z* (electron-impact ionization), 248 (2) M^+, 231 (32) $[PhCHCH_2I]^+$, 121 (100) $[PhCH \cdot OH \cdot CH_2]^+$, 107 (95) $[PhCHOH]^+$.

(S)-1-Phenylethanol-2-S-thiophosphate **2**. Bis-tri-*n*-octylammonium inorganic thiophosphate (250 μmol) and (*S*)-2-iodo-1-phenylethanol (74 mg, 300 μmol) were stirred in dimethylformamide (2 ml) in the dark under nitrogen for 20 h. The solution was poured into triethylammonium bicarbonate buffer (50 ml, 50 mM, pH 9). The suspension was diluted with water (70 ml) and applied to an ion-exchange column (1.5 × 30 cm). A gradient of triethylammonium bicarbonate buffer (25–250 mM, pH 8) was applied at 40 ml h^{-1} over 20 h. The title compound was eluted as the bis-triethylammonium salt at 100 mM buffer (65.4 mg, 60%), $[\alpha]_D^{20}$ +12.5° (methanol, $c = 0.53$, $l = 1$). $\delta_P(CD_3OD)$ +14.9 ppm, $^3J_{PSCH} = 13.5$ Hz. $\delta_H(CD_3OD)$: 3.28 (2H, m, H_2 and $H_{2'}$), 5.02 (1H, m, H_1), 7.50 (5H, m, Ar*H*) plus signals for triethylammonium ion.

4-(S)-Phenyl-2,2-dioxo-1,3,2-thiaoxaphospholane **3**. The bis-triethylammonium salt of (*S*)-1-phenylethanol-2-*S*-thiophosphate (150 μmol, 65 mg) was dissolved in methanol (1 ml) and stirred with Dowex 50 cation-exchange resin (pyridinium form). The mixture was filtered and the filtrate evaporated. The residue was dissolved in methanol (1 ml) and stirred with tri-*n*-butylamine (72 μl, 300 μmol) for 20 min. The solvent was removed and the salt dried by coevaporation with dimethylformamide (4 × 3 ml). The residue was dissolved in dimethylformamide (1 ml) and tri-*n*-butylamine (36 μl, 150 μmol) and one equivalent of diphenylphosphorochloridate (31 μl, 150 μmol) added. The solution was stirred for 5 min after which it was added to triethylammonium bicarbonate buffer (10 ml, 100 mM, pH 9). The solvent and buffer were removed and the residue was dissolved in CD_3OD for examination by ^{31}P NMR spectroscopy. The yield was estimated to be approximately 80%. The product was purified by ion-exchange chromatography on a column of DEAE-Sephadex (9 × 300 mm) and a gradient of triethylammonium bicarbonate buffer (5–120 mM, pH 8) was applied at 30 ml h^{-1} over 24 h. The product (40 mg, 80%) was eluted as the triethylammonium salt at 28 mM buffer strength (followed by and just separate from diphenyl phosphate). $\delta_P(CD_3OD)$: +29.3 ppm. $\delta_H(CD_3OD)$: 3.70 ppm (2H, m, H_5 and $H_{5'}$), 5.51 ppm (1H, m, H_4), 7.65 ppm (5H, m, Ar*H*) plus signals for triethylammonium ion.

cis- and trans-4-(S)-Phenyl-2-methoxy-2-oxo-1,3,2-thiaoxaphospholane **4** *and* **5**. The triethylammonium salt of 4-(*S*)-phenyl-2,2-dioxo-1,3,2-thiaoxaphospholane (120 μmol, 40 mg) was dissolved in methanol (1 ml) and stirred with Dowex 50 cation-exchange resin (pyridinium form). The mixture was filtered and the filtrate evaporated. The residue was dried by coevaporation with acetonitrile (4 × 3 ml) and then dissolved in acetonitrile (1 ml). The solution was stirred in the dark for 6 h with a dry ethereal solution of diazomethane. Excess diazomethane and solvent were removed and the residue was dissolved in CD_3CN (0.5 ml) (or some other aprotic NMR solvent). The solution was filtered through dry glass wool into a clean dry NMR tube. $\delta_P(CD_3CN)$: +37.2 ppm (s, ~60%) +39.0 ppm (s, ~40%). $\delta_H(CD_3CN)$: 3.69–3.73 (2H, m, H_5 and $H_{5'}$), 3.81 and 3.82 (3H, both d, both J = 13.4 Hz, *Me*), 5.56 (1H, m, H_4), 7.21 ppm (5H, m, Ar*H*).

cis- and trans-(4R,5S)-4,5,Diphenyl-1,3,2-dioxaphospholan-2-thiol-2-one. Mesohydrobenzoin (214 mg, 1 mmol) was dried by coevaporation with pyridine (4 × 10 ml) and dissolved in pyridine (6 ml). This was added over a period of 2 h to a stirred solution of thiophosphoryl chloride (100 μl, 1 mmol) in pyridine (2 ml) at

50°C, and stirred at this temperature for a further 2 h. Water (50 μl, 2.8 mmol) in pyridine (2 ml) was added and stirring continued for 10 min. The solvent was removed and the residue taken up in water (100 ml) containing some triethylamine so that the resulting suspension was at pH 10. This was diluted with water (500 ml) and applied to an ion-exchange column (2.6 × 60 cm) which had been equilibrated with triethylammonium bicarbonate buffer (30 mM, pH 10). A gradient of triethylammonium bicarbonate buffer (30–300 mM, pH 10) was applied at 50 ml h^{-1} over 24 h. The title compounds were eluted as their triethylammonium salts at about 300 mM buffer, the *trans* isomer (59 mg, 150 μmol, 15%) followed by the *cis* isomer (157 mg, 400 μmol, 40%). δ_P(CD_3OD): *trans* isomer +66.5 ppm, ($^3J_{POCH}$ = 8.0 Hz), *cis* isomer +69.1 ppm ($^3J_{POCH}$ = 6.8 Hz).

cis- and trans-(4R,5S)-4,5-Diphenyl-1,3,2-[1-^{18}O]dioxaphospholan-2-thiol-2-[^{17}O]one 7 and 8. (1*R*,2*S*)-[1-^{18}O]Dihydroxy-1,2-diphenylethane **6** (173 mg, 0.8 mmol) (*8*) was dried by coevaporation with pyridine (4 × 10 ml) and dissolved in pyridine (6 ml). This was added over a period of 2 h to a stirred solution of thiophosphoryl chloride (80 μl, 0.8 mmol) in pyridine (2 ml) at 50°C and stirred at this temperature for a further 2 h. ^{17}O-enriched water (50 μl, 2.8 mmol) in pyridine (2 ml) was added and stirring continued for 15 min. The solvent was removed and the residue taken up in water (200 ml) containing some triethylamine so that the resulting suspension was at pH 10. This was diluted with water (500 ml) and applied to a DEAE-Sephadex column (2.6 × 60 cm) which had been equilibrated with triethylammonium bicarbonate buffer (30 mM, pH 10).

A gradient of triethylammonium bicarbonate buffer (30–500 mM, pH 10) was applied at 60 ml h^{-1} over 24 h. The title compounds were eluted as their triethylammonium salts at 500 mM buffer, the *trans* isomer (52 mg, 131 μmol, 16%) followed by the *cis* isomer (129 mg, 326 μmol, 41%). δ_P(CD_3OD): *trans* isomer, +66.5 ppm ^{18}O isotope shift: 0.043 ppm; *cis* isomer, +69.1 ppm, ^{18}O isotope shift: 0.043 ppm. δ_O(MeOH, external D_2O lock) *trans* isomer, +138 ppm (±8 ppm) *cis* isomer, +144 ppm (±8 ppm).

Treatment of cis- and trans-(4R,5S)-4,5-diphenyl-1,3,2-dioxaphospholan-2-thiol-2-one with sodium in liquid ammonia. Liquid ammonia (20 ml) was distilled from sodium under dry nitrogen. Sodium (60 mg, 2.6 mmol) was added followed by *cis-* or *trans-(4R,5S)-4,5*-diphenyl-1,3,2-dioxaphospholan-2-thiol-2-one (as triethylammonium salts, 79 mg, 200 μmol) suspended in dioxan (2 ml). After 10 min stirring, solid ammonium chloride was added to quench excess sodium. The ammonia was evaporated by a stream of dry nitrogen. The residue was taken up in water (200 ml) and applied to a DEAE-Sephadex column (9 × 300 mm). A gradient of triethylammonium bicarbonate buffer (25–250 mM, pH 9) was applied at 35 ml h^{-1} over 20 h. Inorganic thiophosphate was eluted at 95 mM buffer and isolated as the bis-triethylammonium salt (28 mg, 44%). δ_P(CD_3OD): +45 ppm (bs).

Treatment of cis-(4R,5S)-4,5-diphenyl-1,3,2-[1-^{18}O]dioxaphospholan-2-thiol-2-[^{17}O]one 7 with sodium in liquid ammonia. The treatment was carried out as for the unlabeled compound but using *cis*-(4*R*,5*S*)-4,5-diphenyl-1,3,2-[1-^{18}O]dioxaphospholan-2-thiol-[1^{17}O]one (129 mg, 326 μmol) to give *R* inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **9** (41 mg, 40%). δ_P(CD_3OD): +45 ppm (bs). δ_O(MeOH, external D_2O) +95 ppm (±7 ppm) (bs).

Treatment of trans-(4R,5S)-4,5-diphenyl-1,3,2-[1-^{18}O]dioxaphospholan-2-thiol-2-[^{17}O]one **8** *with sodium in liquid ammonia.* The treatment was carried out as for the unlabeled compound but using *trans*-(4*R*,5*S*)-4,5-diphenyl-1,3,2-[1-^{18}O]dioxaphospholan-2-thiol-2-[^{17}O]one (52 mg, 131 μmol) to give *S* inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **10** (19 mg, 45%). δ_P(CD$_3$OD): +45 ppm (bs). δ_O(MeOH, external D$_2$O lock) +92 ppm (±8 ppm) (bs).

(S)-1-Phenylethanol-2-S-[(R)-^{16}O, ^{17}O, ^{18}O]thioposphate **11**. The title compound was prepared as for the unlabeled material **2** but using *R* inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **9** (60 μmol). The product was isolated as the bis-triethylammonium salt (15 mg, 58%). δ_P(CD$_3$OD): +14.75 ppm, ^{18}O isotope shift: 0.0276 ppm. δ_O(DMF, external D$_2$O): +130 ppm (±7 ppm) (bs).

(S)-1-Phenylethanol-2-S-[(S)-^{16}O, ^{17}O, ^{18}O]thioposphate **12**. The title compound was prepared as for the unlabeled material **2** but using *S* inorganic [^{16}O, ^{17}O, ^{18}O]thiophosphate **10** (50 μmol). The product was isolated as the bis-triethylammonium salt (11 mg, 55%). δ_P(CD$_3$OD): +14.80 ppm, ^{18}O isotope shift: 0.0277 ppm. δ_O(DMF, external D$_2$O): +128 ppm (+8 ppm, bs).

Cyclization of (S)-1-phenylethanol-2-S-[(R)-^{16}O, ^{17}O, ^{18}O]thiophosphate **11**. The cyclization was carried out as in the preparation of 4-(*S*)-phenyl-2,2-dioxo-1,3,2-thiaoxaphospholane but using (*S*)-1-phenylethanol-2-S[(*R*)-^{16}O, ^{17}O, ^{18}O]thiophosphate (30 μmol). (It is important to use just one equivalent of diphenylphosphorochloridate as an excess will lead to some loss of isotope.) The product was purified by DEAE-Sephadex chromatography and isolated as its triethylammonium salt (7.1 mg, 75%). δ_P(CD$_3$OD): +29.1 ppm, ^{18}O isotope shift: 0.0298 ppm.

Cyclization of (S)-1-phenylethanol-2-S-[(S)-^{16}O, ^{17}O, ^{18}O]thiophosphate **12**. The cyclization was carried out as above but using (*S*)-1-phenyl-ethanol-2-*S*-[(*S*)-^{16}O, ^{17}O, ^{18}O]thiophosphate (25 μmol) to give the product as the triethylammonium salt (6.3 mg 80%). δ_P(CD$_3$OD): +28.95 ppm, ^{18}O isotope shift: 0.0296 ppm.

Methyl esters of the cyclized (S)-1-phenylethanol-2-S-[(R)-^{16}O, ^{17}O, ^{18}O]thiophosphate. The methyl esters were obtained as in the preparation of unlabeled *cis*- and *trans*-4-(*S*)-phenyl-2-methoxy-2-oxo-1,3,2-thiaoxaphospholane but using the product of the cyclization of (*S*)-1-phenylethanol-2-*S*-[(*R*)-^{16}O, ^{17}O, ^{18}O]thiophosphate. The products were dissolved in CD$_3$CN : DMF (3 : 1, v/v, 0.4 ml) and filtered through dry glass wool into a clean dry NMR tube. δ_P: *trans* isomer, +39.44 ppm—^{18}O isotope shifts: Me—●—P=O, 0.0219 ppm; Me—O—P=●, 0.0488 ppm; Me—●—P=●, 0.0704 ppm. *cis* isomer, +37.74 ppm—^{18}O isotope shifts: Me—●—P=O, 0.0248 ppm; Me—O—P=●, 0.0489 ppm; Me—●—P=●, 0.0739 ppm.

Methyl esters of the cyclized (S)-1-phenylethanol-2-S-[(S)-^{16}O, ^{17}O, ^{18}O]thiophosphate. The methyl esters were obtained as in the preparation of unlabeled *cis*- and *trans*-4-(*S*)-phenyl-2-methoxy-2-oxo-1,3,2,thiaoxaphospholane but using the product of the cyclization of (*S*)-1-phenylethanol-2-*S*-[(*S*)-^{16}O, ^{17}O, ^{18}O]thiophosphate. The products were dissolved in CD$_3$CN : DMF (7 : 2, v/v, 0.4 ml) and filtered through dry glass wool into a clean dry NMR tube. δ_P: *trans* isomer, +39.59 ppm—^{18}O isotope shifts: Me—●—P=O, 0.0219 ppm; Me—O—P=●; 0.0484 ppm; Me—●—P=●, 0.0704 ppm. *cis* isomer, +37.92 ppm—

^{18}O ppm isotope shifts: Me—●—P=O, 0.0240 ppm; Me—O—P=●, 0.0483 ppm; Me—●—P=●, 0.0723 ppm.

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