Synthesis of Chiral [^{16}O,^{17}O,^{18}O]Phosphate Esters

By **Paul M. Cullis** and **Gordon Lowe,*** The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY

A stereospecific route for the synthesis of chiral [^{16}O,^{17}O,^{18}O]phosphate monoesters of known absolute configuration is described.

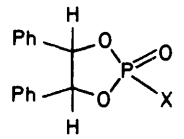
THE widespread occurrence of phosphate esters in living systems and the central importance of enzyme-catalysed phosphoryl transfer reactions in cellular energetics and metabolism, make phosphoryl transferases of particular mechanistic interest. Stereochemical analysis, of course, has long been recognised as a powerful method for elucidating the mechanism of chemical- and enzyme-catalysed reactions. However, although the substituents in tetraco-ordinated phosphorus compounds are tetrahedrally disposed, in phosphate monoesters the phosphorus atom is a *pro-pro*-chiral centre. In order to be able to investigate the stereochemical course of phosphoryl transfer reactions, this centre must be made chiral. One approach, which has attracted much attention, is to use a phosphorothioate analogue of the natural phosphate ester made chiral by stereospecific incorporation of ^{18}O.[1] Phosphorothioate analogues are, however, invariably poorer substrates for enzymes than the natural phosphate esters and in some cases are not substrates at all. It seemed conceivable, therefore, that phosphorothioate analogues may not necessarily follow the same stereochemical course as the natural substrate.

Since oxygen exists as three stable isotopes, namely ^{16}O, ^{17}O, and ^{18}O, and since ^{18}O is currently available at an enrichment in excess of 99 atom % as water and ^{17}O at an enrichment of *ca.* 50 atom % as water, it is feasible to prepare [^{16}O,^{17}O,^{18}O]phosphate monoesters for stereochemical studies.

Our synthetic strategy was constrained by the following considerations. Firstly, the route should be general so that any chiral [^{16}O,^{17}O,^{18}O]phosphate monoester could be made. Secondly, the route should enable ^{17}O and ^{18}O to be incorporated from isotopically labelled water. Thirdly, the absolute configuration of the chiral [^{16}O,^{17}O,^{18}O]phosphate ester should follow from the method of synthesis. These considerations led us to adopt the 2-substituted 2-oxo-4,5-diphenyl-1,3,2-dioxaphospholans (1; X = OR) as the molecular framework within which to incorporate the three oxygen isotopes, since catalytic hydrogenolysis of the benzylic–oxygen bonds should release the chiral [^{16}O,^{17}O,^{18}O]-phosphate monoester without perturbing any of the phosphorus–oxygen bonds.

Ukita *et al.*[2] have shown that when *meso*-hydrobenzoin is treated with phosphorus oxychloride in pyridine, the dioxaphospholan (1a) is obtained as a crystalline material. This reacted with alcohols in pyridine to give cyclic phosphate triesters (1; X = OR) and, in particular, with methanol to give the 2-methoxy-dioxaphospholan (1b). Although no spectroscopic evidence for these compounds was presented we were able to confirm, by ^1H and ^{31}P n.m.r. spectroscopy, that only a single diastereoisomer of both the 2-chloro- and 2-methoxy-dioxaphospholans (1a) and (1b) was formed.

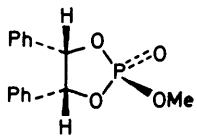
The 2-methoxy-dioxaphospholan (1b) can also be prepared by transesterification of *meso*-hydrobenzoin with trimethyl phosphite followed by oxidation of the cyclic phosphite with ozone.[3] This was shown to be the *trans*-diastereoisomer (2) by *X*-ray crystallography. The ^1H n.m.r. data reported by Newton and Campbell for this diastereoisomer [3] differs substantially from our own data for the 2-methoxy-dioxaphospholan obtained by Ukita's method, which together with the difference in melting points reported by Newton and Campbell (m.p. 74—75 °C) and Ukita (m.p. 101—102 °C) led us initially to assign the *cis*-configuration to the diastereoisomer obtained by Ukita's method.[4] However, we have recently recognised that the ^1H n.m.r. data of Newton and Campbell are in error, and by direct comparison of the ^1H and ^{31}P n.m.r. spectra have established that the 2-methoxy-dioxaphospholan prepared by Ukita's method is identical with that prepared by the method of Newton and Campbell and must therefore be the *trans*-diastereoisomer (2).[5] Newton and Campbell purified the triester (2) by sublimation, whereas Ukita recrystallised the triester from methanol–light petroleum. We have found that *rapid, low temperature* (—78 °C) recrystallisation of Ukita's triester from methanol–hexane gives crystals of m.p. 74—75 °C, in agreement with the melting point obtained



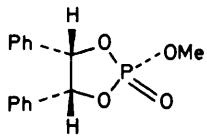
(1)

a ; X = Cl

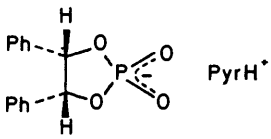
b ; X = OMe



(2)



(3)



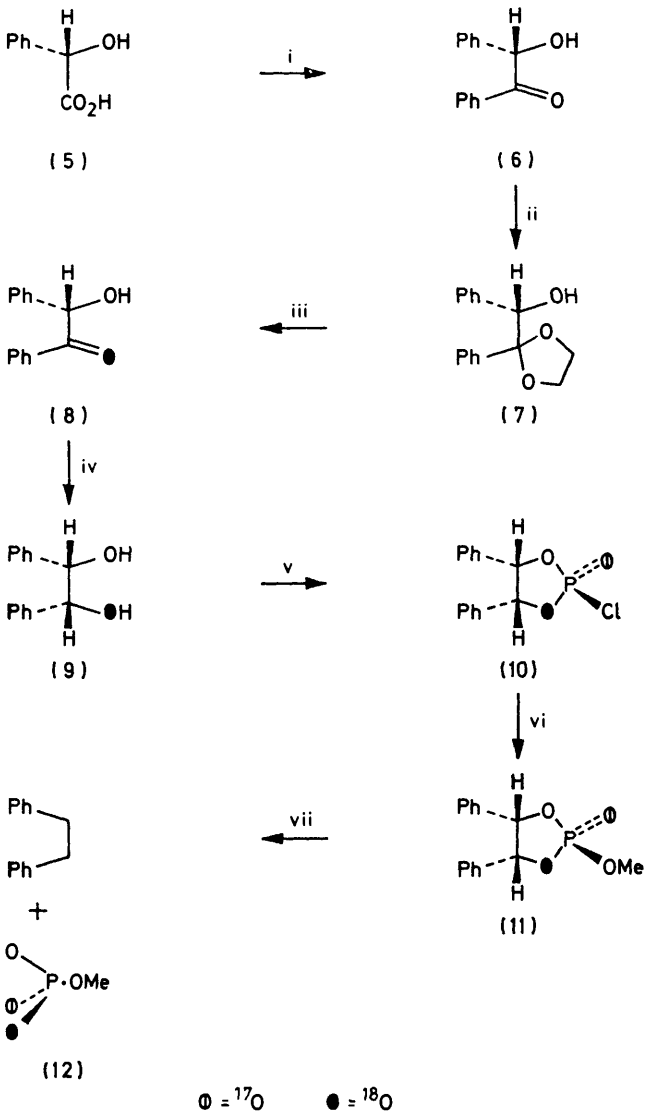
(4)

by Newton and Campbell,[3] but if Ukita's triester is kept in methanol at room temperature for several hours, ring-opening occurs to give dimethyl 1-(2-hydroxy-1,2-diphenylethyl)phosphate as the sole product, m.p. 101—103 °C. It seems likely that the product, m.p. 101—102 °C, reported by Ukita *after recrystallisation from methanol–light petroleum* was this acyclic triester.

In order to establish that the *cis*- and *trans*-2-methoxy-dioxaphospholans (2) and (3), were resolved by ^1H and ^{31}P n.m.r. spectroscopy, the pyridinium salt of the cyclic phosphate diester (4),[6] was treated with diazomethane. As expected, both the *trans*- (2) and *cis*-diastereoisomers (3) were formed, and the assignments in the ^1H and ^{31}P n.m.r. spectra were made by adding to the mixture the authentic *trans*-diastereoisomer (2), prepared by the method of Newton and Campbell.[3] The ratio of *trans*- to *cis*-isomers was 2 : 1.

It was also found, by ^1H and ^{31}P n.m.r. spectroscopy, that when *meso*-hydrobenzoin reacts with phosphorus oxychloride in tetrahydrofuran (THF) with only 2 equiv. of base present, both diastereoisomers of the 2-chloro-dioxaphospholan (1a) are formed. The configuration of the 2-substituted dioxaphospholans (1) can be assigned with some confidence because the diamagnetic anisotropy of the P=O group causes the ring protons *cis* to the P=O group to resonate at lower field than protons *trans* to the P=O group in the ^1H n.m.r. spectrum.[7] On the basis of this assignment, the ratio of the *cis*- to *trans*-2-chloro-dioxaphospholans (1a) is *ca.* 3 : 2. It seems that when only 2 equiv. of base are present, kinetic control leads to the predominant formation of the *cis*-diastereoisomer, but in the presence of an excess of pyridine, reversible ring-opening of the cyclic phosphorochloridate (1a) is possible leading to the thermodynamically more stable *trans*-diastereoisomer. Similar observations have been made in the preparation of the diastereoisomers of 2-methoxy-4,5-diphenyl-1,3,2-dioxaphospholan-2-thione.[8] The mixture of *cis*- and *trans*-2-chloro-dioxaphospholans reacts with methanol in the presence of a further equivalent of base to give the *cis*- and *trans*-2-methoxy-dioxaphospholans (3) and (2), respectively, in the same ratio of 3 : 2, implying that the reaction proceeds with retention of configuration. Indeed, this is the expected result for an exocyclic displacement at phosphorus in a five-membered cyclic phosphate derivative.[9] The simplest mechanism that gives rise to retention of configuration is the adjacent attack requiring a pseudorotation of the pentaco-ordinate intermediate. Because of the strong preference for five-membered rings to span apical–equatorial positions and because groups approach to and depart from the pentaco-ordinate intermediate preferentially from apical positions, it is likely that all nucleophilic displacement reactions with the 2-chloro-dioxaphospholan (1a) will proceed with retention of configuration.

Adaptation of the route developed by Ukita *et al.*[2] to the synthesis of chiral [^{16}O,^{17}O,^{18}O]phosphate monoesters is outlined in the Scheme. (*R*)- and (*S*)-Benzoin have been prepared by a variety of methods, but we have found that treatment of (*S*)-mandelic acid (5) with phenyl-lithium to form (*S*)-benzoin (6) represents the most direct and efficient method hitherto reported. The experimental conditions are somewhat critical and the



SCHEME *Reagents:* i, PhLi; ii, $HOCH_2CH_2OH$, *p*-$MeC_6H_4SO_3H$; iii, H_2[^{18}O]-dioxan–*p*-$MeC_6H_4SO_3H$; iv, $LiAlH_4$; v, $P^{17}OCl_3$, C_5H_5N; vi, MeOH, C_5H_5N; vii, H_2, Pd–C

best results were obtained when the solution of (*S*)-mandelic acid was allowed to fall in drops directly into the stirred solution of phenyl-lithium at −78 °C. If allowed to run down the sides of the reaction vessel the higher local concentration of mandelic acid leads to production of some 1,2-dihydroxy-1,1,2-triphenylethane, presumably by acting as a proton source. Allowing the reaction a further 2 h at room temperature was also essential to obtained yields of the order of 75% of the (*S*)-benzoin (6).

Although (*S*)-mandelic acid and (*S*)-benzoin were shown to be configurationally stable under conditions

which would allow the acid-catalysed ^{18}O-exchange from [^{18}O]water into the carboxy- and keto-groups, the dilution of isotope that would accompany this exchange made incorporation of ^{18}O into (*S*)-benzoin by hydrolysis of its acetal (7) more attractive. It was established that the acid-catalysed acetalisation of (*S*)-benzoin with ethylene glycol and the acid-catalysed hydrolysis of the acetal back to (*S*)-benzoin could be achieved without loss of chirality. The experimental conditions for the hydrolysis of the acetal are critical. The procedure outlined in the Experimental section represents the mildest conditions necessary to hydrolyse completely the acetal and it has been found that prolonged heating causes extensive racemisation.

Acid-catalysed hydrolysis of the acetal (7) using [^{18}O]-water (99.5 atom %) gave the ethan[1-^{18}O]one (8). The atom % ^{18}O) in pyridine, compound (9) gave the 2-[^{17}O]oxo-[1-^{18}O]-1,3,2-dioxaphospholan (10).

Methanolysis of the cyclic phosphorochloridate (10) in pyridine gave the 2-[^{17}O]oxo-[1-^{18}O]-1,3,2-dioxaphospholan (11) as a crystalline product which, on hydrogenolysis over palladium-on-charcoal, gave methyl [(*S*)-^{16}O,^{17}O,^{18}O]phosphate and 1,2-diphenylethane.

The *trans*-2-methoxy-dioxaphospholan (2) was also reductively cleaved by sodium in liquid ammonia to give methyl phosphate in good yield. This method is a useful alternative to catalytic hydrogenolysis, since it is difficult to achieve totally anhydrous conditions for hydrogenolysis, even when the palladium-on-charcoal catalyst is dried over phosphorus pentaoxide in a vacuum desiccator.

The dioxaphospholan (10), as expected, proves to be a general reagent for the preparation of chiral [^{16}O,^{17}O,-^{18}O]phosphate esters. The Table lists the compounds that have, to date, been prepared; full details of their synthesis will appear elsewhere. It should also be noted that it is possible to synthesize [(*R*)-^{16}O,^{17}O,^{18}O]phosphate monoesters by using either (*S*)-[^{17}O]benzoin and phosphorus [^{18}O]oxychloride or (*R*)-mandelic acid.

Reaction between the nucleophiles shown and (2*R*,4*S*,5*R*)-2-chloro-2-[^{17}O]oxo-[1-^{18}O]-1,3,2,-dioxaphospholan (10) followed by hydrogenolysis or cleavage with sodium in liquid ammonia gave the products shown, which indicates the generality of the method for synthesising [(*S*)-^{16}O,^{17}O,^{18}O]phosphate esters (P. M. Cullis, R. L. Jarvest, G. Lowe, and B. V. L. Potter, unpublished results)

Nucleophile	Product	Yield based on $P^{17}OCl_3$ (%)
Methanol	Methyl [(*S*)-^{16}O,^{17}O,^{18}O]phosphate	80
Cyanoethylphosphate	Inorganic [(*S*)-^{16}O,^{17}O,^{18}O]pyrophosphate	50
1,2,3,4-Tetra-*O*-acetyl-β-D-glucose	Glucose 6-[(*S*)-^{16}O,^{17}O,^{18}O]phosphate	55
2′,3′-Diacetyladenosine	Adenosine 5′-[(*S*)-^{16}O,^{17}O,^{18}O]phosphate	51
Adenosine-5′-diphosphate	Adenosine 5′-[γ-(*S*)-^{16}O,^{17}O,^{18}O]triphosphate	22
1,2-Dipalmitoyl-*sn*-glycerol	*sn*-Glycerol-3-[(*S*)-^{16}O,^{17}O,^{18}O]phosphate	60
p-Nitrobenzyl 3-*O*-triphenylmethyl-*R*-glycerate	2-[(*S*)-^{16}O,^{17}O,^{18}O]phospho-(*R*)-glycerate	32

configurational stability of (*S*)-benzoin under the conditions used for the hydrolysis of the acetal (7) effectively excludes the possibility of ^{18}O-exchange into the hydroxy-group. This, however, was confirmed by reducing the ethan[1-^{18}O]one (8) with lithium aluminium deuteride; the mass spectrum of the resultant [^{2}H,^{18}O]hydrobenzoin showed a molecular-ion peak at *m/z* 217 and base peaks of equal intensities at *m/z* 107 and 108 ($PhCH{=}O^{+}H$ and $[PhCH_2OH]^{+\cdot}$) and 110 and 111 ($PhC^{2}H{=}^{18}O^{+}H$ and $[PhCH^{2}H^{18}OH]^{+\cdot}$), which clearly indicates that the ^{2}H and ^{18}O were borne by the same carbon atom and hence that (2*S*)-[^{18}O]benzoin was exclusively labelled in the carbonyl group.

Reduction of (*RS*)-benzoin with lithium aluminium hydride at 0 °C is a classic example of asymmetric induction and gave exclusively *meso*-hydrobenzoin.[10] Reduction of (*S*)-benzoin with lithium aluminium hydride at 0 °C, or with sodium borohydride, likewise gave excellent yields of *meso*-hydrobenzoin. No circular dichroism was shown by these products down to λ 230 nm, indicating that they were free of (1*S*,2*S*)-1,2-dihydroxy-1,2-diphenylethane. Reduction of the ethan[1-^{18}O]one (8) with lithium aluminium hydride at 0 °C gave the [1-^{18}O]dihydroxy-ethane (9) which retained chirality solely because of isotopic substitution. On treatment with phosphorus [^{17}O]oxychloride (derived from phosphorus pentachloride and 1 equiv. of water containing 44.0 atom % ^{17}O; 1.8 atom % ^{16}O; and 54.2

EXPERIMENTAL

M.p.s were determined on a Koefler block and are uncorrected. ^{1}H N.m.r. spectra were recorded on a Perkin-Elmer R32 spectrometer. ^{31}P N.m.r. spectra were recorded on a Bruker WH90 spectrometer; by convention, resonances downfield from the reference, trimethyl phosphate, are assigned positive chemical shifts. Rotations were measured on a Perkin-Elmer 241 polarimeter. Circular dichroic spectra were measured on a Jasco J 40 CS spectrometer. Mass spectra were recorded on a Varian MAT CH 7 mass spectrometer.

(S)-*Benzoin* (6).—A solution of phenyl-lithium in ether was prepared from freshly distilled bromobenzene (40 g, 0.25 mol) and lithium wire (3.8 g, 0.55 mol). This solution was cooled to −78 °C and (*S*)-mandelic acid (8.2 g, 54 mmol) in dry tetrahydrofuran (THF) (50 ml) was added as drops with vigorous stirring in an atmosphere of dry nitrogen. The mixture was allowed to warm to room temperature and stirred for a further 2 h. The solution was poured onto a mixture of ice (300 g) and solid ammonium chloride (40 g) with vigorous stirring, and the organic layer was collected. The aqueous layer was further extracted with dichloro-

methane (2 × 100 ml) and the combined organic extract was washed with aqueous sodium hydrogencarbonate and brine, dried, and evaporated to give a creamy white solid (8.4 g, 74%). Careful recrystallisation from methanol gave (*S*)-benzoin (6) as white needles, m.p. 132—133 °C, $[\alpha]_D^{20}$ +117° (acetone, *c* 0.95, *l* 1) [lit.,[11] m.p. 132—133 °C, $[\alpha]_D^{20}$ +118.4° (acetone, *c* 2.5, *l* 1)]; δ_H {[$^2H_6$]dimethyl sulphoxide (DMSO)} 6.2 (2-d, AB, CHOH) and 7.2—8.1 (10 H, m, Ar-H) (Found: C, 79.0; H, 5.9. Calc. for $C_{14}H_{12}O_2$: C, 79.2; H, 5.7%).

2-Phenyl-2-(S-α-hydroxybenzyl)-1,3-dioxolan (7).—Compound (7) was prepared in the same way as the racemic material.[12] (2*S*)-Hydroxy-1,2-diphenylethanone (1 g, 4.72 mmol), ethane-1,2-diol (0.47 g, 7.6 mmol), and toluene-*p*-sulphonic acid (30 mg, 0.16 mmol) in dry benzene (30 ml) were heated under reflux in a Dean–Stark apparatus and the azeotropically distilled water collected over a period of 16 h. The cooled solution was washed with aqueous sodium hydrogencarbonate and water, and the organic layer was dried and evaporated to give a white, crystalline solid (0.9 g, 80%). Recrystallisation from benzene and hexane gave the dioxolan (7) as white needles, m.p. 138—139.5 °C (*cf.* the racemic compound, lit.,[12] m.p. 144.1—144.5 °C); $[\alpha]_D^{20}$ −12.0° (acetone, *c* 1, *l* 1); δ_H ([$^2H_6$]DMSO) 3.6—4.0 (4 H, m, CH_2), 4.78 (1 H, d, CH), 5.44 (1 H, d, OH), and 7.14 and 7.20 (10 H, 2 s, Ar-H) (Found: C, 75.0; H, 6.3. $C_{16}H_{16}O_3$ requires C, 75.0; H, 6.3%).

(2S)-*Hydroxy-1,2-diphenylethan*[1-^{18}O]*one* (8).—The dioxolan (7) (0.5 g, 1.95 mmol) and anhydrous toluene-*p*-sulphonic acid (40 mg, 0.23 mmol) in dry dioxan (0.8 ml) and [^{18}O]water (0.4 ml, 99.5 atom %) were sealed in a hydrolysis tube under nitrogen. The tube was heated at 90 °C for 3.5 h after which the contents were transferred to a flask attached to a vacuum line and the dioxan–water mixture recovered for re-use. The solid residue was dissolved in dichloromethane (20 ml) and the solution was washed with aqueous sodium hydrogencarbonate and water. Evaporation of the dried organic layer yielded a white, crystalline solid (0.4 g, 97%). Recrystallisation from benzene and hexane gave the ethanone (8) as needles, m.p. 132—133 °C, $[\alpha]_D^{20}$ +116° (acetone, *c* 1, *l* 1).

Reduction of (2S)-*Benzoin* (6).—(a) $LiAlH_4$: *using the method of Pohoryles* et al.[10] Solid lithium aluminium hydride (40 mg, 1.17 mmol) was added in portions to a suspension of (2*S*)-hydroxy-1,2-diphenylethanone (212 mg, 1 mmol) in dry ether (10 ml) at 0 °C. The mixture was stirred for 2 h after which the excess of reagent was destroyed by addition of ether saturated with water. The precipitated salts were filtered off and the filtrate was washed with water, dried ($MgSO_4$), and evaporated to give a white, crystalline solid (175 mg, 81%). Recrystallisation from benzene gave *meso*-hydrobenzoin as white plates, m.p. 134—135.5 °C which possessed no measurable circular dichroism.

(b) $NaBH_4$. Solid sodium borohydride (40 mg, 1 mmol) was added in portions to a suspension of (2*S*)-hydroxy-1,2-diphenylethanone (0.212 g, 1 mmol) in ethanol (2 ml). The suspension was shaken at room temperature until all the solid had dissolved and then the solution was heated at 50 °C for 10 min. Dilution with water (8 ml) gave white plates of *meso*-hydrobenzoin (0.17 g, 80%), m.p. 134 °C, which possessed no measurable circular dichroism; δ_H ($CDCl_3$) 2.78 (2 H, s, OH), 4.78 (2 H, s, CH), and 7.2 (10 H, s, Ar-H).

(1R,2S)-1,2-[1-^{18}O]*Dihydroxy-1,2-diphenylethane* (9) was obtained by reduction of the ethan[1-^{18}O]one (8) using either of the above procedures. The c.d. spectrum has been reported previously.[13]

(1R,2S)-1,2-[1-^{2}H,1-^{18}O]*Dihydroxy-1,2-diphenylethane* was obtained by reduction of the ethan[1-^{18}O]one (8) with lithium aluminium deuteride employing the above general procedure. The mass spectrum had *m/z* 217(M^+) and base peaks of equal intensity at *m/z* 107 and 108 (PhCH=O^+H and [$PhCH_2OH$]$^{+\bullet}$) and 110 and 111 ($PhC^2H{=}^{18}O^+H$ and [$PhCH^2H^{18}OH$]$^{+\bullet}$). *meso*-Hydrobenzoin has *m/z* 214 (M^+) and base peaks of equal intensity at *m/z* 107 and 108.

2-Chloro-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholan (1a).—(a) trans-*2-Chloro-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholan.*—The *trans*-diastereoisomer was prepared by the method of Ukita *et al.*[2] *meso*-1,2-Dihydroxy-1,2-diphenylethane (1.5 g, 7 mmol), dissolved in dry pyridine (30 ml), was added as drops to a rapidly stirred solution of freshly distilled phosphorus oxychloride (1.75 g, 11.4 mmol) in dry pyridine (10 ml) during 2 h. The solution was stirred for a further 1 h at 0 °C, then kept at room temperature for 30 min before the solvent and the excess of reagent were evaporated under reduced pressure. The residue was extracted with benzene (2 × 10 ml) and the insoluble pyridinium chloride centrifuged off. The benzene solution was slowly added to light petroleum (b.p. 40—60 °C; 50 ml) and a white, crystalline material was obtained on cooling. The supernatant liquid was decanted and the crystals of *trans*-(1a) dried in a vacuum desiccator over P_2O_5 (1.48 g, 70%); δ_H (C_6D_6) 5.35 (2 H, d, J_{HP} 7.6 Hz, H-4 and H-5) and 6.7—7.2 (10 H, m, Ar-H); δ_P (THF, D_2O lock) +15.26 p.p.m.

(b) cis- *and* trans-*Chloro-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholan* (1a). *meso*-1,2-Dihydroxy-1,2-diphenylethane (214 mg, 1 mmol) in dry THF (2 ml) was added as drops to a stirred solution of phosphorus oxychloride (153 mg, 1 mmol) and pyridine (158 mg, 2 mmol) in THF (1 ml). After 2 h at room temperature the supernatant liquid was separated from the precipitated pyridinium hydrochloride to give *cis*- and *trans*-(1a); δ_H (C_6D_6) 5.3 (d, J_{HP} 7.6 Hz, *trans*-CH), 5.6 (d, J_{HP} 7.6 Hz *cis*-CH), and 6.7—7.2 (m, Ar-H); δ_P (THF, D_2O lock) +15.26 (*trans*) and +15.04 p.p.m. (*cis*).

Purification of trans-*2-Methoxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholan* (2).—The *trans*-dioxaphospholan (2) was obtained crystalline by the literature procedures of Ukita *et al.*[2] and of Newton and Campbell.[3] This material can, with care, be recrystallised from methanol and hexane, provided the compound is not left in solution for extended periods (see below). The solid was dissolved at room temperature in a small volume of methanol saturated with hexane, and the solution filtered. The solution was cooled to −78 °C to afford white prisms which can be isolated by rapid filtration or centrifugation, m.p. 74—75 °C (lit.,[3] m.p. 74—75 °C after sublimation).

Dimethyl 1-(*2-Hydroxy-1,2-diphenylethyl*)*phosphate.*—Dimethyl 1-(2-hydroxy-1,2-diphenylethyl)phosphate is obtained when the *trans*-dioxaphospholan is dissolved in methanol at room temperature and left for *ca.* 5 h. Evaporation of the methanol yielded a crystalline material which was recrystallised from benzene–hexane to give the *dimethyl phosphate* as white needles, m.p. 101—103 °C; δ_H ($CDCl_3$) 3.12 (1 H, br s, OH), 3.34 (3 H, d, J_{PH} 11 Hz, Me), 3.37 (3 H, d, J_{PH} 11 Hz, Me), 4.91 (1 H, d, *J* 6 Hz, C*H*OH), 5.38 (1 H, dd, *J* 11, 6 Hz, CHOP), and 7.18 (10 H, s, Ar-H); δ_P ($CHCl_3$, D_2O lock) −2.04 p.p.m. (Found: C, 59.9; H, 6.1; P, 9.3. $C_{16}H_{19}O_5P$ requires C, 59.6; H, 5.9; P, 9.6%).

Phosphorus [^{17}O]*Oxychloride.*—The oxychloride was prepared by the method of Laulicht *et al.*[14] [^{17}O]Water (0.091

ml; 44.0 atom % ^{17}O, 1.8 atom % ^{16}O; 54.2 atom % ^{18}O) was added as drops to solid phosphorus pentachloride (1.07 g, 5.1 mmol) cooled in ice. The flask was allowed to warm to room temperature after the last addition, then the clear solution was heated under reflux for 30 min. Distillation afforded phosphorus [^{17}O]oxychloride as a clear liquid, b.p. 105—106 °C (0.57 g, 3.7 mmol, 73%).

(2R,4S,5R)-2-*Chloro*-2-[^{17}O]*oxo*-4,5-*diphenyl*-[1-^{18}O]-1,3,2-*dioxaphospholan* (10).—The [1-^{18}O]Dihydroxy-ethane (9) (216 mg, 1 mmol) in dry pyridine (4 ml) was added to a cold solution of phosphorus [^{17}O]oxychloride (94 μl, 1.05 mmol) during 2 h with vigorous stirring. The mixture was stirred for a further 30 min at 0 °C and then for 30 min at room temperature. The pyridine solution of the cyclic phosphorochloridate (10) was used without further purification.

(2R,4S,5R)-2-*Methoxy*-2-[^{17}O]*oxo*-4,5-*diphenyl*-[1-^{18}O]-1,3,2-*dioxaphospholan* (11).—Anhydrous methanol (42 μl, 1 mmol) in dry pyridine (1 ml) was added to the solution of the cyclic phosphorochloridate (10) (1 mmol) at 0 °C during a period of 20 min. After 1 h the solvent was removed by evaporation and the residue was partitioned between benzene and water. The organic extract was washed with aqueous sodium hydrogencarbonate and evaporated to give a white, crystalline solid (11) (294 mg quantitative); δ_H ($CDCl_3$) 3.95 (3 H, d, J_{HP} 11.6 Hz, Me), 5.78 (2 H, d, J_{HP} 7.9 Hz, CH), and 6.9—7.5 (10 H, m, Ar-H); δ_P ($CHCl_3$, D_2O lock) +13.4 p.p.m.

Methyl [(S)-^{16}O,^{17}O,^{18}O]*Phosphate* (12).—Palladium-on-charcoal (50 mg, 10% metal, dried over P_2O_5 in a vacuum desiccator) in ethyl acetate was reduced with hydrogen prior to introducing the dioxaphospholan (11) (294 mg, 1 mmol) dissolved in ethyl acetate (5 ml). The solution was vigorously shaken with hydrogen for 15 min and then more palladium-on-charcoal (50 mg, 10% metal) was added. Uptake of hydrogen continued for *ca.* 1.5 h after which the catalyst was filtered off through a glass-fibre filter paper and washed with hot ethyl acetate (3 × 15 ml). The ethyl acetate washings were combined, the solution was evaporated, and the clear gum partitioned between water and ether. The aqueous layer was neutralised to pH 8.0 with aqueous sodium carbonate before being lyophilised to give methyl [(*S*)-^{16}O,^{17}O,^{18}O]phosphate as a white powder (113 mg, 95%).

Methyl Phosphate.—The phospholan (2) (290 mg, 1 mmol) in dry THF (5 ml) was added to a solution of sodium (*ca.* 46 mg, 2 mmol) in dry liquid ammonia (50 ml) under dry nitrogen. The solution was stirred for 10 min, then quenched with a slight excess of solid ammonium chloride and the liquid ammonia evaporated with a stream of nitrogen. The solid residue was extracted with water (5 × 20 ml) and this solution was applied, at pH 7.6, to a column of DEAE-sephadex A25 (*ca.* 40 ml. 1 × 20 cm) which had been previously equilibrated with aqueous triethylammonium hydrogencarbonate buffer (50 mM) at pH 7.6. The column was eluted with a linear gradient of increasing buffer concentration (50—400 mM) and the eluate was collected in fractions. Those fractions containing methyl phosphate were identified by t.l.c. using an ammonium molybdate spray and were pooled and evaporated to give the bis-(triethylammonium) salt. The bis-sodium salt of methyl phosphate was obtained by treatment with an excess of Dowex 50W (Na^+ form); δ_H (D_2O) 3.5 (d, J_{HP} 11.8 Hz, Me); δ_P (D_2O) + 1.9 p.p.m.

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