Stereochemical Studies of Reactions of Phosphate and Thiophosphate Esters

A Thesis submitted for the Degree of

Doctor of Philosophy

by

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in the

Faculty of Science

of the

Department of Chemistry

at the

University of Leicester

September 1988

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To Mom and Dad

STATEMENT

The accompanying thesis submitted for the degree of Doctor of Philosophy entitled "Stereochemical Studies of Reactions of Phosphate and Thiophosphate Esters" is based on work conducted by the author in the Department of Chemistry of the University of Leicester mainly during the period between October 1983 and September 1986.

All the work recorded in this thesis is original unless otherwise acknowledged in the text or by references.

None of the work has been submitted for another degree in this or any other University.

Signed: ...A. Iagrossi..... Date: .August 1988

ACKNOWLEDGEMENTS

I would like to thank Dr. Paul Cullis for his help, encouragement and consideration shown over the past years.

I am grateful to the research students, the academic staff and the technicians in the Department of Chemistry for their help.

I would like to thank Miss V. Orson-Wright for the typing and Mrs. C. A. Crane for the diagrams in this thesis.

Finally, I would like to thank the Science and Engineering Research Council for financial support.

ABBREVIATIONS AND SYMBOLS

$S_N(P)$	=	nucleophilic substitution
t.b.p	=	trigonal bipyramid
spy	=	square pyramid
ψ	=	pseudorotation
ATP	=	Adenosine triphosphate
^{31}P nmr	=	phosphorus nuclear magnetic resonance
ppm	=	parts per million
ADP	=	Adenosine diphosphate
RNA	=	Ribonuclease
E	=	Enzyme
$POCl_3$	=	phosphorus oxychloride
Pd	=	palladium
C	=	charcoal
AMPS	=	Adenosine monophosphate
TMSI	=	Trimethylsilyliodide
O-Alk	=	O-alkyl
O	=	oxygen
Se	=	Selenium
S	=	Sulphur
PEP	=	phosphoenol pyruvate
chlorocompound	=	2-chloro-1,3,2-oxazaphospholidine
$Bu_3NH^+Cl^-$	=	Tributylammonium
PO_3	=	monomeric metaphosphate
SO_3	=	sulphur trioxide
EDTA	=	Tetraacetic acid disodium salt
P_1	=	pyrophosphate
pAp	=	Adenosine 2',5'-bisphosphate
ppAp	=	Adenosine-2'-phospho-5'-diphosphate
PIX	=	positional isotope exchange
^tBuOH	=	tertiary butanol
CH_3CN	=	acetonitrile
D.N.A.	=	Deoxyribonucleic acid
B^{nuc}	=	Brønstead coefficient for the nucleus
B^{lg}	=	Brønstead coefficient for the leaving group

ABBREVIATIONS AND SYMBOLS (Continued) ....

L	=	Laevo
D	=	Dextro
EtOH	=	Ethanol
MeOH	=	Methanol
Cl	=	Chlorine
F	=	Fluorine
OMe	=	Methoxide
OEt	=	Ethoxide
SEt	=	Ethane thiol
SPr	=	Propane thiol
$\Delta S^{\ddagger}$	=	entropy change
NMe_2	=	Dimethyl amine
Bz	=	Benzyl
Ar	=	Aryl
Ph	=	Phenyl
Et	=	Ethyl
Bu^t	=	tertiary butyl
Me	=	methyl
Ac	=	Acetyl
THF	=	Tetrahydrofuran
TCA	=	Trichloroacetic acid
S	=	chemical shift measured in parts per million (ppm)
Δ	=	denotes reaction proceeds under thermal conditions
^{31}P n.m.r.	=	phosphorus nuclear magnetic resonance spectroscopy
^{1}H n.m.r.	=	proton nuclear magnetic resonance spectroscopy
M^+	=	molecular ion
t.l.c.	=	thin layer chromatography
r.t.	=	retention time
R_f	=	retention factor
Hz	=	Hertz
KHz	=	Kilohertz
M	=	molar
°C	=	degrees Centigrade
s	=	singlet

ABBREVIATIONS AND SYMBOLS (Continued) ....

d	=	doublet
t	=	triplet
m	=	multiplet
q	=	quartet
i.r.	=	infra-red
u.v.	=	ultra violet
cms	=	centimetres
cm^3	=	cubic centimeters

PUBLICATIONS

Reprinted from the Journal of the American Chemical Society, 1986, *108*, 7869.

Thiophosphoryl-Transfer Reactions: A General Synthesis and Configurational Analysis of O-Substituted [^{16}O,^{18}O]Thiophosphates

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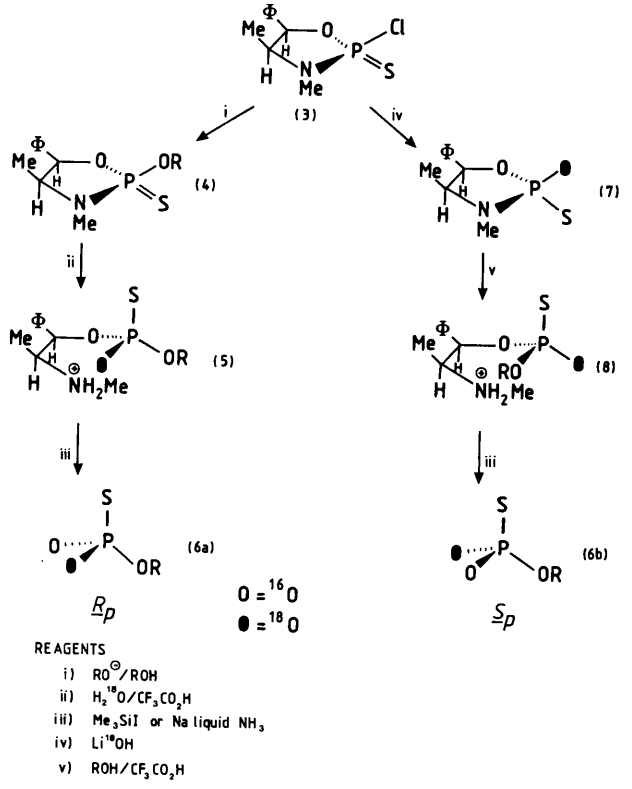
[^{16}O,^{18}O]Thiophosphate (**1**) and [^{16}O,^{17}O,^{18}O]phosphate (**2**) esters have been utilized extensively to determine the stereochemical course of many enzyme-catalyzed thiophosphoryl-[1] and

phosphoryl-transfer[2] reactions. Although the stereochemical courses of some simple chemical phosphoryl-transfer reactions have recently been determined,[3,4] hitherto *simple thiophosphoryl*-transfer reactions have not been studied. With existing methods these would in fact be difficult to determine. Such studies would be of interest since (i) the stereochemical course of enzyme-catalyzed thiophosphoryl-transfer reactions has frequently been assumed to be the same as for the natural phosphoryl-transfer reaction and it would be pertinent to determine whether these reactions are indeed stereochemically equivalent[5] and (ii) thiophosphate monoesters have been reported to react more rapidly via a dissociative reaction than the corresponding phosphate esters.[6] We report here the first simple chemical configurational analysis of structures such as **1** (R = alkyl or aryl)[7] together with general synthetic routes to simple [^{16}O,^{18}O]thiophosphate monoesters (**1**).[8]

Our two general routes to isotopically chiral [^{16}O,^{18}O(or ^{17}O)]thiophosphate monoesters of either the R_P or S_P absolute configuration are shown in Scheme I. By analogy with the previously published route(s) to [^{16}O,^{17}O,^{18}O]phosphate esters,[9]

Scheme I



(1) Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423. Frey, P. A. *Tetrahedron* **1982**, *38*, 1541.

(2) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877. Lowe, G. *Acc. Chem. Res.* **1983**, *16*, 244. Gerlt, J. A.; Coderre, J. A.; Mehdi, S. *Adv. Enzymol.* **1983**, *55*, 291.

(3) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 1438. Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4911. Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 6126.

(4) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 6721. Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 1298.

(5) The demonstration for a number of enzymes that phosphoryl and thiophosphoryl transfer proceed with the same stereochemical course (see ref 1 and 2) would suggest that within the constraints of the enzyme active site these two reactions are equivalent.

(6) Breslow, R.; Katz, I. *J. Am. Chem. Soc.* **1968**, *90*, 7376.

(7) Two configurational analyses have been reported for AMPS ^{18}O and other nucleoside [^{18}O]thiophosphates: the first relies on the stereospecific enzyme-catalyzed phosphorylation of the *pro-R/S* oxygen as the key step (Sheu, K.-F. R.; Frey, P. A. *J. Biol. Chem.* **1977**, *252*, 4445); the second method has assigned the absolute configurations of the *O,S*-dimethyl nucleoside triesters by relating these to the *O*-methyl nucleoside diesters which have been assigned on the basis of the known stereoselectivity of snake venom phosphodiesterase (Cummins, J. H.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1985**, 851). Neither method was suitable for our proposed study.

(8) Previous syntheses of isotopically chiral thiophosphate monoesters based on the *meso*-hydrobenzoin route (Cullis, P. M.; Lowe, G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2317. Jarvest, R. L.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1979**, 364) have been reported but not extensively applied. Similarly [γ-^{16}O,^{18}O,S]ATP and [^{18}O]AMPS have been synthesized by routes that would not easily be extendible to simple thiophosphate esters.

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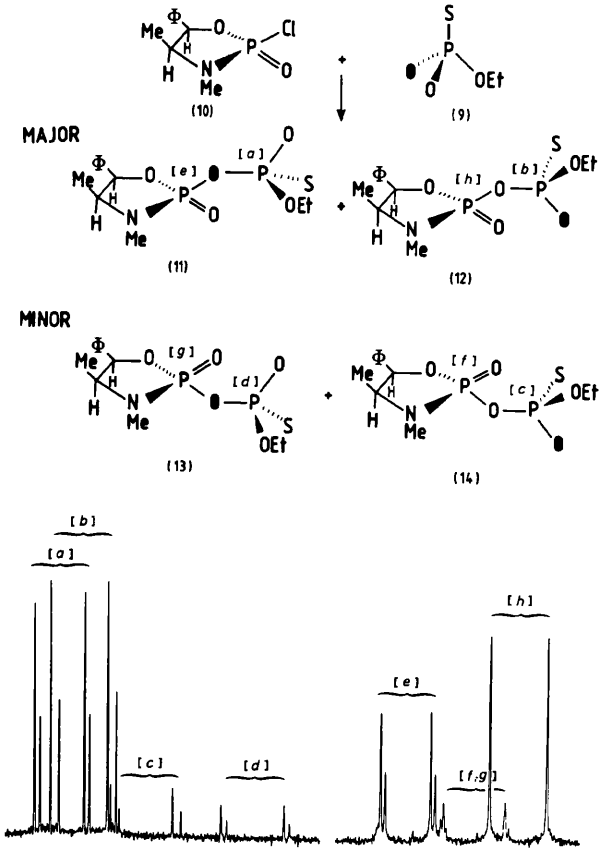

Figure 1. Stereochemical analysis of ethyl (S_P)-[^{16}O,^{18}O]thiophosphate by ^{31}P NMR spectroscopy of the product following reaction with *cis*-2-chloro-3,4(*S*)-dimethyl-5(*S*)-phenyl-1,3,2-oxazaphospholidin-2-one. The spectrum was recorded on a Bruker AM-300 at 121.5 MHz and processed with Gaussian multiplication (Gaussian broadening 0.1 Hz, line broadening −0.3 Hz). The assignments are as shown with the downfield resonances (thiophosphoryl center) at ca. +46 ppm and the upfield resonance (1,3,2-oxazaphospholidine center) at ca. +7 ppm.[12]

these syntheses exploit the stereocontrolled displacement reactions of 2-substituted 1,3,2-oxazaphospholidine-2-thiones, which have established precedent in the work of Inch et al.[10]

The major objective has been the development of a general method for the configurational analysis of isotopically chiral thiophosphate monoesters. During the course of our work on the stereochemistry of phosphoryl transfer from P^1,P^1-disubstituted pyrophosphates,[4] we synthesized the unlabeled diastereomeric pyrophosphates corresponding to **11** and **12**. These were readily distinquished by high-field ^{31}P NMR spectroscopy and form the basis of the configurational analysis reported here. S_P-*O*-Ethyl [^{16}O,^{18}O]thiophosphate (**9**) (^{18}O enrichment ca. 33%) was synthesized by the route shown in Scheme I. The absolute configuration follows from the synthesis. Reaction of **9** with the *cis*-2-chloro-1,3,2-oxazaphospholidin-2-one (**10**) derived from (−)-ephedrine gave rise to the pyrophosphate derivatives **11**–**14**. The high-field ^{31}P NMR spectrum together with the assignments are shown in Figure 1. Resonances corresponding to centers **e** and **h** can be unambiguously assigned since the 1,3,2-oxazaphospholidine phosphorus center is attached to ^{18}O in diastereosomer **11** but not in diastereoisomer **12**, hence only one set of resonances will be split by the stereospecific incorporation of ca. 33% ^{18}O. On the basis of the bond-order dependence of the ^{18}O shift[11] on the thiophosphoryl signal, resonances **a** can be assigned to the diastereoisomer **11** in which the ^{18}O is located in the bridging position and resonances **b** can be assigned to the diastereoisomer **12**. The additional minor resonances seen in Figure 1 are due to structures **13** and **14**, which are epimeric at the ring phosphoryl center with respect to **11** and **12**.[13] R_P-*O*-Ethyl [^{16}O,^{18}O]thiophosphate would give rise to a ^{18}O shift on **h** rather than **e** and the magnitude of the ^{18}O shifts on **a** and **b** would be reversed. The downfield ^{31}P resonances arise from diastereoisomer **11** (in which the new chiral center at the thiophosphoryl position has the R_P configuration) while the upfield ^{31}P NMR resonances arise from diastereoisomer **12** (in which the new chiral center has the S_P configuration). We have established that this assignment holds for **6a** (R = *p*-nitrophenyl) and **6b** (R = ethyl), and it may hold for a wide range of R groups. The above assignments form the basis of our method for studying the stereochemical course of simple thiophosphoryl-transfer reactions.[14] The above analysis strategy is potentially general and may allow extension to the study of a range of hydrolysis reactions leading to phosphorus acids of the type $R^1R^2PO_2^-$ ($R^1 \neq R^2$).[15]

Acknowledgment. This work was supported by a grant from the SERC.

(9) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2558. The extension of the published route to isotopically chiral phosphate monoesters to the synthesis of *thio*phosphate monoesters is *non*trivial; the thiophosphorochloridate is significantly less reactive, the acid ring opening step can lead to competing loss of sulfur, and finally the removal of the ephedrine framework is difficult. Full details will be published elsewhere.

(10) Cooper, D. B.; Hall, C. R.; Harrison, J. M.; Inch, T. D. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1969.

(11) Lowe, G.; Potter, B. V. L.; Sproat, B. S.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1979**, 733. Cohn, M.; Hu, A. *J. Am. Chem. Soc.* **1980**, *102*, 913.

(12) The ^{31}P NMR data from the spectrum shown in Figure 1 are as follows: diastereoisomer **11** δ ($CDCl_3$) +7.14 (d, J_{PP} = 25.9 Hz, 1,3,2-oxazaphospholidin-2-one, ^{18}O shift 2.28 Hz), +46.39 (d, J_{PP} = 25.9 Hz, R_P thiophosphoryl center, ^{18}O shift 2.84 Hz); diastereoisomer **12** δ ($CDCl_3$) +6.65 (d, J_{PP} = 29.7 Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{PP} = 29.7 Hz, S_P thiophosphoryl center, ^{18}O shift 4.46 Hz).

(13) The trans diastereoisomers **13** and **14** apparently do not arise from trace amounts of the trans chloro compound analogous to **10** but are due to an epimerization reaction.

(14) Cullis, P. M.; Iagrossi, A., following paper in this issue.

(15) Trippett, S.; White, C. *J. Chem. Soc., Chem. Commun.* **1984**, 251.

Reprinted from the Journal of the American Chemical Society, 1986, *108*, 7870.

Thiophosphoryl-Transfer Reactions: Stereochemical Course of Solvolysis of *p*-Nitrophenyl Thiophosphate in Protic Solvent and the Possible Role of Thiometaphosphate

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Received May 28, 1986

There is much current interest in monomeric metaphosphate as a possible intermediate in nucleophilic displacement reactions of monosubstituted phosphate esters[1] and, in particular, in relation to enzyme-catalyzed phosphoryl-transfer reactions.[2] Stereochemical,[3,4] kinetic,[5,6] and thermodynamic[7] evidence suggests that metaphosphate is so reactive that it does not have a significant lifetime in *protic* solvents (although many other three-coordinate P(V) compounds have appreciable stabilities[8,9]). In contrast,

(1) Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313.
(2) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877. Lowe, G. *Acc. Chem. Res.* **1983**, *16*, 244.
(3) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 1438. Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4911.
(4) Calvo, K. *J. Am. Chem. Soc.* **1985**, *107*, 3690.
(5) Skoogs, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1983**, *105*, 3356. Skoogs, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597.
(6) Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1983**, *105*, 3357. Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591.
(7) Ramirez, F.; Maracek, J.; Minore, J.; Srivastava, S.; le Noble, W. *J. Am. Chem. Soc.* **1986**, *108*, 348.
(8) Regizt, M.; Maas, G. *Top. Curr. Chem.* **1981**, *97*, 71–120.
(9) Roesky, H. W.; Ahlrichs, R.; Brode, S. *Angew. Chem., Int. Ed., Engl.* **1986**, *25*, 82.

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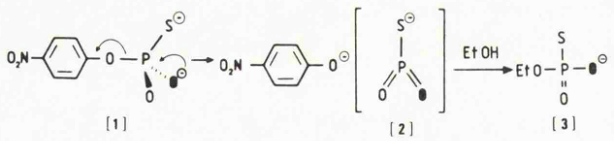
Scheme I



stereochemical studies on analogous phosphoryl-transfer reactions in *aprotic* solvents have found them to proceed with extensive racemization[10-12] which leaves open the possibility of an intermediate with an appreciable lifetime. Further to our studies on monomeric metaphosphate[10,11] we have been interested in the properties and lifetime of the closely related *thio*metaphosphate, this being the closest relative of metaphosphate itself. We report here one of *the first stereochemical investigations of a simple thiophosphoryl-transfer reaction.*

Thiophosphate monoesters undergo nucleophilic displacement reactions more rapidly than their oxy counterparts, in marked contrast to the corresponding di- and triesters.[13] This enhanced reactivity has been explained in terms of a facile dissociative breakdown to give monomeric thiometaphosphate, Scheme I. Utilizing isotopically chiral *p*-nitrophenyl [^{16}O,^{18}O]thiophosphate (**1**) we have sought stereochemical evidence pertinent to this point. *p*-Nitrophenyl (*R*)-[^{16}O,^{18}O]thiophosphate (**1**) (ca. 75% ^{18}O enrichment at the labeled site) was synthesized by the route described in the preceding paper. The absolute configuration followed from the synthesis and the enantiomeric purity (≥90% R_P) was independently established by our general method of analysis, Figure 1C.[14] The dianion (100 mM)[15] in ethanol was solvolyzed at 50 °C for 3 h. The product ethyl [^{16}O,^{18}O]thiophosphate (**3**) was isolated by ion-exchange chromatography and subjected to the stereochemical analysis described in the accompanying paper. Since an ^{18}O shift is clearly evident on both sets of upfield resonances,[16] Figure 1A, one can conclude that *the product was largely racemic*, as indicated by the ^{31}P NMR resonance assignments shown. The enantiomeric excess in the product ethyl [^{16}O,^{18}O]thiophosphate (**3**) can be quantified from the relative intensities of the resonances in Figure 1A and corresponds to thiophosphoryl transfer occurring with ca. 80% racemization and a 20% excess of the S_P configuration which would arise from thiophosphoryl transfer occurring with inversion of configuration. Independent experiments established that (i) ethyl (*S*)-[^{16}O,^{18}O]thiophosphate does not racemize under the solvolysis conditions, Figure 1B, and (ii) the starting material, *p*-nitrophenyl (*R*)-[^{16}O,^{18}O]thiophosphate, reisolated from a solvolysis reaction carried out to 50% completion (50 °C, 1.5 h), had not racemized (within experimental error), Figure 1C.[14] It is therefore clear that the *racemization arises during the thiophosphoryl-transfer reaction.*

The simplest interpretation of this result is that the reaction proceeds via a dissociative reaction involving the monomeric thiometaphosphate intermediate **2**. Furthermore, in contrast to analogous phosphoryl-transfer reactions in protic solvent in which the putative metaphosphate intermediate is so reactive that the reaction follows a concerted preassociative mechanism,[3,5-7] the thiometaphosphate intermediate must be sufficiently long-lived to allow partial loss of stereochemical integrity.

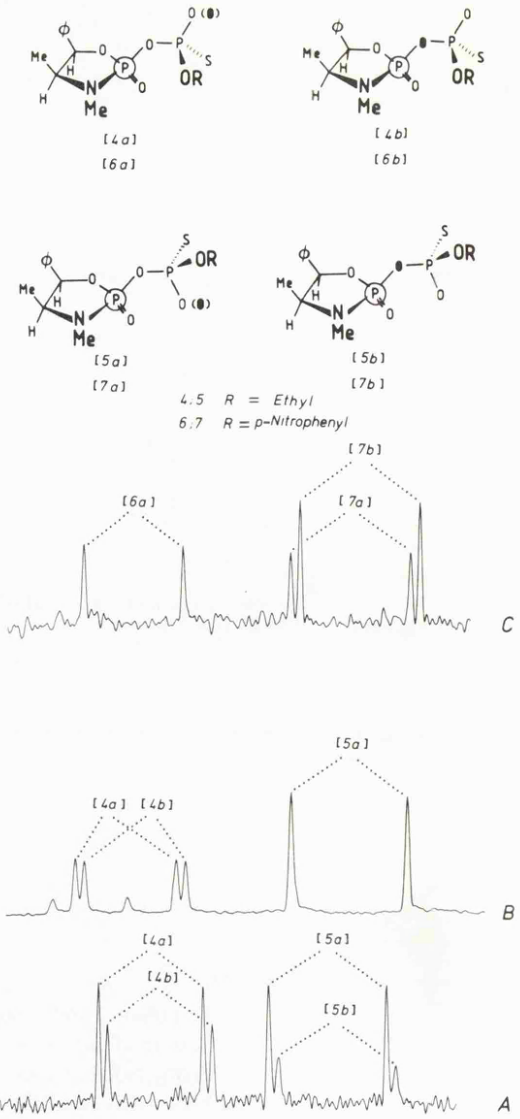


Figure 1. ^{31}P NMR spectra showing the 1,3,2-oxazaphospholidin-2-one resonances (resonances e and h in the preceeding paper) at ca. +7 ppm of the products from the stereochemical analysis of (A) ethyl [^{16}O,^{18}O]thiophosphate obtained from the solvolysis of *p*-nitrophenyl (R_P)-[^{16}O,^{18}O]thiophosphate dianion in ethanol at 50 °C, (B) ethyl (S_P)-[^{16}O,^{18}O]thiophosphate (ca. 50% enriched at the ^{18}O site) after being subjected to conditions identical with the solvolysis reaction, and (C) *p*-nitrophenyl (R_P)-[^{16}O,^{18}O]thiophosphate reisolated from the solvolysis reaction. The spectra were recorded on a Bruker AM-300 at 121.5 MHz and processed with Gaussian multiplication (GB 0.1 Hz; LB −0.5 Hz).

Note Added in Proof. Professor Berkovic has recently reported preliminary observations of partial racemization during the hydrolysis of *p*-nitrophenyl thiophosphate monoanion (Domanico, P.; Mizrahi, V.; Berkovic, S. J. In *Mechanisms of Enzymatic Reactions: Stereochemistry*; Frey, P. A., Ed.; Elsevier: Amsterdam, 1986; pp 127–137).

Acknowledgment. This work was supported by the Science and Engineering Research Council (UK). We thank David Turner for help in obtaining NMR spectra and Martin Harger for much helpful discussion. We thank Professor Berkovic for providing details of their related studies.

(10) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 6721.
(11) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 1298.
(12) Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 6126.
(13) Breslow, R.; Katz, I. *J. Am. Chem. Soc.* **1968**, *90*, 7376.
(14) The spectrum in Figure 1C is actually of starting material reisolated from a thiophosphoryl-transfer reaction; however, the spectrum for the starting material analyzed directly from the synthesis was identical. Although we believe there to be essentially no **6b** present, the lower limit on this is determined by the signal-to-noise ratio. A conservative estimate of the enantiomeric excess is therefore 90 ± 10%. The departure of the ratio of **7a** to **7b** from 1:3 arises from dilution of the isotope during the synthesis; no attempt to quantify this has been made since the configurational analysis does not depend on absolute intensities.
(15) *p*-Nitrophenyl (*R*)-[^{16}O,^{18}O]thiophosphate was dissolved as its triethylammonium salt and the solution buffered by addition of sodium bicarbonate. ^{31}P NMR chemical shift arguments support the proposal that the dianion is present in solution and the leaving group exists as the phenolate anion as judged by the UV/visible spectrum. Preliminary results with the corresponding tetrabutylammonium salt indicate a similar degree of racemization thus tending to exclude nucleophilic participation of the counterion as a possible source of the observed racemization.
(16) The racemization can also be demonstrated on the downfield resonances corresponding to the thiophosphoryl phosphorus center (see preceeding article) since these are all split into three due to the presence of ^{18}O in the bridging and nonbridging positions of *both* diastereoisomers.

Epimerisations and Non-stereospecific Reactions of 1,3,2-Oxazaphospholidin-2-ones and -2-thiones

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cis-2-Chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (**1a**) reacts with *O*-ethyl thiophosphate with *ca.* 10% inversion and 90% retention of configuration and with fluoride ion with complete loss of stereochemistry, and (**1a**) and the corresponding -2-thione (**2a**) are epimerised to the more stable *trans*-isomers by pyridine and other nucleophilic catalysts; these reactions formally require an unexpected in-line exocyclic displacement at a phosphorus centre held in a five-membered ring.

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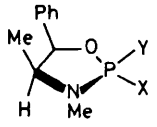
The stereochemistry of displacement reactions of 2-substituted 1,3,2-oxazaphospholidin-2-ones (X = O) and -2-thiones (X = S) derived from (−)-ephedrine have been extensively studied by Inch and co-workers.[1,2] It is generally accepted that such systems are configurationally stable and that exocyclic displacement reactions at phosphorus held in a five-membered ring proceed with retention of configuration because of the strong preference for the ring to be placed axial–equatorially in the pentaco-ordinate intermediate.[3] We report here that *cis*-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (**1a**) reacts non-stereospecifically with *O*-ethyl thiophosphate (90% retention and 10% inversion). This reaction has recently been used in the synthesis of [^{16}O,^{17}O,^{18}O]thiopyrophosphates[4] and the configurational analysis of isotopically chiral *O*-alkylthiophosphate.[5] Furthermore, compound (**1a**) and the corresponding -2-thione (**2a**) are epimerised by nucleophilic catalysts, and in particular pyridine, in a reaction that formally requires an exocyclic displacement that proceeds with *inversion* of configuration. This has important implications for the synthesis of isotopically chiral phosphate[6] and thiophosphate[5] monoesters and for the use of the phosphorochloridates (**1**) and (**2**) as chiral derivatising agents in the determination of the enantiomeric excess of chiral alcohols and amines,[7] and [^{16}O,^{18}O]thiophosphate monoesters.

Compound (**1a**) can be prepared as the major diastereoisomer (*cis* : *trans ca.* 10 : 1) by the reaction of (−)-ephedrine with phosphoryl chloride in the presence of triethylamine and can be isolated in pure form either by silica gel chromatography or by recrystallisation.[1] Reaction of the pure (**1a**) with *O*-ethyl thiophosphate bis(tributylammonium) salt in dioxane gave a mixture of two major diastereoisomers (**3a** and **b**) that arise because of the generation of a new chiral centre at the thiophosphoryl phosphorus, together with two minor diastereoisomers arising from partial loss of stereochemical control at the oxazaphospholidine phosphoryl centre. Careful analysis of the purity of (**1a**) has established that this does *not* arise from a contaminating amount of (**1b**) and the epimerisation occurs during the coupling reaction. In view of the absolute stereochemical control usually observed in the exocyclic displacement reactions of such systems we have looked for the origins of this loss of stereochemical control. The phosphorochloridate (**1a**) was observed to be completely configurationally stable in solution in the presence of tributylamine. Furthermore, the coupling reaction was monitored by ^{31}P n.m.r. spectroscopy and at 50% reaction there was no evidence for the epimerisation of (**1a**). There are two alternative explanations for this result: either the initial nucleophilic displacement reaction occurs non-stereospecifically, or the products (**3a** and **b**) epimerise during the course of the reaction. Samples taken from a coupling reaction after 24 and 48 h showed closely similar relative amounts of the minor diastereoisomers (**3c** and **d**), suggesting that the loss of stereochemical integrity occurs in the displacement reaction.

When the above reaction was carried out in pyridine the products (**3a** and **b**) and (**3c** and **d**) were produced in comparable amounts. Under these conditions we have established that the loss of stereochemical control arises from competing epimerisation of the starting material (**1a**). In contrast with a previous report,[2] we observe that in pyridine the pure *cis*-material (the kinetic product) epimerises to the *trans*-compound (the thermodynamic product). The equilibration of (**1a**) can be monitored directly by ^{31}P n.m.r. spectroscopy ($t_{\frac{1}{2}}$ *ca.* 12 h at room temperature) [Figure 1; δ(^{31}P), pyridine, 24.0 p.p.m. *trans*; 19.54 p.p.m. *cis*]. The



(**1a**) X = O; Y = Cl
(**1b**) X = Cl; Y = O
(**2a**) X = S; Y = Cl
(**2b**) X = Cl; Y = S
(**3a** and **b**) X = O; Y = OP(O)(S)OEt
(**3c** and **d**) X = OP(O)(S)OEt; Y = O
(**4a**) X = O; Y = F
(**4b**) X = F; Y = O

(**9**) X or Y = O or 

(**10**) X or Y = S or OMe

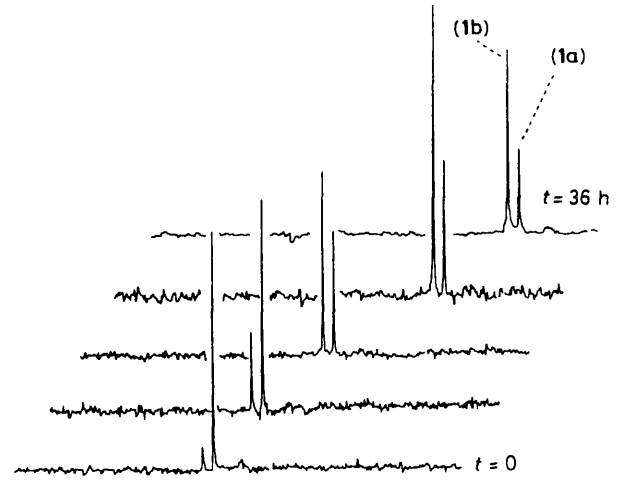
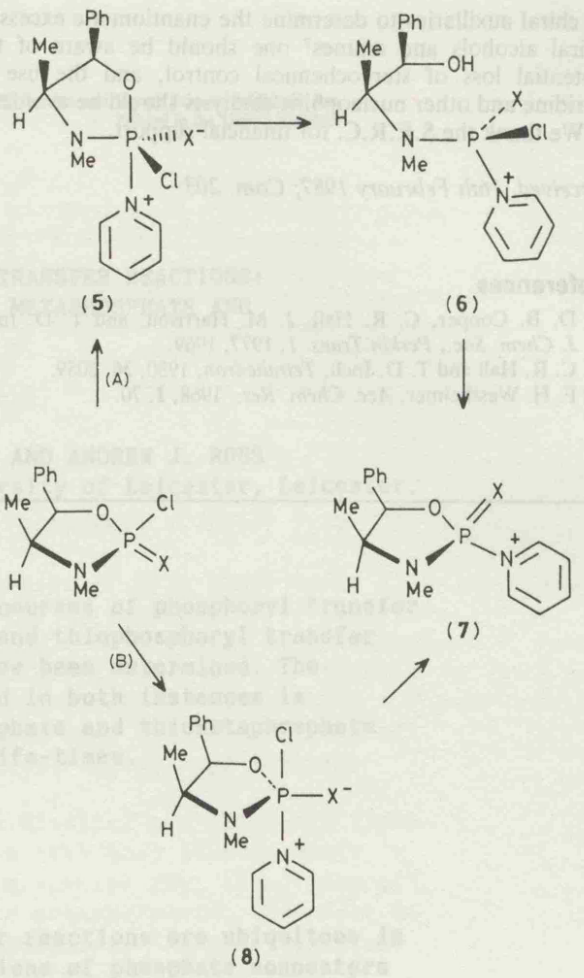


Figure 1. The ^{31}P n.m.r. spectra of the epimerisation reaction of the *cis*-compound (**1a**) in anhydrous pyridine.

thermodynamic mixture favours the *trans*-material by *ca.* 2:1 and is obtained starting from either the pure *cis*-(**1a**) or pure *trans*-(**1b**). When (−)-ephedrine and phosphoryl chloride reacted in pyridine the *trans*-product (**1b**) was obtained as the major isomer directly. The isolated material is identical with the minor isomer reported by Inch [m.p. 110—111 °C; δ(^{1}H) $CDCl_3$: 0.80 (3H, d, *J* 7 Hz), 2.65 (3H, d, *J* 13 Hz), 3.70 (1H, ddq, *J* 7 and 7 Hz†), 5.54 (1H, dd, *J* 7 and 7 Hz), and 7.3 (5H, ArH)]. Similarly the *cis*-1,3,2-oxazaphospholidine-2-thione (**2a**) is obtained as the major isomer on reaction of (−)-ephedrine with thiophosphoryl chloride in benzene in the presence of triethylamine. Treatment of the isolated pure *cis*-compound (**2a**) with anhydrous pyridine led to the thermodynamic mixture of epimers at phosphorus with the *trans*-compound favoured by *ca.* 3:1. This reaction was considerably slower ($t_{\frac{1}{2}}$ *ca.* 16 h at 50 °C) than the corresponding reaction of (**1a**), as would be expected from the known difference in reactivity of trisubstituted thiophosphates as compared to phosphates in associative nucleophilic substitution reactions.[8] With exocyclic substituents other than halogen no such epimerisations were observed. However, in seeking other examples of exocyclic displacement reactions not involving amine nucleophiles that proceed non-stereospecifically we have observed that when either (**1a**) or (**1b**) is treated with one equivalent of tetrabutylammonium fluoride in tetrahydrofuran (THF) identical mixtures of the epimeric cyclic fluorides (**4a**) and (**4b**) were produced. The reaction is instantaneous and it was not possible to determine whether the epimers arise in the first displacement step or as a result of epimerising the initial product.

The mechanism of these epimerisation reactions is of considerable interest. Presumably in the case of pyridine the amine must be acting as a nucleophile since no other nucleophile is present. We cannot rigorously exclude the possibility that a trace of base hydrochloride is formed and that this is responsible for the epimerisation. However, the chloro compound (**1a**) does *not* epimerise in anhydrous acetone in the presence of LiCl. Furthermore, when lutidine was used in place of pyridine the rate of the epimerisation was considerably reduced. The lowest energy pathway for a direct exocyclic displacement at phosphorus held within a five-membered ring will be the adjacent mechanism involving a pseudorotation step; however this leads to retention of configuration at phosphorus.[2,3] The mechanism for the epimerisation must therefore be multistep and two principal pathways for this type of reaction can be considered (Scheme 1). Firstly, a mechanism involving initial ring opening, pathway (A), could account for our observations, as has been suggested for the epimerisation of the cyclic phosphorochloridate[9] and thiophosphorochloridate[10] derived from *meso*-hydrobenzoin. Alternatively, a direct in-line displacement, *via* the pentaco-ordinate intermediate (**8**), would invert the configuration at phosphorus, pathway (B), and would eventually give the epimeric chloro compound after displacement of the pyridinium ligand by chloride *via* the normal adjacent displacement (with retention).

Since no intermediates that were detectable by ^{31}P n.m.r. spectroscopy accumulated during the reaction it is difficult to distinguish between these two pathways. A pathway involving diequatorial placement of the five-membered ring would be unusual because of the presumed increased strain in the phosphorane. However, the energetic cost of moving a five-membered ring from axial–equatorial to the diequatorial position in a phosphorane plus the energy associated with



Scheme 1

moving the lone pairs on the ring heteroatoms from their preferred orientation in the equatorial plane has been estimated to be only *ca.* 20 kcal mol^{-1} (cal = 4.184 J) and this in part can be offset by the relative apicophilicities of the other substituents, *i.e.* the chlorine.[11] The possibility of direct in-line displacement, pathway (B), should not therefore be discounted.

With other nucleophilic catalysts such as *N*-methylimidazole and *N*,*N*-dimethylaminopyridine epimerisation was also observed with both (**1a**) and (**2a**); however, the appearance of other resonances in the ^{31}P n.m.r. spectra make firm mechanistic conclusions difficult. The ^{31}P chemical shifts of these additional resonances suggest species in which the 1,3,2,-oxazaphospholidine ring is still intact. The most reasonable structures would be (**9**) in which the nitrogen nucleophile is still attached; support for this was obtained by demonstrating that addition of methanol led to formation of the cyclic triester (**10**) as a mixture of epimers in the case of (**2a**) with *N*-methylimidazole.[12]

The observation of exocyclic displacement reactions at phosphoryl and thiophosphoryl centres held in a five-membered ring proceeding with inversion of configuration, albeit at comparatively slow rates (with the exception of fluoride ion), is unexpected. Importantly, when using such systems to generate isotopic chirality at phosphorus,[4—6] particularly when the reaction involves a very weak nucleophile, the stereochemical integrity of the intermediates should not be assumed. Furthermore, in advocating the use of such systems

† This coupling constant appears to be incorrectly assigned in the original publication.[1]

as chiral auxillaries to determine the enantiomeric excess of chiral alcohols and amines[7] one should be aware of the potential loss of stereochemical control, and the use of pyridine and other nucleophilic catalysts should be avoided.

We thank the S.E.R.C. for financial support.

Received, 16th February 1987; Com. 203

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Phosphorus and Sulfur, 1987, Vol. 30, pp. 559–562
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PHOSPHORYL AND THIOPHOSPHORYL TRANSFER REACTIONS: STEREOCHEMICAL IMPERATIVES FOR METAPHOSPHATE AND THIOMETAPHOSPHATE

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Abstract The stereochemical courses of phosphoryl transfer reactions in aprotic solvents and thiophosphoryl transfer reactions in protic solvent have been determined. The extensive racemisation observed in both instances is discussed in terms of metaphosphate and thiometaphosphate intermediates of significant life-times.

INTRODUCTION

Enzyme catalysed phosphoryl transfer reactions are ubiquitous in metabolism. Studies on model reactions of phosphate monoesters have suggested a dissociative mechanism involving monomeric metaphosphate.[1,2] However, stereochemical investigations of a large number of enzyme catalysed phosphoryl transfer reactions[3] and of the solvolysis of aryl phosphates in aqueous solutions[4] have shown these to proceed with clean inversion of configuration, which would apparently rule out a "free" metaphosphate. We report here some of the first stereochemical studies of phosphoryl transfer reactions in aprotic solvents that proceed with substantial racemisation of configuration.[5,6] We also report methods for the synthesis and configurational analysis of chiral [^{16}O,^{18}O]thiophosphate monoesters. Using these methods we have determined the stereochemical course of the first simple thiophosphoryl transfer reaction.

RESULTS AND DISCUSSION

It has been shown that adenosine 5'-diphosphate trianion will phosphorylate even hindered alcohols such as ^{t}BuOH at appreciable rates particularly in acetonitrile solvent. We have studied the stereochemistry of this reaction using [β-^{16}O,^{17}O,^{18}O]ADP, Figure 1.[6] The synthesis[7] and configurational analysis[8] were achieved

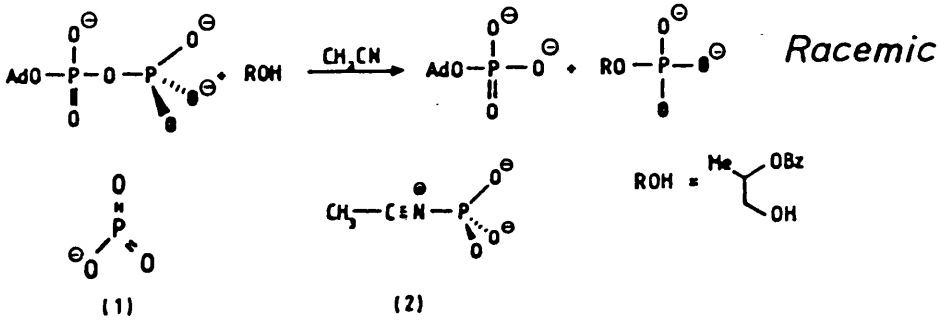

FIGURE 1 Stereochemistry of Phosphoryl Transfer from ADP.

by the published methods and the results of this study have indicated that the phosphoryl transfer is accompanied by almost complete racemisation of configuration. This result would accord with a "free" metaphosphate intermediate (1). However, there is an alternative explanation namely that a solvent coordinated zwitterion such as (2) is involved and that the racemisation of configuration arises because of multiple phosphoryl transfer reactions.

P^1,P^1-Disubstituted pyrophosphate dianions are unique in their reactivity amongst pyrophosphates in that they spontaneously decompose to phosphate diester and a species that shows many of the properties expected of monomeric metaphosphate. In order to study the stereochemistry of such phosphoryl transfer reactions P^1-O-ethyl-P^1-thio-P^2[^{16}O,^{17}O,^{18}O] pyrophosphate has been synthesised by the literature procedures.[5,7] Upon methylation with a variety of methylating agents, the P^1,P^1-disubstituted pyrophosphate is generated in situ, and as the dianion, spontaneously decomposes. In the presence of an alcohol, the corresponding phosphate ester and O-ethyl-S-methyl thiophosphate are obtained in good yield. Using 2-O-benzyl-(*S*)-propane-1,2-diol the phosphate ester was obtained, Figure 2, and the isotopic configurational analysis was achieved by the literature procedure[8] using high field ^{31}P NMR spectroscopy.

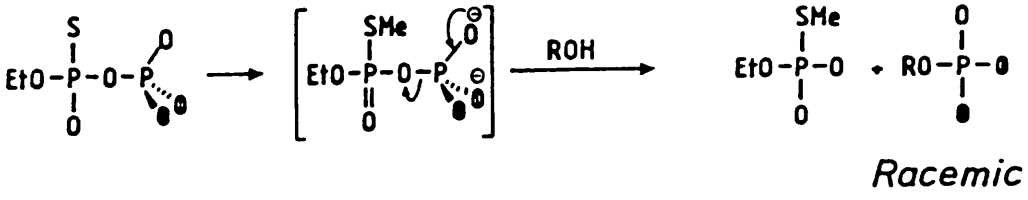

FIGURE 2 Phosphoryl Transfer from a P^1,P^1-Pyrophosphate Diester.

When the phosphoryl transfer reaction was conducted in dichloro-

methane the isolated product showed considerable racemisation of configuration at phosphorus with a small amount of excess inversion of configuration.[5] We have recently shown that the same reaction carried out in acetonitrile occurs with ***complete racemisation***.

Breslow[9] has reported that thiophosphates react more readily via a dissociative mechanism than the corresponding phosphates. We have sought to investigate the stereochemistry of a simple thiophosphoryl transfer reaction to compare with the results of the phosphoryl transfer reactions. We have synthesised O-ethyl S_p[^{16}O,^{18}O]thiophosphate (3) and O-p-nitrophenyl R_p[^{16}O,^{18}O]-thiophosphate (4) by variants of the general synthesis. Analysis of the absolute configuration of (3) and (4) has been achieved as shown in Figure 3. The position of the ^{18}O in (6a) and (6b) is located by the upfield shift on the ^{31}P NMR resonance. The ^{31}P NMR spectrum together with the assignments for the analysis of O-ethyl S_p[^{16}O,^{18}O]thiophosphate are shown in Figure 3.

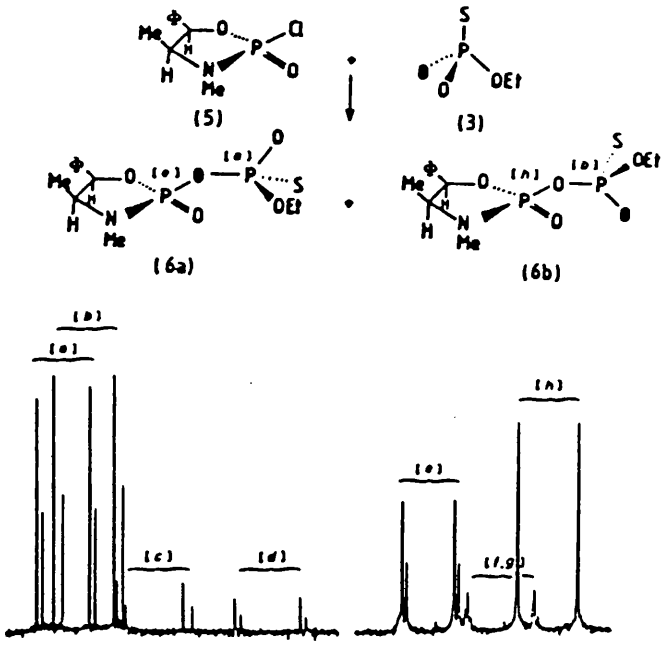

FIGURE 3 Configurational Analysis of Ethyl [^{16}O,^{18}O]thiophosphate.

The stereochemical course of the solvolysis of R_p[^{16}O,^{18}O]p-nitrophenyl thiophosphate dianion in ethanol has been determined,

Figure 4. The resulting ethyl thiophosphate, *via* the above analysis (Figure 3), can be shown to be largely racemic. The appropriate controls confirmed that this racemisation arises during the thiophosphoryl transfer step.

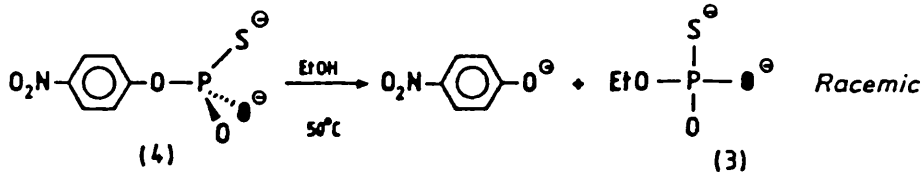

FIGURE 4 Solvolysis of p-Nitrophenyl [^{16}O,^{18}O]thiophosphate.

CONCLUSION

Stereochemical evidence would support a metaphosphate intermediate in phosphoryl transfer reactions conducted in aprotic solvents only. In contrast thiophosphoryl transfer reactions appear to proceed with a considerable loss of stereochemical integrity even in protic solvents which would suggest the involvement of a thiometaphosphate intermediate and that such an intermediate is a longer lived intermediate that the parent metaphosphate.

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Stereochemical Studies of Reactions of Phosphate and Thiophosphate Esters

Anna Iagrossi

ABSTRACT

Nucleophilic substitution reactions involving phosphate monoesters have been investigated. Two general syntheses of O-alkyl or O-aryl [^{16}O,^{18}O] thiophosphate monoesters are reported. An independent and general method for the determination of the enantiomeric excess of isotopically chiral thiophosphate monoesters has been developed and the absolute configurations of the diastereoisomers of (2R)-O-(O-ethyl thiophosphoryl)-3,4S-dimethyl-5S-phenyl-1,3,2-oxazaphospholidin-2-one have been assigned.

The solvolysis of p-nitrophenyl [R-^{16}O,^{18}O] thiophosphate in ethanol gives rise to ethyl [^{16}O,^{18}O] thiophosphate with a large degree of racemisation of configuration (~80%). This observation is consistent with the formation of a thiometaphosphate intermediate of finite lifetime which is then trapped by ethanol with accompanying loss of stereochemical integrity. This study provides the first direct evidence for a monomeric thiometaphosphate in protic solvent.

During the course of developing the stereochemical analysis, it was noted that O-ethyl thiophosphate reacts with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one with ca. 10% inversion and 90% retention of configuration. This system also reacts with fluoride ion with complete loss of stereochemistry. Cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one and the corresponding 2-thione are epimerised to the more stable trans isomers by pyridine and other nucleophilic catalysts. These reactions require an in-line exocyclic displacement at a phosphorus centre held in a five-membered ring.

Nucleophilic substitution at di- and tri-esters have also been studied. The stereochemical course of the hydrolysis of the 1,3,2-dioxaphosphorinan-2-one system involving good leaving groups such as chloride and fluoride occur with inversion of configuration via an in-line mechanism, whereas hydrolysis of this system involving poor leaving groups occurs with retention of configuration via a pseudorotation mechanism.

CONTENTS

EXPERIMENTAL

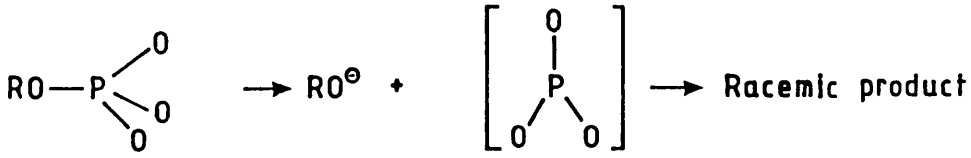
CHAPTER 1

General Introduction

Reactions at Phosphorus

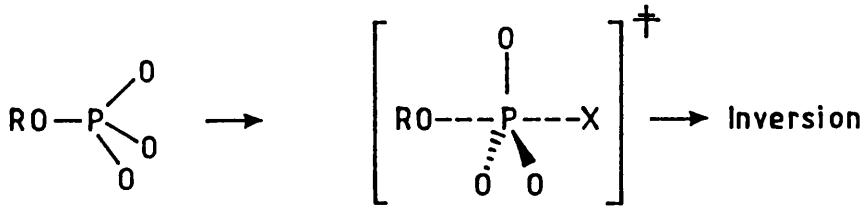
Nucleophilic displacement reactions at phosphorus are widespread in Chemistry and Biochemistry. In considering reactions of this type, four fundamental[1,2,3] mechanisms need to be examined. These four mechanisms along with their cryptic stereochemistries are illustrated in Figure 1.1.

(1a) Dissociative reaction via monomeric metaphosphate



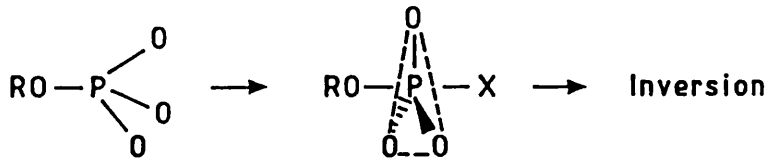
SN1(P)

(2a) Associative reaction via a pentacoordinate transition state



SN2(P)

(2b) In-line addition-elimination mechanism via a pentacoordinate intermediate



(2c) Adjacent addition-elimination mechanism involving a pseudo-rotation

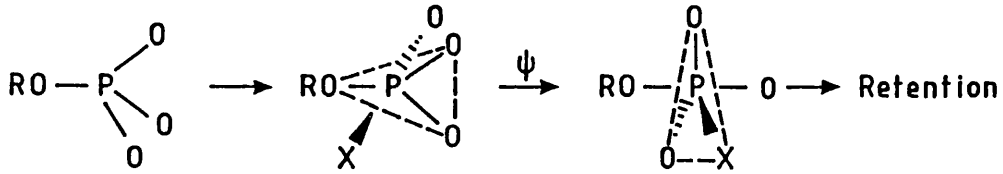


FIGURE 1.1 Nucleophilic reactions at phosphorus.

The first of these (1a) is a dissociative process. The SN1(P) mechanism in which the leaving group RO^- is expelled from the monoester

in the rate limiting step, produces the planar electrophilic monomeric metaphosphate as a reactive intermediate. Being planar monomeric metaphosphate can be captured by the nucleophile at either face in a second step to produce a racemic product.

There are three associative mechanisms. The first (2a) is a concerted SN2(P) mechanism in which the nucleophile attacks from the side opposite the leaving group and displaces it in a single step via a pentacoordinate transition state. The stereochemical outcome of this mechanism is inversion of configuration.

Both of the other associative mechanisms (2b + 2c) involve true intermediates; they differ in the stereochemistry of the intermediates.

Mechanism (2b) involves an attack of the nucleophile opposite the leaving group to form a pentacovalent intermediate. This pentacovalent intermediate has the nucleophile and leaving group in the apical positions and the other three substituents in the equatorial plane. The leaving group can depart directly from its apical position to form the product with inversion of configuration.

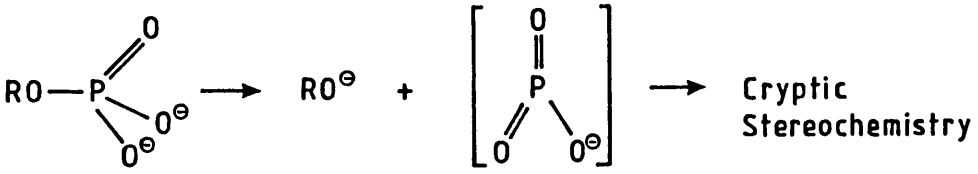
In mechanism (2c) the attacking nucleophile approaches in an apical position with the leaving group in an adjacent equatorial position to form the initial intermediate shown. The expulsion of the leaving group must be preceded by a pseudorotatory rearrangement to form a second isomeric intermediate. This intermediate now has the two apical groups of the first intermediate in equatorial positions. The leaving group is now apical and so it satisfies the laws of microscopic reversibility and can depart. The stereochemical outcome of this mechanism is retention of configuration at phosphorus.

Metaphosphate

Much evidence has accumulated in favour of the dissociative mechanism for reactions of phosphate monoesters. The key enzyme catalysed phosphoryl transfers, represent reactions of this type and it is pertinent to enquire whether these proceed via a monomeric metaphosphate intermediate.

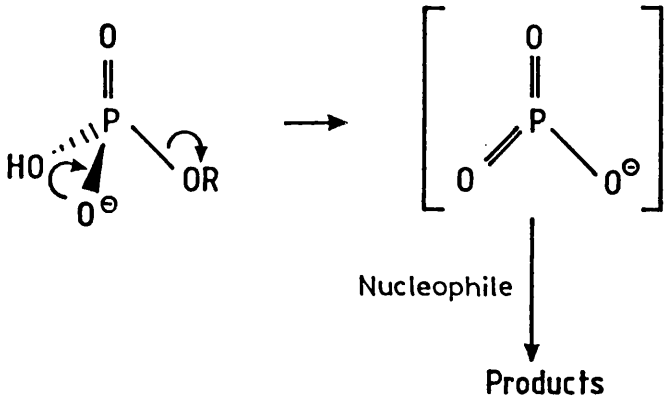
A brief review of monomeric metaphosphate will be discussed here (a more in depth discussion will be presented later in Chapter 4).

In 1955 monomeric metaphosphate was first postulated as an intermediate of the hydrolysis of monoesters of phosphoric acid in an aqueous medium as shown below.



Monomeric metaphosphate, a planar electrophilic ion, was proposed as an intermediate which could be trapped by a nucleophile to give products.

Reports from the laboratories of Westheimer[4] and Bunton[5] showed that many alkyl and aryl monoesters of phosphoric acid exhibit a characteristic bell-shaped pH rate profile. The rate of hydrolysis of phosphoric monoester is maximal near pH 4 as illustrated in Figure 1.2. This is where the monoanionic form of the ester is the predominant species as shown and the mechanisms in Figure 1.2 was proposed to account for this.



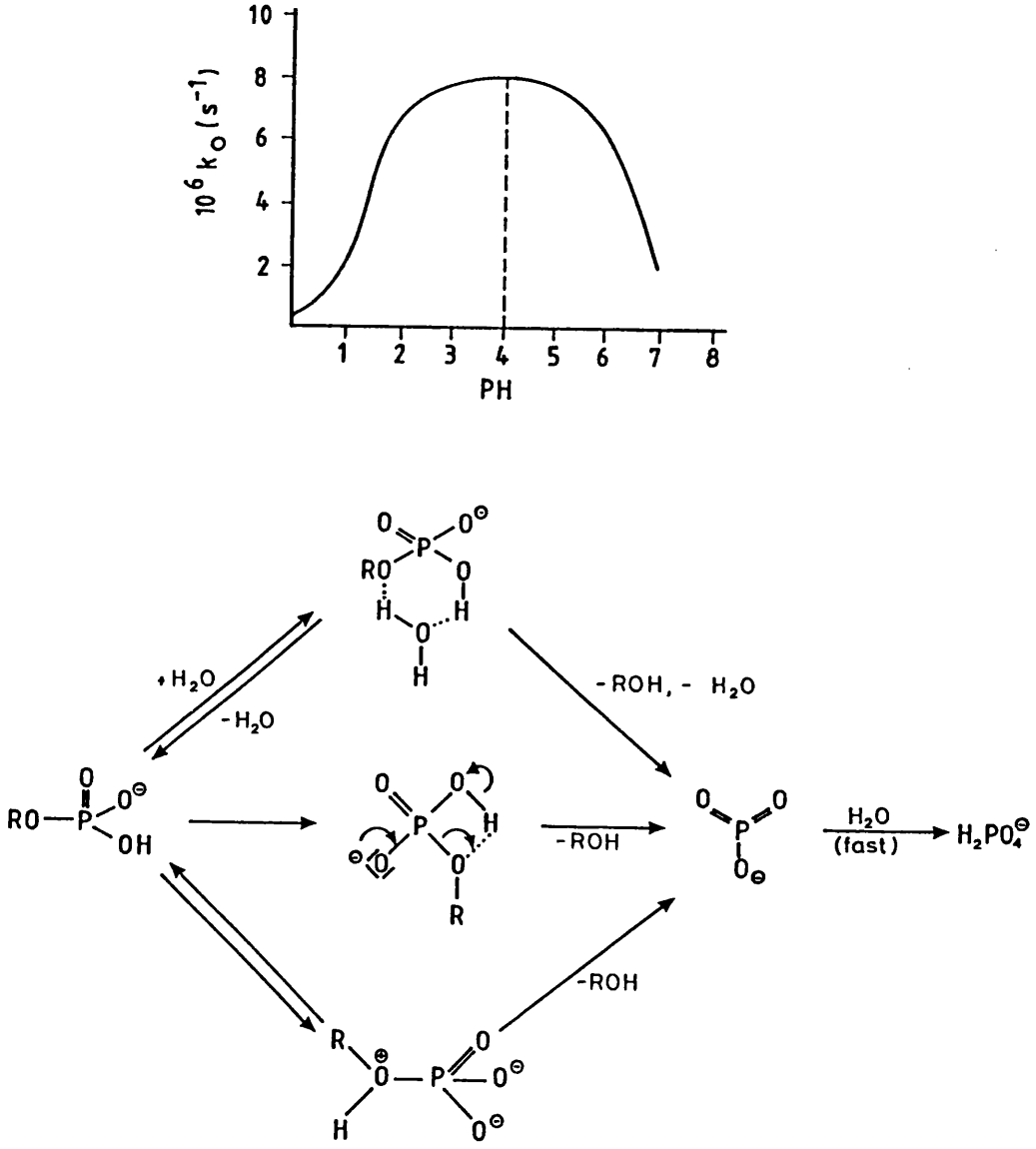
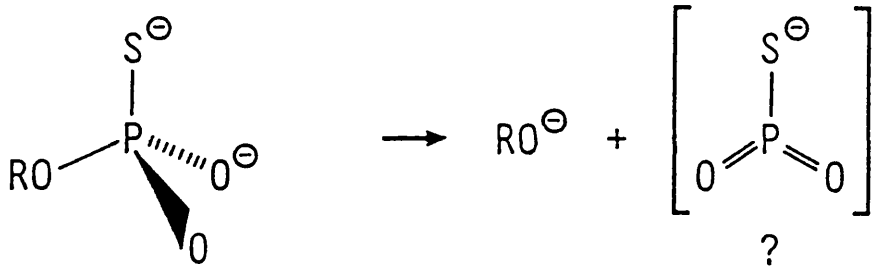


FIGURE 1.2 Hydrolysis of simple phosphate monoesters.

Since then much evidence in favour of monomeric metaphosphate has been cited. However, considerable controversy remains concerning the lifetime of the intermediate.

Reported evidence by Breslow[49] and Roesky[50] suggest that thiometaphosphate could be a more long lived species than metaphosphate [see Figure 1.3].

Breslow has presented evidence that thiophosphate monoesters react more



Evidence for existence of a long-lived thiometaphosphate:

(cf. reverse reactivity of corresponding di- and tri- esters)

Note: $PS_3^{\ominus}$ has been isolated!

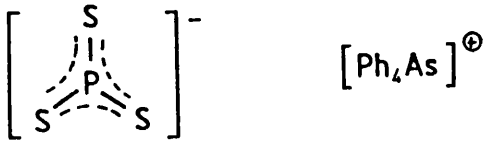
FIGURE 1.3 Evidence for the existence of thiometaphosphate by Breslow, Katz and Roesky.

rapidly via a dissociative reaction than the corresponding phosphate ester.

This is in contrast to the reduced reactivity of thiophosphate di- and tri esters with respect to the corresponding phosphates.

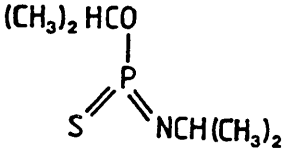
It is well known that the reaction of triesters follows an addition elimination mechanism in which the P=O (P=S) bond order decreases in the transition state and the charge on oxygen (sulphur) increases. This suggests that the monoester reacts via an elimination addition sequence in which the P=O (P=S) bond order increases in the transition state and the charge on oxygen (sulphur) diminishes. The effects themselves probably reflect mainly the lesser electronegativity of sulphur compared with oxygen.

Roesky has recently isolated a PS_3^- ion as the salt below.



This would imply that replacement of oxygen by sulphur produces a species of greater kinetic stability implying that thiometaphosphate may be a longer-lived species than metaphosphate.

Many other 3-coordinate P(V) structures are known, some have even been isolated and characterised. Extra stability can be conferred by the attachment of bulky groups on the ligands attached to phosphorus, e.g. alkoxyiminothiophosphoranes. Obviously such species are only indirectly of relevance to monomeric metaphosphate itself.

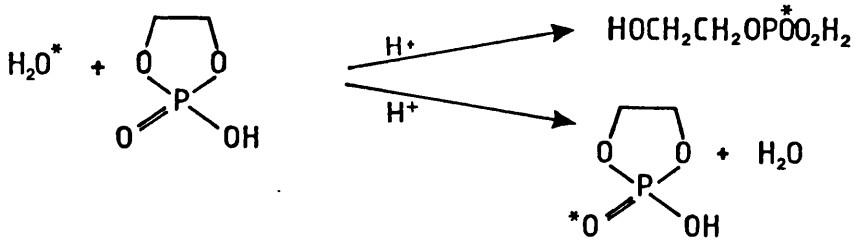


Pentacoordinate Intermediates

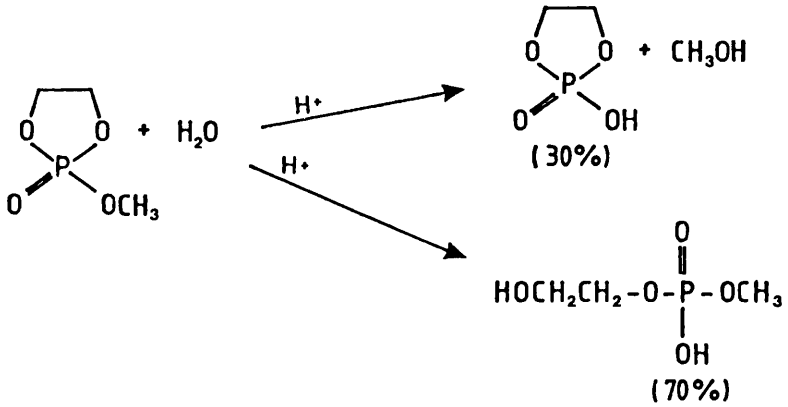
Pentacoordinate intermediates have also been proposed for nucleophilic reactions at phosphorus particularly di- and tri-substituted. Westheimer has contributed enormously to our understanding of such intermediates.

Westheimer and his co-workers have found that the hydrolysis in acid or base of five-membered cyclic esters of phosphoric acid proceed ca. 10^6 times faster than those of their acyclic analogues.[6-9] By contrast, the rates of hydrolysis of six- and seven-membered cyclic phosphates are comparable with those of acyclic phosphates.

A key finding[10] concerning these processes is that the acid hydrolysis of hydrogen ethylene phosphate is accompanied by rapid oxygen exchange into unreacted hydrogen ethylene phosphate as illustrated.



The label is distributed among the oxygen atoms of the phosphoryl and hydroxyl groups [oxygen exchange proceeds ~20% as fast as hydrolysis]. Hydrolysis in the above reaction occurs ~10^8 times faster than that for hydrogen dimethyl phosphate, thus the oxygen exchange is ~2×10^7 times faster than the hydrolysis of dimethyl phosphate. A similar result[13] was obtained for the hydrolysis of methyl ethylene phosphate. The reaction in acid is one million times faster[11,12] than that for trimethyl phosphate resulting in cleavage of the methoxy group in addition to ring cleavage. This hydrolysis is illustrated below.



Although ring strain[13-16] can account for the rapid opening of the ring in cyclic phosphates (the heat of hydrolysis of methyl ethylene phosphate exceeds that of trimethyl phosphate by ~5 kcal/mol)[13,14] it cannot account for the increased rate of displacement of the exocyclic substituent.

Westheimer proposed that the enormously accelerated hydrolysis external to the ring proceeds with "pseudorotation" between bipyramidal intermediates.[12,17,18]

Stephen Berry[19] introduced the concept of pseudorotation and the Berry pseudorotation. Although both Berry and Turnstile pseudorotations are stereochemically equivalent, the Berry pseudorotation is the most generally accepted and this is illustrated in Figure 1.4 and described below.

The mechanism of pseudorotation can be seen to be the attack of a nucleophile at a position adjacent to the leaving group to form a pentacovalent intermediate with trigonal bipyramidal geometry. This intermediate has two apical groups (one of which is the nucleophile) and three equatorial groups (one of which is the leaving group). Axial departure of the leaving group is expected on the grounds of microscopic reversibility. In order for this to occur, a pseudorotation step was proposed which involved the exchange of two equatorial ligands (which includes the leaving group) with two axial ligands by a non-dissociative process. The apical atoms become equivalent to two of the equatorial atoms by deforming the trigonal bipyramid to the square pyramid structure. Further deformation produces a new trigonal bipyramid in which the new apical groups were previously equatorial. After the pseudorotation the axis of the trigonal bipyramid is 90° to the original axis. The pivot ligand remains equatorial throughout the operations. Westheimer rationalised

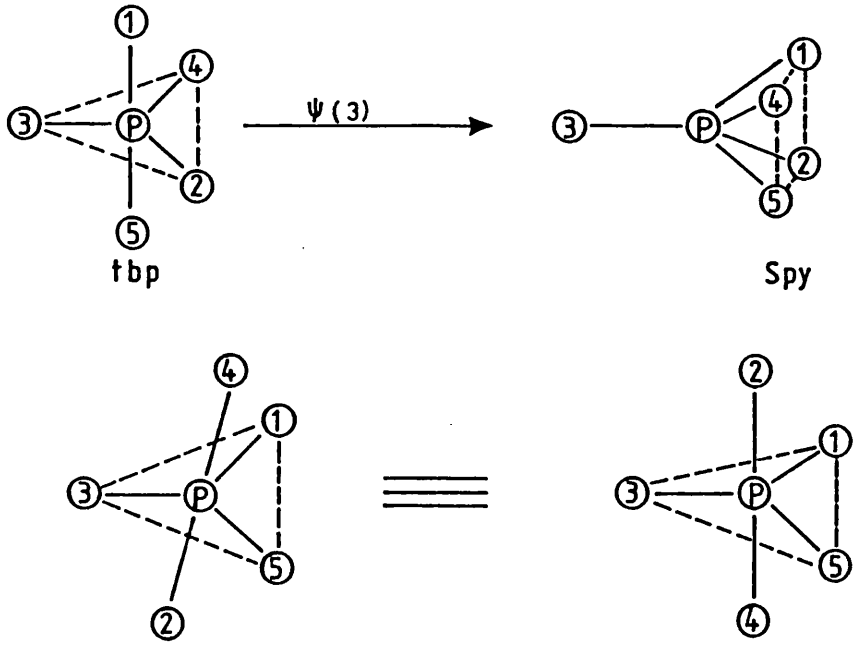


FIGURE 1.4 The Berry pseudorotation mechanism.

all of his observations including the rate acceleration of the hydrolysis of esters by making four assumptions about the intermediate.[12,17]

[1] It is energetically unfavourable for carbon atoms to occupy apical sites at the expense of oxygen or other electronegative atoms. Electronegative atoms preferentially occupy the axial position.

[2] Five-membered rings prefer to occupy apical-equatorial positions rather than diequatorial.

[3] Pseudorotation is facile provided conditions [1] and [2] are maintained.

[4] Attacking groups enter an apical position and leaving groups ultimately depart from an apical position.

The pseudorotation process that occurs in the hydrolysis of methyl ethylene phosphate incorporates the above points and is illustrated in Figure 1.5.

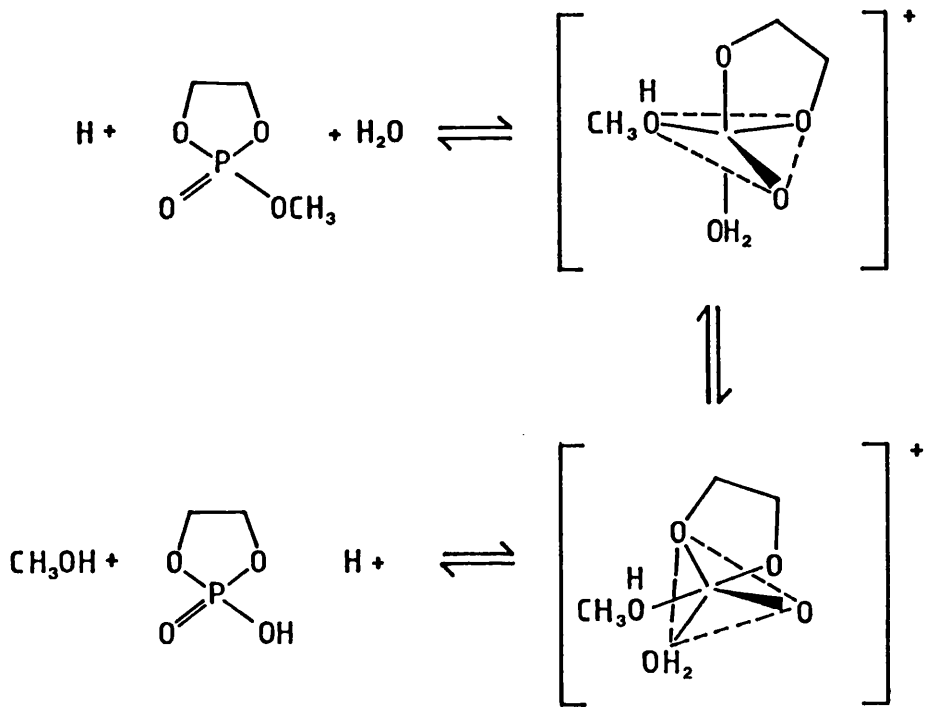


FIGURE 1.5 Pseudorotation of methyl ethylene phosphate.

Westheimer considered various other mechanistic possibilities not involving pseudorotation, subject to the following constraints.

(i) A single mechanism should account for ring opening, exocyclic cleavage and exchange.

(ii) Methanol and ethanol are sufficiently similar to water to imply that an alcohol molecule can occupy an analogous geometrical position to a water molecule in an intermediate.

It has been suggested that water molecules might both enter and leave during ^{18}O exchange into hydrogen ethylene phosphate from equatorial positions. Although this possibility satisfies the laws of microscopic reversibility, it is however inconsistent with the chemistry (e.g. if both entering and leaving groups were equatorial then the ring would not open upon hydrolysis). This is contrary to experimental evidence.

An alternative mechanism would allow a water molecule to enter from an apical position and an alcohol molecule to depart by an equatorial

position and vice versa. This is again inconsistent with ring cleavage hydrolysis. Therefore, experimental data provides evidence that pseudo-rotation may accompany the hydrolysis of phosphate esters.

Factors affecting the reaction pathway

Nucleophilic substitution at phosphorus mono-, di- and tri-esters can proceed through several mechanisms, as outlined previously. Which pathway is followed can be affected by many factors. The major factors[20-22] affecting reaction pathway are listed below (a more in-depth discussion is given in Chapter 6). These factors are as follows:

[1] The nature of the nucleophile - can alter the stereochemical pathway.

[2] The nature of the leaving group (its apicophilicity) - more apicophilic[23] groups such as F prefer axial orientations within a compound compared to less apicophilic groups such as OMe.

[3] The presence of ring structures - can affect the reaction pathway. There is precedent in the literature that shows that 4-membered[23] rings prefer axial-equatorial orientations within a structure while seven-membered rings can occupy both axial-equatorial and equatorial-equatorial positions. The presence of small rings will tend to dominate the reaction pathway.

[4] Stereoelectronic effects - the orientation of lone pairs on heteroatoms directly attached to phosphorus can influence the stability of adjacent[24,25] bonds and thus affect the reaction pathway.

[5] Reaction conditions - can have a dramatic effect on the reaction pathway. This can be caused by pH change, solvent change, etc.

Such factors can have a considerable effect on the reaction pathway, as indicated by the stereochemical course.

Enzyme Catalysed Reactions at Phosphorus

Enzyme catalysed reactions of phosphates and thiophosphates have been extensively studied over the past decade. The thiophosphoryl transfer reactions studied during the course of this work represents models of the widespread enzyme catalysed phosphoryl transfer. Such reactions are central to the energy balance of all organisms. These reactions are involved in cellular control mechanisms at every level, e.g. ion transport, muscle action and driving thermodynamically unfavourable reactions.

The classes of enzymes that catalyse displacements at phosphorus centres are illustrated in Figure 1.6.

The enzymes that handle phosphoric monoesters fall into three categories:-

(i) Phosphokinases. - These catalyse phosphoryl transfer from ATP to an acceptor nucleophile.[29]

(ii) Phosphatases. - These enzymes catalyse the transfer of the phosphoryl group of phosphate ester to water which acts as the acceptor molecule (e.g. ATPases transfer the γ phosphoryl group of ATP to water).[27,28]

(iii) Mutases. - In this case the phosphoryl group is transferred to an acceptor molecule which is another functional group on the donor molecule, in an apparently intramolecular reaction.

The enzymes that handle phosphoric diesters are either hydrolytic (e.g. nucleases) or nucleotidyl transfer catalysts.

Investigation of the mechanism of enzyme-catalysed reactions at the phosphorus locus is at three levels:-

[1] The first concerns the definition of the number and kind of reaction intermediates.

[2] The second involves the kinetics of the reactions.

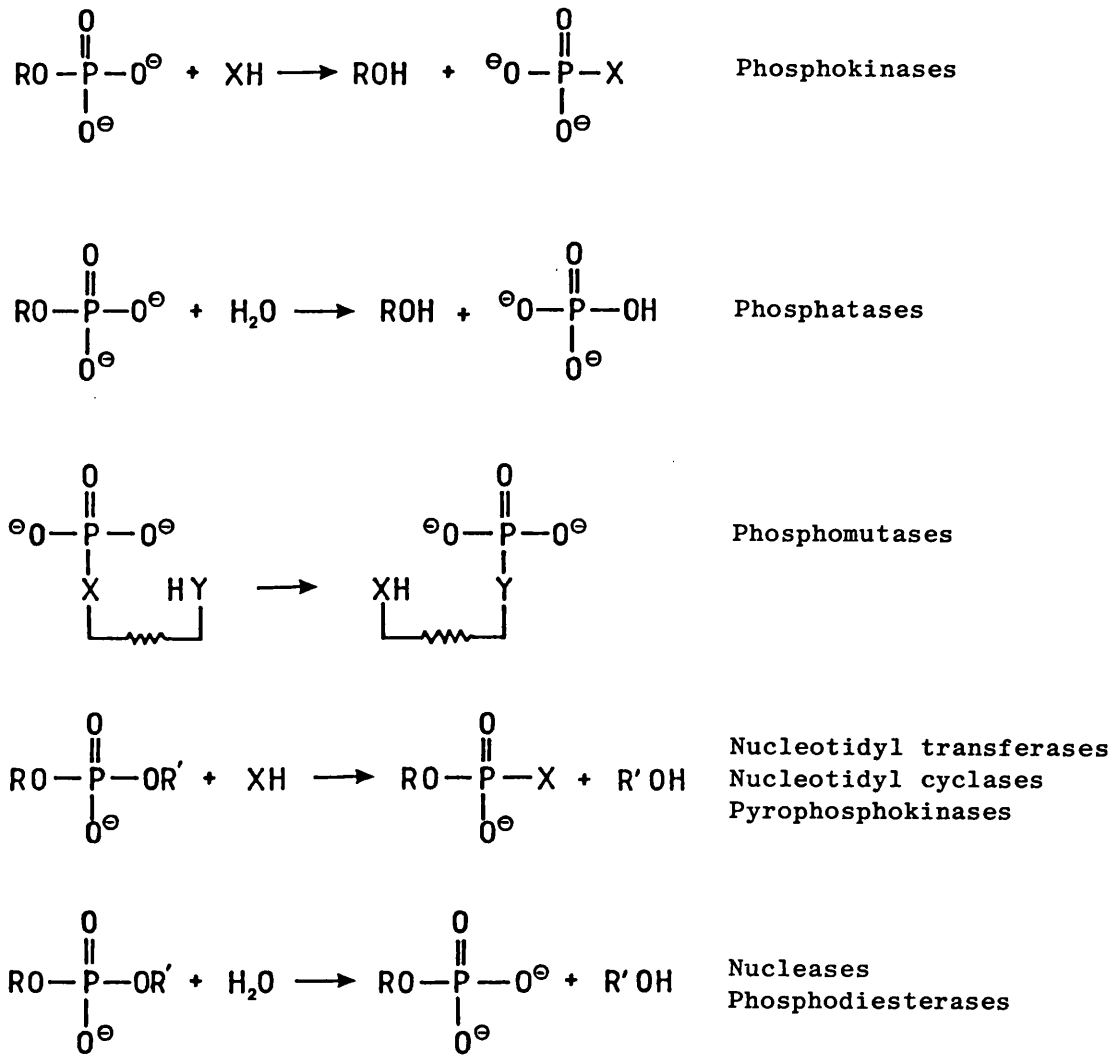


FIGURE 1.6 Enzyme catalysed reactions at phosphorus.

[3] The third relates to the stereochemistry of the reaction. Both kinetic and stereochemical information can provide powerful evidence concerning the mechanism of the reaction.

Kinetic investigations have provided considerable insight into various aspects of phosphoryl transfer but it is often difficult to make the fundamental distinction between a direct displacement and a double displacement involving a phosphoenzyme intermediate. The isolation of the covalently-bound phosphoenzyme intermediate and the demonstration that it is kinetically competent is very good evidence that the inter-

mediate is important during catalysis. Unfortunately, the isolation may not be possible and certainly will not be possible in the case of the transient intermediates that may form part of the sequential mechanism.

Therefore, stereochemical information has played an important part in the understanding of enzyme phosphoryl transfer.

Isotopic Chirality

Stereochemical analysis has long been recognised as a powerful mechanistic tool in chemistry and enzymology. However, despite the widespread occurrence of phosphate esters and anhydrides and their central importance to all living systems, the methodology for investigating the stereochemical course of the enzyme-catalysed phosphoryl transfer reactions has been developed only recently.[30,31]

Phosphate esters have been greatly studied due to their importance in many metabolic processes, however the inherent achirality of phosphate esters meant that stereochemical information could not be deduced. The illustration below shows that phosphate and thiophosphate mono and diesters are achiral due to the equivalence of the oxygens.

monoester diester

This problem was overcome via the use of three stable isotopes of oxygen, i.e. ^{16}O, ^{17}O, ^{18}O. The introduction of isotopes into monoesters and diesters is illustrated in Figure 1.7.

For phosphate monoesters all three oxygen isotopes are needed to confer chirality on the molecule while for thiophosphate monoesters only two stable isotopes are required. In the diester case, two stable oxygen

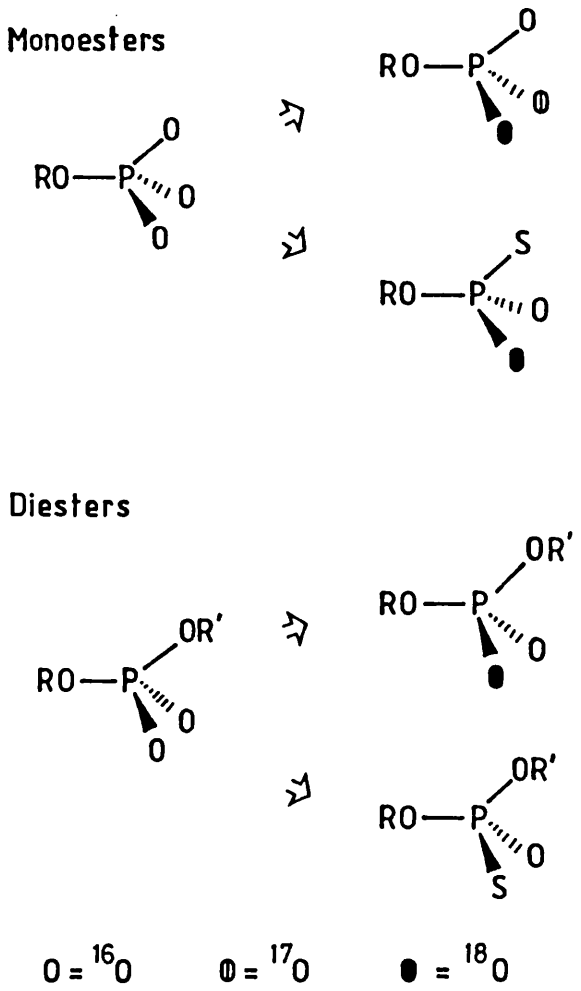


FIGURE 1.7 Isotopic chirality of phosphate and thiophosphate mono and diesters.

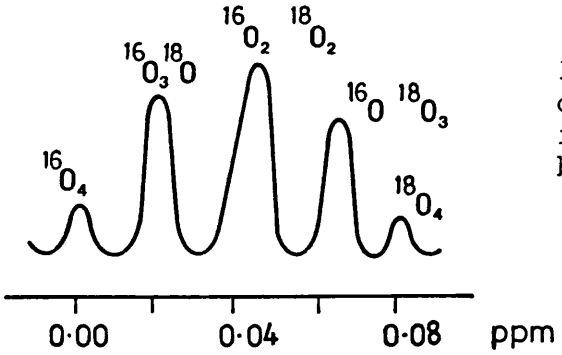
isotopes produce chirality in the phosphate diester and one isotope in the thiophosphate diester.

Oxygen is the lightest element to exist naturally as three stable isotopes ^{16}O, ^{17}O, ^{18}O. By 1970's ^{17}O and ^{18}O were available as water and dioxygen at enrichment that made it feasible to synthesise chiral [^{16}O,^{17}O,^{18}O] phosphate and thiophosphate monoester. Currently, ^{17}O is available at about 50% ^{17}O and ^{18}O in excess of 99 atom % ^{18}O as water.

The isotopic substitution does not, of course, perturb either the chemistry or the enzymology of the substrate.

The effects of oxygen isotopes directly bonded to phosphorus in ^{31}P nmr spectroscopy was independently described by the laboratories of Cohn,[32] Lutz[33] and Lowe.[34] These laboratories reported that ^{18}O directly bonded to phosphorus caused an upfield shift of the ^{31}P nmr resonance. This effect is relatively small, approximately 0.02 ppm per bond between ^{18}O and ^{31}P in inorganic phosphate. This effect is easily distinguished via high-field ^{31}P nmr.

Inorganic phosphate, randomly labelled with 50% enriched ^{18}O, produced five resonances that could be distinguished in the ^{31}P nmr. This spectrum is illustrated below. This demonstrated that the magnitude of the upfield perturbation in chemical shift was approximately additive increasing as the number of ^{18}O's attached to phosphorus increased. Other investigations by Lowe[35] and Cohn[36] also proved that the magnitude of the upfield shift depended on the bond order between ^{18}O and ^{31}P. The greater the bond order the larger the perturbation.

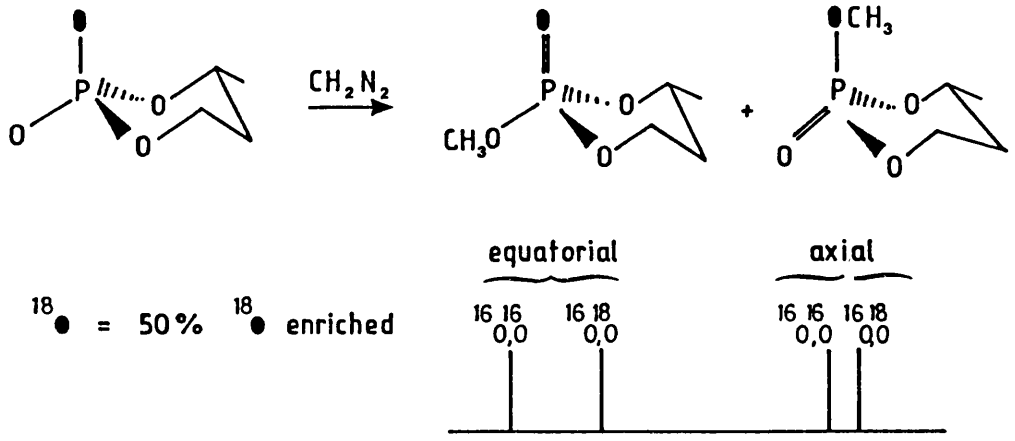


145.7 MHz ^{31}P nmr spectrum of randomly ^{18}O-labelled inorganic phosphate reported by Cohn.

FIGURE 1.8 ^{31}P nmr of inorganic phosphate.

For the series inorganic phosphate, monomethyl phosphate, dimethyl phosphate and trimethyl phosphate the values 0.020, 0.024, 0.029 and 0.035 ppm respectively[35] were obtained as ^{18}O perturbations resulting from labelling of one of the phosphoryl oxygens. Similar data was obtained by Cohn for labelled adenine nucleotides.[36]

This led to the use of ^{18}O perturbations in determining the configurations of $^{16}O,^{18}O$ chiral phosphodiesters. Cohn found that no configurational information could be obtained by measuring the ^{18}O perturbations on the ^{31}P nmr resonances of the phosphodiesters with identical upfield perturbation for each diastereomer of cyclic [$^{16}O,^{18}O$] dAMP.[26] The chemical non-equivalence and diastereotopic nature of the phosphoryl oxygen are enhanced by the ring structure, so that upon alkylation a mixture of diastereomeric esters are produced. These are shown below.



A mixture of equatorial and axial esters are formed. Alkylation allows the isotopic identity of the diastereotopic oxygens to be ascertained. The larger isotope perturbation being associated with the doubly bonded ^{18}O shift.

^{17}O nucleotides

Independent reports by Tsai[37] and Lowe[35] made observations concerning the spectral properties of phosphates in which ^{17}O was directly bonded to phosphorus. The properties of ^{17}O bonded to phosphorus are discussed below.

The ^{17}O nucleus is quadrupolar and has a spin of $5/2$. The direct spin-spin coupling of this quadrupolar oxygen nucleus to the dipolar ^{31}P nucleus is accompanied by a rapid and effective relaxation of the ^{31}P

nucleus causing the ^{31}P nmr resonance to be greatly broadened. The one bond coupling can be obscured by this quadrupolar effect,[38] however in some cases the ^{31}P resonance of the ^{17}O containing material can be resolved thus appearing as six, equally spaced lines of equal intensity,[39] as for [^{17}O] $POCl_3$. Thus the only effect that ^{17}O has on directly bonded ^{31}P nuclei is extensive line broadening.

These two nmr effects have been elaborated into a configurational analysis of [^{16}O,^{17}O,^{18}O] chiral phosphate esters, using ^{31}P nmr spectroscopy [see Chapter 3].

Enzyme Stereochemistry

Many of the enzymes representing each of the enzyme classes shown in Figure 1.6 have been studied from a stereochemical viewpoint.

A summary of experimentally determined stereochemical courses of enzymic phosphoryl and thiophosphoryl transfer is given in Table 1.1.

It is to be noted that the many enzymes investigated using both natural and thiophosphate substrates have shown the same stereochemical course from the two methods. These include methionyl tRNA synthetase,[40,41] adenylate cyclase,[42,43] glycerol kinases,[44-47] pyruvate kinase[44-46,48] and many others. It therefore seems valid to use thiophosphates as stereochemical probes. The majority of the enzymes proceed with inversion of configuration of the transferred phosphoryl moiety.

The simplest interpretation of this result is that a single phosphoryl transfer step is involved. Phosphokinases catalyse phosphoryl transfer with inversion of configuration; the only known exception to this is nucleoside diphosphate kinase, for which the phosphoryl transfer is observed with retention of configuration. Phosphomutases and some phosphatases are also known to proceed with retention of configuration. A phospho-enzyme has been implicated in these reactions from classical studies.

TABLE 1:1

Stereochemical course of enzymic phosphoryl and thiophosphoryl transfer reactions

Enzyme	Method	Stereochemical Course	References
PHOSPHOKINASES			
Acetate kinase	2	Inversion	51
Adenosine kinase	1	"	52
Adenylate kinase	1	"	53
Creatine kinase	2	"	54
Glucokinase	2	"	55
Glycerol kinase	1,2	"	44,45,46,47
Hexokinase	1,2	"	44,51,56
Nucleosidediphosphate kinase	1	Retention	57
Nucleoside phosphotransferase	1	"	58
Phosphofructokinase	2	Inversion	59
Polynucleotide kinase	1,2	"	60,61,46
Pyruvate kinase	1,2	"	44,45,46,48
Ribulose phosphate kinase	1	"	62
PHOSPHATASE			
Acid phosphatase	2	Retention	63
Alkaline phosphatase	2	"	64
Mitochondrial ATPase	1	Inversion	65
Myosin ATPase	1	"	66
Sarcoplasmic reticulum ATPase	1	Retention	67
Elongation factor G GTPase	1	Inversion	68
Elongation factor T GTPase	1	"	69
Thermophilic bacterium PSE ATPase	1	"	70
Pyrophosphatase	1	"	71
Glucose-6-phosphatase	2	Retention	72
Snake venom-5'-nucleotidase	1	Inversion	73

TABLE 1:1 (Continued) ....

Enzyme	Method	Stereochemical Course	References
MUTASES			
Phosphoglucomutase	2	Retention	74
Phosphoglycerate mutase (muscle)	2	"	75
Phosphoglycerate mutase (wheat germ)	2	"	75

Method 1: [^{18}O]-thiophosphate;
Method 2: [^{16}O,^{17}O,^{18}O] phosphate.

Overall retention of configuration would accord with a mechanism which involved two in-line displacements.

$$E-XH + A-\textcircled{P} \rightleftharpoons E-X-\textcircled{P} + AH$$

$$E-X-\textcircled{P} + B-H \rightleftharpoons E-XH + B-\textcircled{P}$$

The phosphate group $\textcircled{P}$ is transferred from molecule A to molecule B through the intermediate $E-X-\textcircled{P}$.

Although these enzyme-catalysed phosphoryl transfer reactions proceed with in-line geometry, this does not necessarily imply an associative reaction. It would be reasonable to assume that an enzyme would direct a reaction involving a reactive intermediate such as metaphosphate and that in-line transfer would be ensured by the appropriate placement of the nucleophile.

Introduction; aims of the thesis

The chemical basis of phosphoryl transfer reactions has been reviewed by Benkovic.[2] There is much evidence to support the contention that phosphate monoesters react mainly through a dissociative pathway involving monomeric metaphosphate [see Chapter 4 for a detailed discussion]. It has been suggested that such an intermediate could also participate in enzyme-catalysed phosphoryl transfer reactions. However, there remains considerable controversy concerning the lifetime of such an intermediate, particularly in aqueous solution and whether a truly free intermediate can exist.

Stereochemistry is recognised as a fundamental probe of mechanism. The isotopically chiral phosphate monoesters, originally developed to study the enzyme-catalysed reactions, have recently been used to address the problem of the existence or otherwise of monomeric metaphosphate. Despite the fact that thiophosphoryl transfer reactions have been used as stereochemical probes for enzyme-catalysed phosphoryl transfer reactions,

there have been few investigations of simple chemical thiophosphoryl transfer reactions. The work of Katz and Breslow[49] has shown that thiophosphate monoesters react more rapidly than the corresponding phosphate ester. This has been interpreted in terms of a more facile dissociative reaction for the thiophosphate ester. Thus thiometaphosphate may be a longer-lived intermediate than metaphosphate.

The major aim of this work is to probe this by studying the stereochemical course of simple thiophosphoryl transfer reactions. The results of such studies would provide information pertinent to the mechanistic pathways for thiophosphoryl transfer and would provide an insight into the use of thiophosphates in enzyme catalysed reactions.

Such a study poses two problems:

[1] The stereocontrolled synthesis of [^{16}O,^{18}O (or ^{17}O)] thiophosphate esters;

[2] The independent configurational analysis of such species.

These two problems will be fully discussed and developed in later chapters.

Although the main interest of this work lies in the stereochemical course of transfer reactions at phosphorus monoesters, there is also considerable interest in the stereochemical course of reactions which involve di- and tri-esters.

The 1,3,2-dioxaphosphorinan-2-one provides an ideal system within which to study the stereochemical course of hydrolyses of di- and tri-phosphoryl esters.

CHAPTER 2

Thiophosphoryl Transfer Reactions: The Synthesis of Isotopically Chiral Thiophosphate Esters

INTRODUCTION

In order to study the stereochemical courses of enzyme catalyzed phosphoryl and nucleotidyl transfer reactions, much effort has gone into the development of methods for the stereospecific synthesis of isotopically chiral phosphate and thiophosphate esters.[45,76-79]

The first oxygen chiral phosphate esters were reported in 1978, when two basic strategies were reported for the synthesis of chiral [^{16}O,^{17}O,^{18}O] phosphate monoesters (phosphate esters and anhydrides). One reported by Knowles[45] and coworkers at Harvard and the other by Lowe[76] and coworkers at Oxford.

The Harvard synthesis is illustrated in Figure 2.1 and is based on the studies of Inch et al.[80] The starting cyclic adduct was prepared in high yield by the reaction of (-)ephedrine with [^{17}O] phosphorus oxychloride in the presence of triethylamine giving an epimeric mixture of five-membered ring phosphoramidic chlorides.

The mixture was reacted directly with a variety of nucleophiles (in this illustration 2-benzyl-(S)-propane-1,2-diol was used), to afford a separable mixture of cyclic phosphoramidic esters. [Inch has assigned the configurations of the phosphoramidic chlorides and several esters derived from alcoholysis of the chlorides.] The assignments were based on the observed deshielding of protons in a 1,3 cis relationship to the phosphoryl group.[80]

The stereochemical course of many nucleophilic displacements of the exocyclic substituent in 5-ring phosphoryl compounds such as 1,3,2-oxaza-phospholidin-2-one and -2-thione have been established. The absolute configurations of the phosphoramidic esters follows from the configuration of the chloride since there is a good precedent for assuming that the displacement reaction occurs with retention of configurations.[80] The

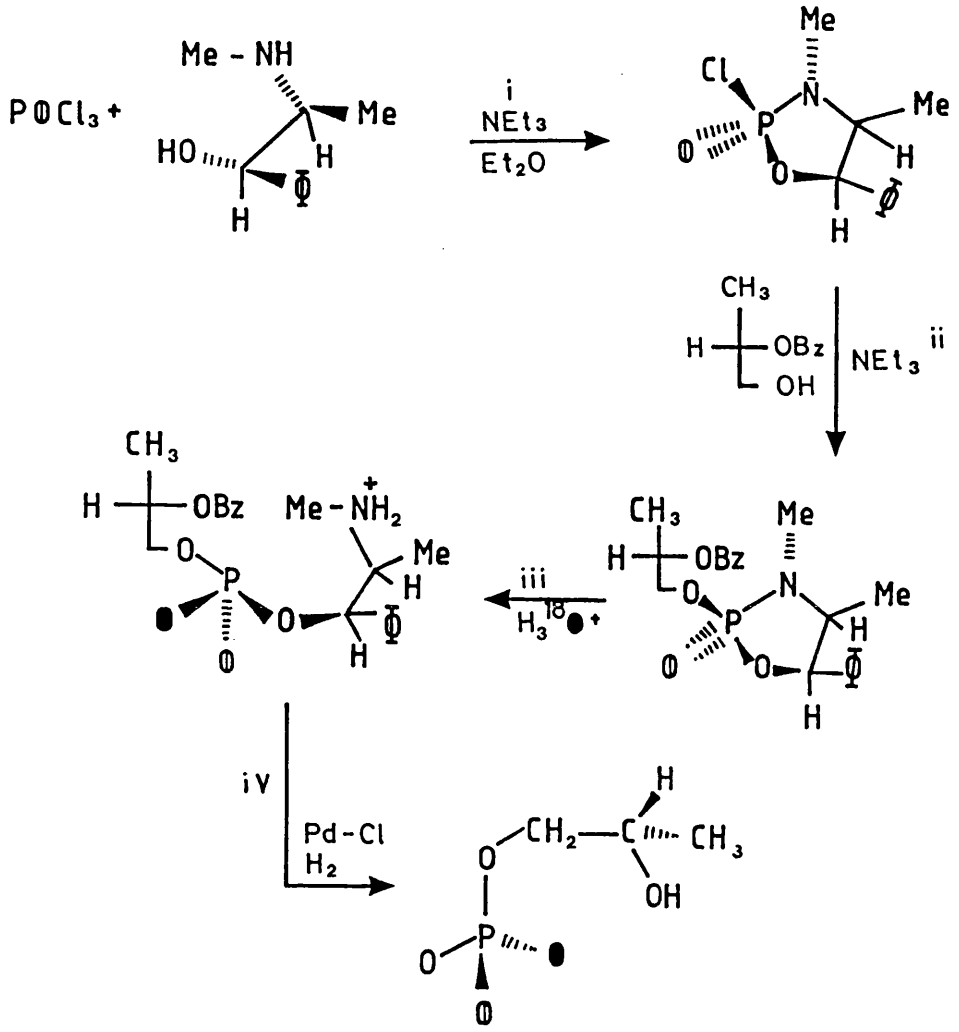


FIGURE 2.1 The Harvard route to the Rp diastereomer of [1-^{16}O,^{17}O,^{18}O]phospho-(S)-1,2-propane diol reported by Knowles et al.

^{18}O is introduced in the next step by hydrolyzing the P-N bond of the major phosphoramide ester, under acidic conditions in $H_2{}^{18}O$. The stereochemical course of P-N ring opening is known to proceed with inversion[80] of configuration at the phosphorus.

Catalytic hydrogenolysis of the acyclic $^{17}O,^{18}O$ chiral diester removes the ephedrine moiety by C-O cleavage of the benzylic linkage as well as the benzyl group. This results in the formation of 1-[$^{16}O,^{17}O,^{18}O$]-phospho-1,2-(S)-propane diol. The synthesised ester was predicted to have the Rp configuration at phosphorus which was later supported by the analytical methods. This method is general for phosphate esters and anhydrides.[81,64]

The alternative enantiomer can be produced by the same routes by reversing the order in which ^{17}O and ^{18}O are introduced.

The Oxford route is illustrated in Figure 2.2. This differs from Knowles' method[45] in that it does not involve the introduction of oxygen isotopes in a hydrolysis reaction involving a nucleophilic displacement at phosphorus. The key intermediate is (1R,2S) [1-^{18}O]-dihydroxy-1,2-diphenylethane.

The synthesis started from (S)-mandelic acid which is reacted with phenyl lithium to form (S)-benzoin. Acid catalyzed hydrolysis of the corresponding ethylene ketal in $H_2{}^{18}O$ introduces the isotope in the carbonyl oxygen. Reduction of this material gives the key intermediate (the stereospecifically labelled meso hydrobenzoin) which is chiral by virtue of the isotopic substitution. The second isotope is introduced by reacting ^{17}O-enriched phosphorus oxychloride with isotopically chiral meso-hydrobenzoin. The chloride was treated with methanol to give the corresponding five-membered ring methyl triester. Catalytic hydrogenolysis releases the [$^{16}O,^{17}O,^{18}O$] methyl ester of Sp configuration.

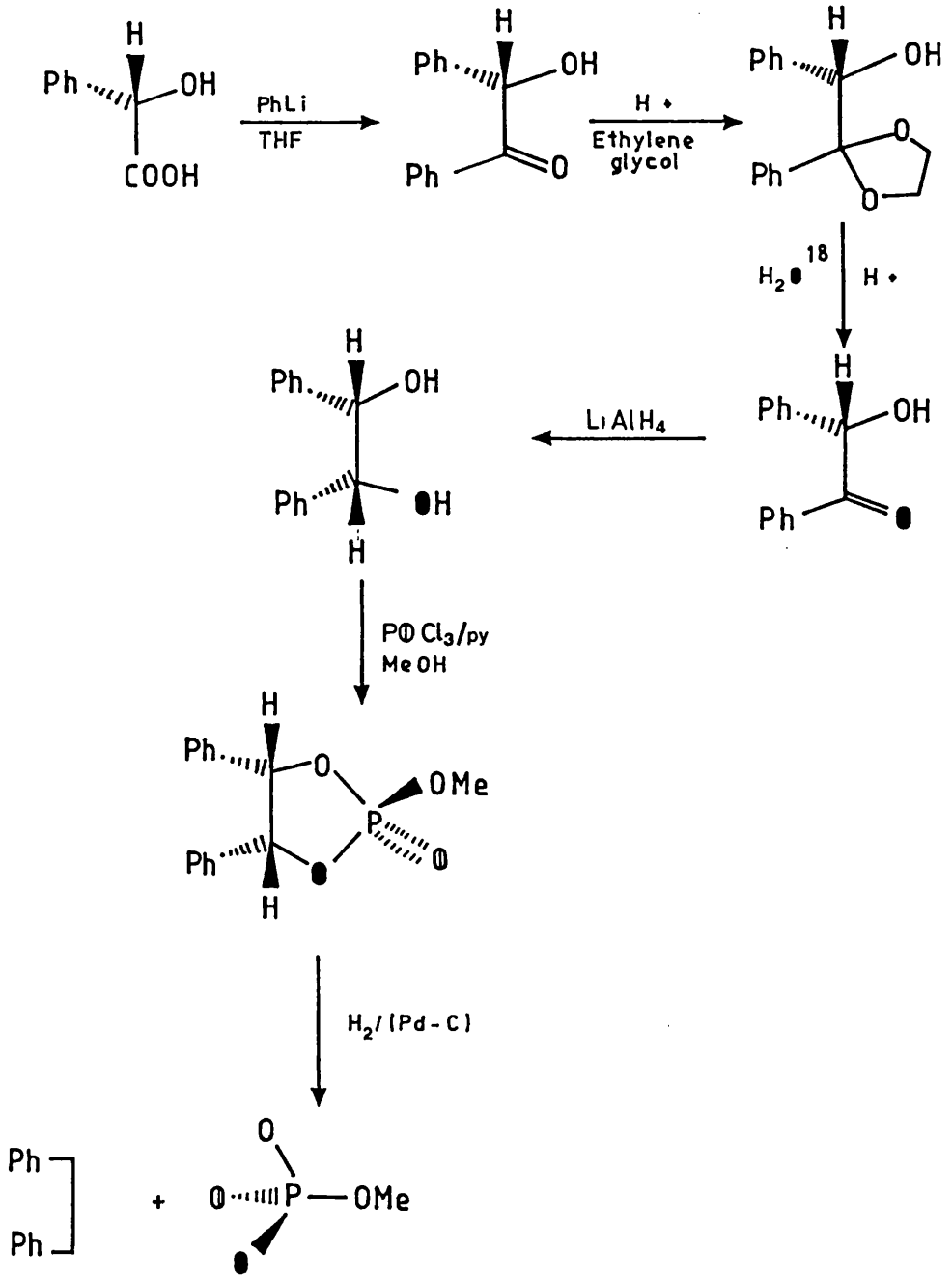


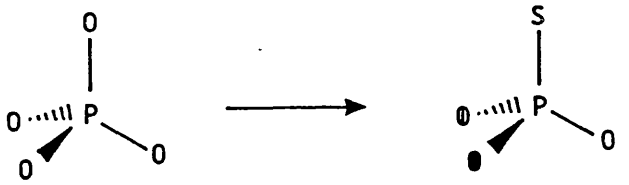
FIGURE 2.2 The Oxford route to the Sp enantiomer of methyl[^{16}O,^{17}O,^{18}O]phosphate reported by Lowe et al.

The Rp configuration can be formed by reversing the introduction of the isotopes or preparing the compound from (R)-mandelic acid. This is also a general route for phosphate esters and anhydrides.

Both syntheses have in common the use of a five-membered ring to introduce chirality at phosphorus. Many displacement reactions involving such five ring phosphoryl compounds have been shown to proceed stereospecifically, thus providing a good precedent for the stereocontrolled introduction of isotopes.

Thiophosphates as analogues of natural Substrates

In order to probe the stereochemistry of enzymatic reactions, particularly phosphatases, thiophosphate analogues of naturally occurring substrates have been extensively used.



(1) (2)

Phosphatases lead to inorganic phosphate (1) as the product. In order to study the stereochemistry of such reactions the chiral inorganic [^{16}O,^{17}O,^{18}O] thiophosphate (2) shown above must be exploited.

Early doubt about the validity of stereochemical results derived from thiophosphate analogues are apparently unsubstantiated. From the experimental evidence discussed in Chapter 1. It can be seen that both the natural and thiophosphoryl analogues follow the same stereochemical course.[42,43,82-85] These results allow some confidence to be placed in the results for phosphatases in which the use of thiophosphates is the only way of determining the stereochemical course.

Thiophosphates generally react at much slower rates than phosphates and for some enzymes, e.g. phosphomutases,[86] thiophosphates may not serve

as substrates; however, based on the studies to date thiophosphates can be used as reliable stereochemical probes for enzyme catalyzed reactions.

The work described in this chapter will cover methods for the synthesis of isotopically chiral thiophosphate monoesters, therefore it is appropriate to review the existing methods reported in the literature.

Thiophosphates at the diester (O,O diester) level are chiral in the conventional sense. At the monoester level the centre is prochiral and substitution of one oxygen with either ^{17}O or ^{18}O gives a chiral monoester. The diastereoisomers of thiophosphate diesters have, in many cases, been separable by ion exchange chromatography.

The substitution of oxygen by sulphur may be expected to alter several key substrate properties, any one of which may be responsible for the general slower reactivity with respect to the corresponding oxy substrates.

[1] Intrinsic reactivity in the uncatalysed reaction.
[2] Steric properties.
[3] pKa.
[4] Metal ion coordination.
[5] Hydrogen bonding.

Synthesis of isotopically chiral thiophosphates

(a) nucleotide thiophosphate

(1) For terminal thiophosphoryl group

i.e. AMPS, ADPβS, and ATPγS.

A scheme for the synthesis of the above was devised by Richard and Frey,[77] as illustrated in Figure 2.3. It begins with AMPS [$^{18}O_2$], (prepared by the reaction of adenosine with $PSCl_3$ followed by work up with $H_2{}^{18}O$) which reacts with diphenyl phosphorochloridate to form a diastereomeric mixture of labelled diphenyl esters of ADPαS. Subsequent displacement of diphenyl phosphorochloridate by unlabelled AMP resulted in a mixture of diastereomeric anhydrides of AMP and [$^{18}O_1$]

AMPS. This mixture of diastereoisomers can be separated by ion-exchange chromatography. The pyrophosphate is cleaved enzymatically by attack at the phosphoryl centre by nucleotide pyrophosphate to produce Rp AMPS ^{18}O and Sp AMP ^{18}O as shown.

Rp and Sp diastereoisomers of [β-^{16}O,^{18}O] ADPβS were synthesised as shown in Figure 2.3. The key steps in this reaction are the formation

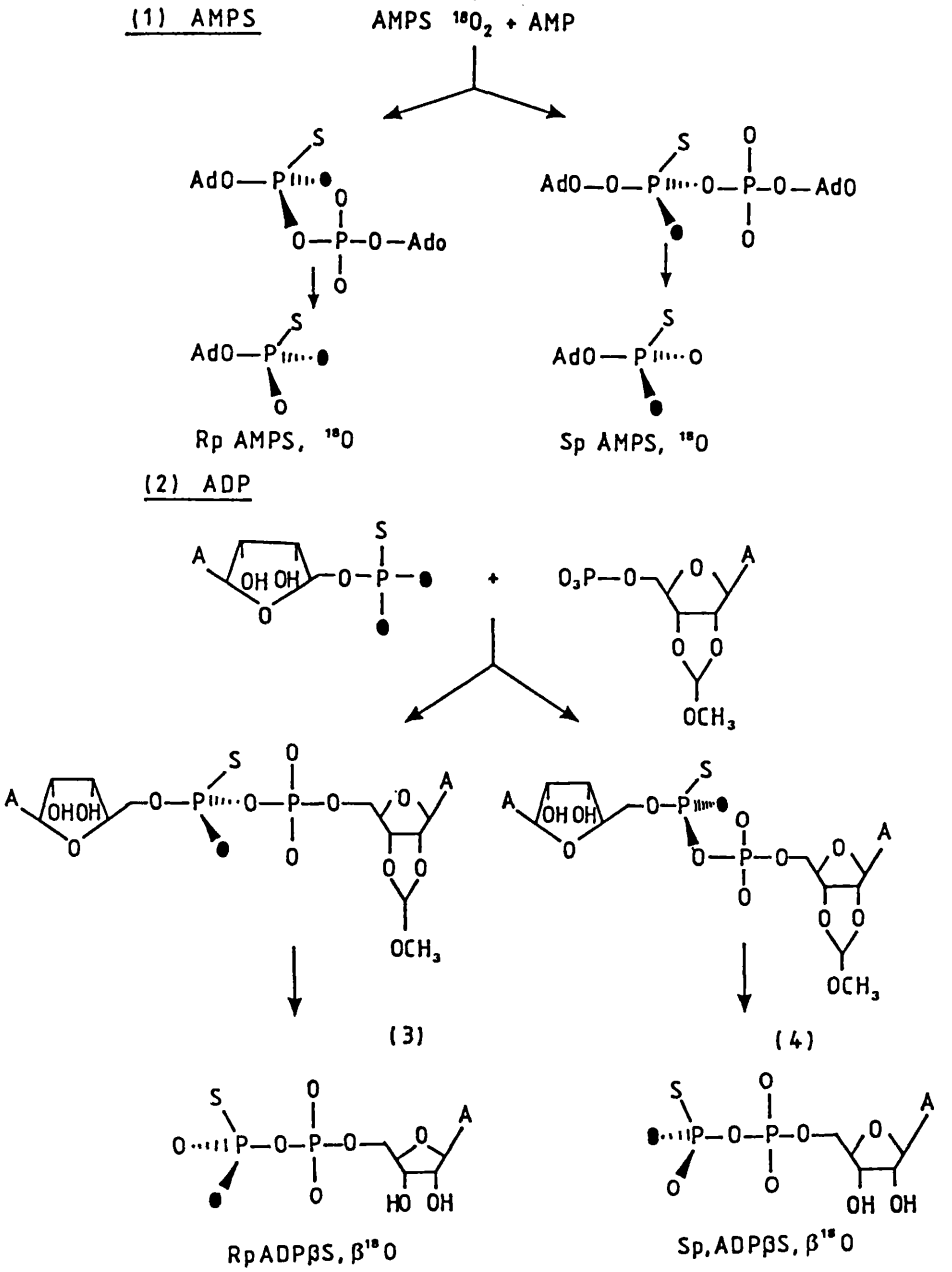


FIGURE 2.3 The external synthesis of:-
(1) the diastereomers of [^{16}O,^{18}O] AMPS;
(2) the diastereomers of [β-^{16}O,^{18}O] ADPβS by Richard and Frey.

of diastereomeric mixed anhydrides (3,4) (by reaction of 2',3'-methoxy methylidene AMP with AMPS via activation with diphenyl phosphorochloridate. Its separation by chromatography and the degradation of the adenosine moiety attached to the thiophosphoryl group occur by periodate cleavage of the unprotected 1,2-diol leading ultimately to Rp ADPβS, $\beta^{18}O$ and Sp ADPβS, $\beta^{18}O$.

ATPγS [γ-^{16}O,^{18}O] can be made in an analogous manner as shown in Figure 2.4.

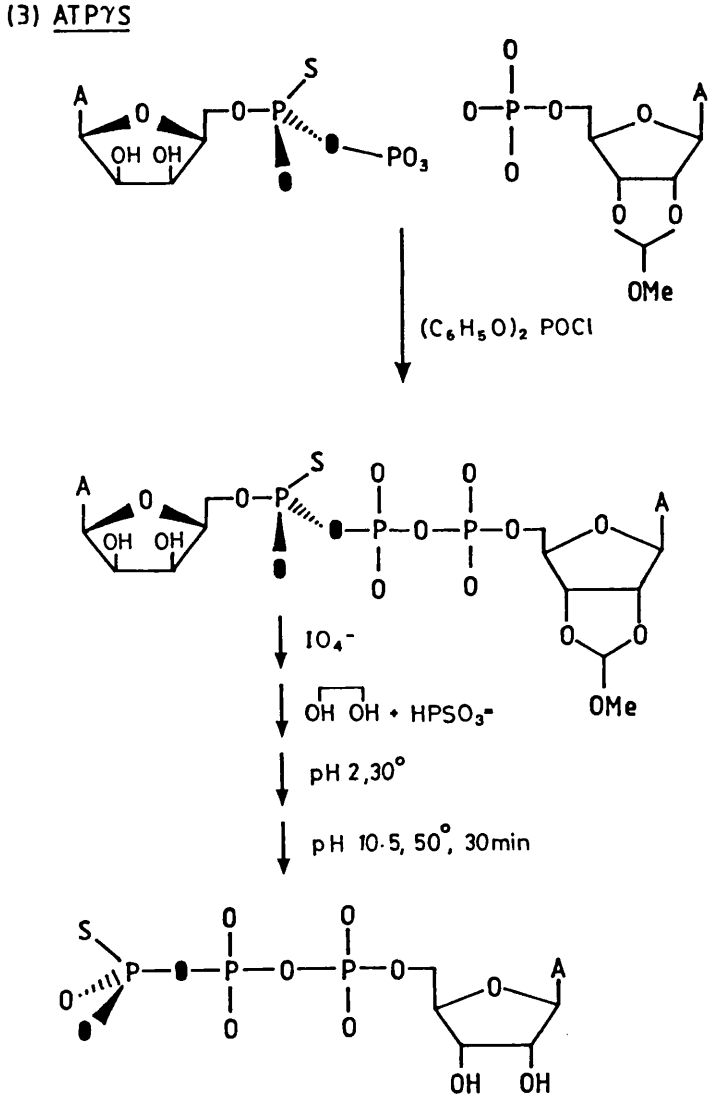


FIGURE 2.4 The external synthesis of the Rp diastereomer of [γ-^{16}O,^{18}O] ATPγS by Richard and Frey.

(2) Internal thiophosphoryl groups

e.g. Rp and Sp ADPαS, ATPαS and ATPβS.

Samples of diastereomerically pure ADP and ATP analogues with internal thiophosphoryl groups can be prepared from the nucleotide analogues with terminal thiophosphoryl groups by exploitation of the stereospecificity of various kinase reactions. A large number of ADP and ATP derivatives can be synthesised in this way.

Sheu and Frey[79] have shown that adenylate kinase and pyruvate kinase on AMPS form Sp ATPαS.

Eckstein and Goody[78] have also described enzymatic reactions that would convert ADPβS into either Rp or Sp isomers of ATBβS. Modifications of these reactions have been reported by various workers and the reactions are illustrated in Figure 2.5. More recently, Stingelin et al.[87] have reported that the 3-phosphoglycerate kinase catalysed phosphorylation of ADPβS also yields the Rp diastereomer of ATPβS as originally reported by Eckstein and Goody.

(b) Monoester [^{18}O] thiophosphates

The synthesis of isotopically chiral thiophosphate monoesters by a route analogous to that reported for isotopically chiral phosphate monoesters has been reported by Jarvest and Lowe.[88] Reaction of the [^{18}O]-labelled meso hydrobenzoin with thiophosphoryl bromide yielded the corresponding cyclic thiophosphate bromidate (5) as shown in Figure 2.6.

Reaction of the bromidate with, for example, alcohols leads to the corresponding cyclic triesters. Removal of the hydrobenzoin framework could not be achieved by hydrogenolysis as in the chiral phosphate synthesis, however sodium in liquid ammonia led to reductive cleavage of the benzylic groups. Significant competing aminolysis of the reactive five-membered ring was observed.[89] Although this route is potentially

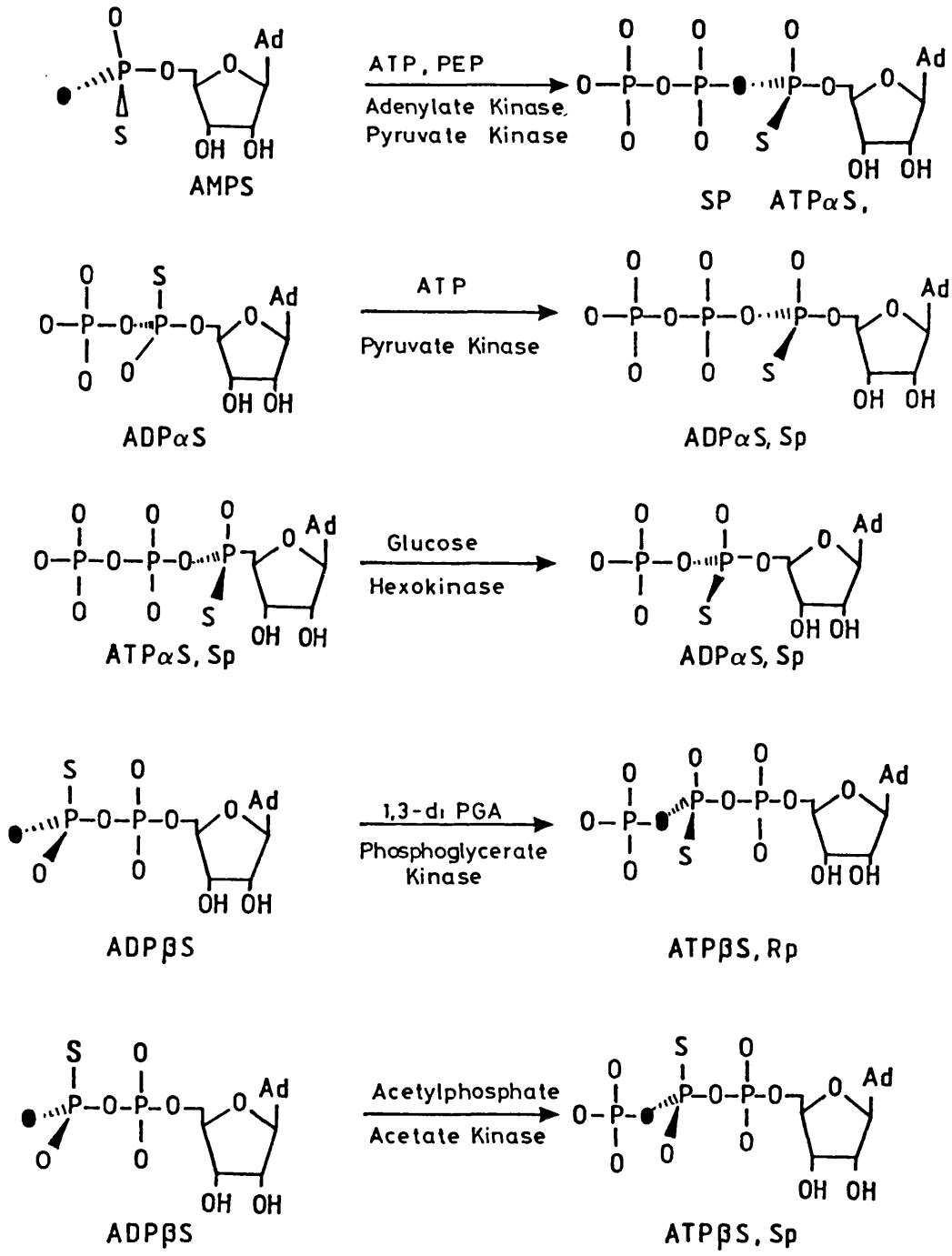
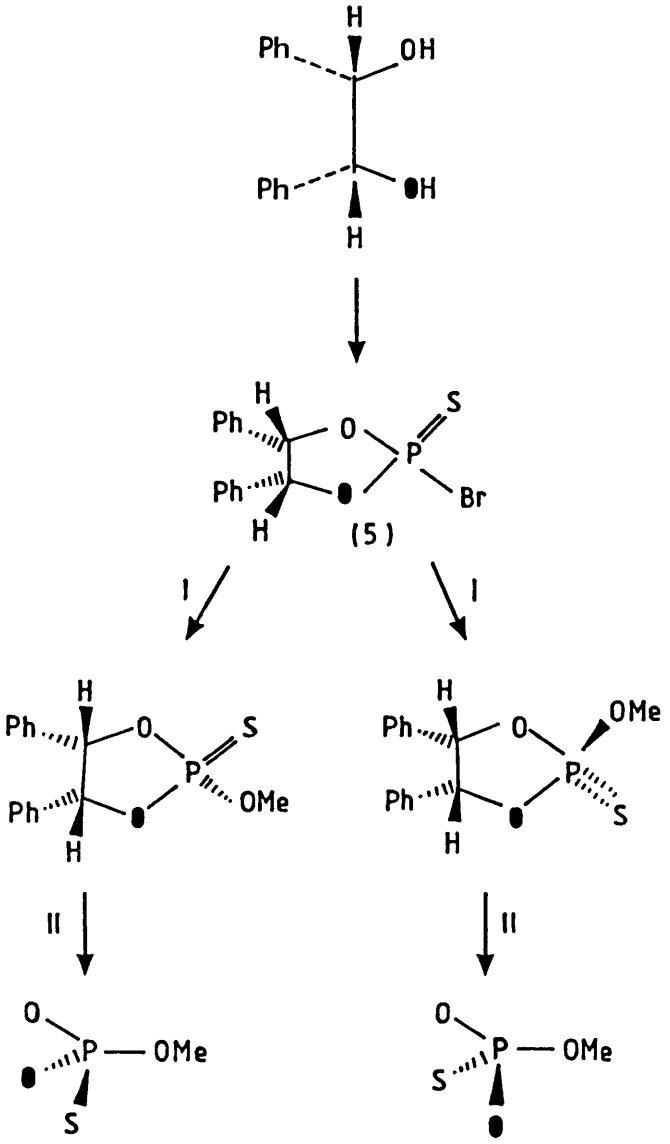


FIGURE 2.5 The internal synthesis of:-
(1) ATPαS, Sp
(2) ADPαS, Rp
(3) ADPαS, Sp
(4) ATPβS, Rp
(5) ATPβS, Sp



I (a) $PSBr_3$ $-C_5H_5N$ (2 equivalents)
(b) MeOH $-NEt_3$
II Na $-liq\,NH_3$

FIGURE 2.6 Synthesis of $^{16}O,^{18}O$ Monoesters by Jarvest and Lowe.

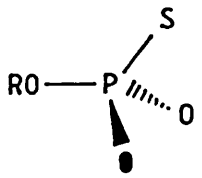
general it has not been widely used, probably because the ^{18}O meso hydrobenzoin is difficult to prepare in large amounts and the final removal of the carbon framework is difficult.

Method for the synthesis of thiophosphate ester

The synthesis of thiophosphates have been reviewed in this Chapter and,

with the exception of the meso hydrobenzoin route in Figure 2.6, the methods described are not general.

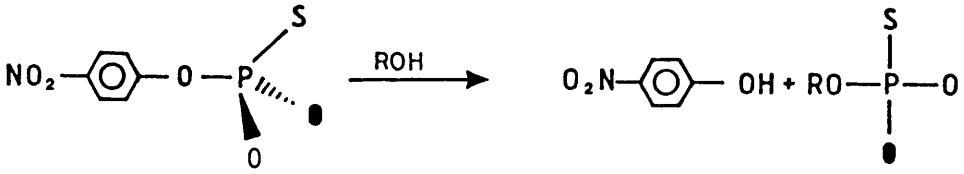
The objective of this study has been the development of a stereospecific general route to isotopically chiral thiophosphate monoesters such as (6). The route should be capable of introducing the isotope in



(6)

a stereocontrolled reaction, in high yield, using enriched water as the source of isotope.

Initially the stereochemical course of the solvolysis of p-nitrophenyl thiophosphate will be studied, following the work of Breslow et al.[49]



(18) (11)

The synthetic route must therefore be capable of synthesis of both enantiomers of (6) where R = aryl or alkyl.

By analogy with the successful routes to [^{16}O,^{17}O,^{18}O] phosphate monoesters [Knowles et al., Cullis and Lowe], synthetic routes involving the thiophosphoryl residue constrained in a five-membered ring were considered since this would provide a number of stereocontrolled reactions for the introduction of ^{18}O.

Two routes were initially considered illustrated in Figure 2.7. In the first of these a P^{III} compound bearing the appropriate ester substituent would be prepared and oxidised to the corresponding thiophosphate with elemental sulphur. This route sought to exploit the higher reactivity of P^{III} chloridites. The second route was directly analogous

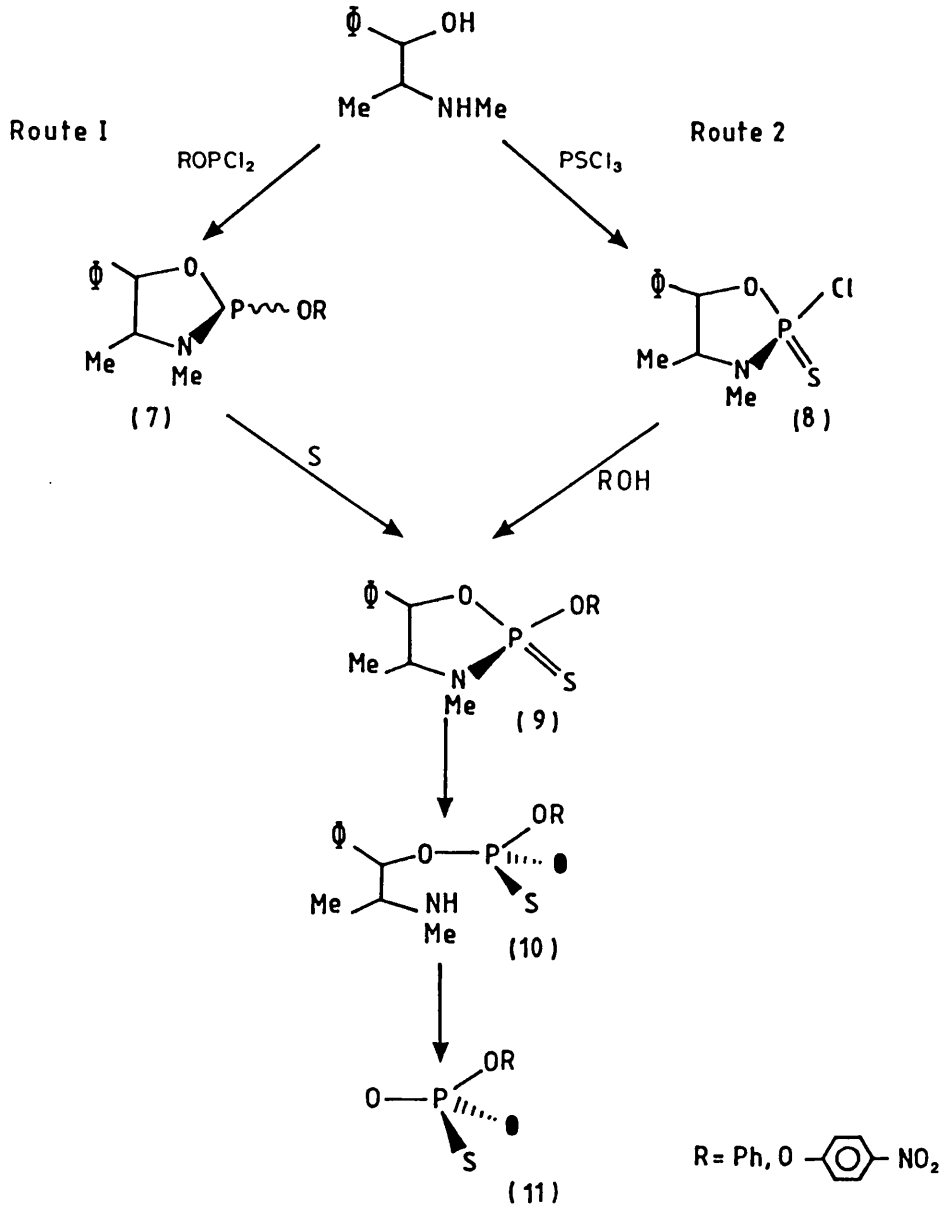


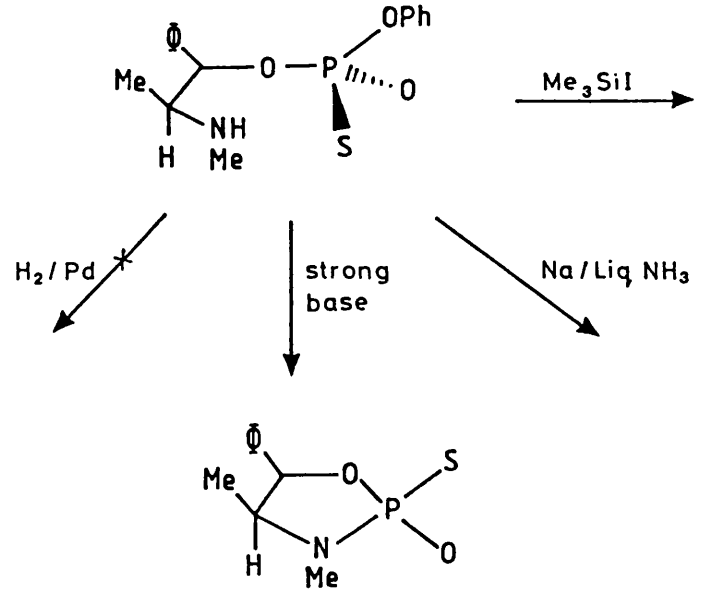
FIGURE 2.7 General synthetic routes for the synthesis of thiophosphate esters.

to the syntheses of [^{16}O,^{17}O,^{18}O] phosphate esters reported by Knowles et al., and based on the original ideas of Inch et al. The key thiophosphorochloridates suffer from the fact that thiophosphorochloridates are significantly less reactive than the corresponding oxychlorides. Both routes converge at the 2-substituted-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione.

The synthesis of Rp-O-phenyl [^{16}O,^{18}O] thiophosphate

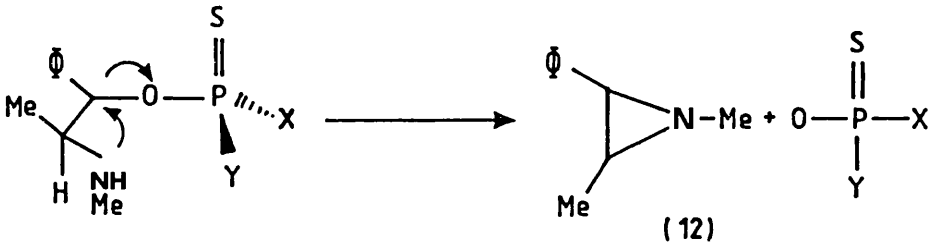
The reaction of (-)ephedrine with $PhOPCl_2$ gave the expected product (7) as a mixture of isomers. Trialkyl phosphites are readily oxidised to the corresponding trialkylthiophosphate with elemental sulphur. This reaction has been shown to proceed with retention of configuration.[50] Treatment of (7) with sulphur yielded 2-phenoxy-3,4-dimethyl-5-phenyl 1,3,2-oxazaphospholidin-2-thione (9) as a mixture of diastereoisomers which were separable by column chromatography. P-N bond cleavage in dioxan/water in the presence of trifluoroacetic acid proceeded smoothly. Using the cis compound (9a) ring opening led to a single diastereoisomer of (10) which is assumed to have the stereochemistry as shown in Figure 2.7 since there is a good precedent for P-N bond cleavage in acid proceeding with inversion of configuration.[80,90,91]

The removal of the ephedrine framework could not be achieved by hydrogenolysis as in the work of Knowles et al. because of the presence of sulphur which leads to poisoning of the catalyst. Alternative procedures are shown.



Inch et al. removed the ephedrine moiety from a variety of thiophos-

phorus acid derivatives by treatment with strong base which led to elimination of the thiophosphoryl component with concomitant aziridine formation[92] (12).

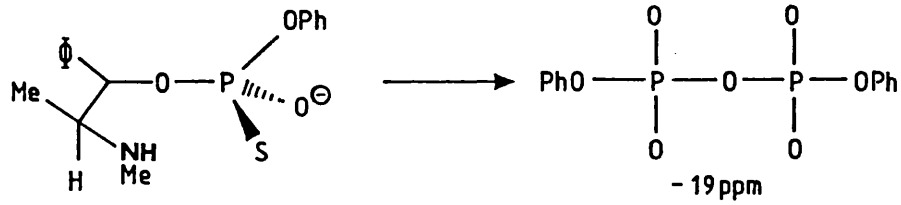


(12)

X = Ph
Y = O

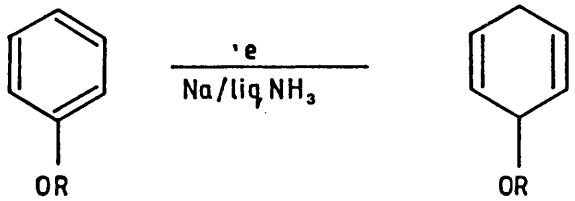
This procedure when applied to the arylthiophosphate led only to the formation of the 5-ring oxazaphospholidin—2-thione as shown in the scheme.

The third method involved reductive cleavage in sodium liquid ammonia. ^{31}P nmr of this reaction showed the formation of a major resonance at -19 ppm (13). This appears to be consistent with the loss of sulphur and formation of a pyrophosphate, as shown below.



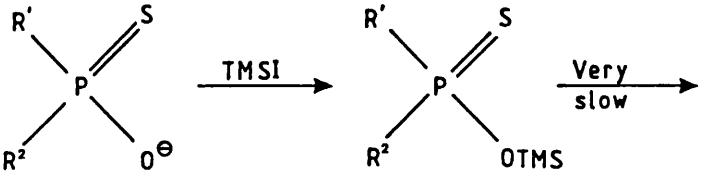
(10) (13)

Sodium in liquid ammonia promotes the Birch reduction which involves a single electron transfer. This electron transfer can reduce the aromaticity of the phenyl group. This method was no longer used.



The use of TMSI to effect C-O bond cleavage is well known in the literature.[93] This reagent was then used to effect this cleavage.

Problems were also encountered in this reaction. After the first reaction on oxygen, no further reaction occurred even with increasing amounts of TMSI and heating. This is understandable in terms of the low philicity of P=S with TMSI as noted by Michalski.[93]



Ideally a route to p-nitrophenyl thiophosphate was required. It is doubtful that p-nitrophenyl [^{16}O,^{18}O] thiophosphate could be made by this route because the p-nitrophenyl phosphorochloridate would be difficult to prepare due to the tendency of P^{III} compounds to abstract oxygen from aryl nitro groups.[94]

The synthesis of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate

The alternative route shown in Figure 2.7 (Route 2) was explored. The thiophosphorochloridate (8) was prepared and characterised as described by Inch.[80] Since the displacement reaction with p-nitrophenoxide proceeded extremely slowly at room temperature, an alternative method for the formation of p-nitrophenyl ester was sought. This is shown in Figure 2.8.

Reaction of p-nitrophenyl dichlorothiophosphate[174] with ephedrine in toluene in the presence of triethylamine, yielded the cyclic triester in ca. 12 hours (14). The product was purified over silica to produce the cyclic ester in good yield. The ^{31}P nmr showed only one resonance, as illustrated in Figure 2.9, suggesting a single diastereoisomer had been obtained. Inch has assigned the configurations of the phosphoramidic chlorides and esters on the observed deshielding of protons in a 1,3 cis relationship of the phosphoryl group. These assignments, of course, required the availability of both epimers of the chloride and ester. The cis and trans literature ^{1}H nmr values for compound (14) were very close such that it was difficult

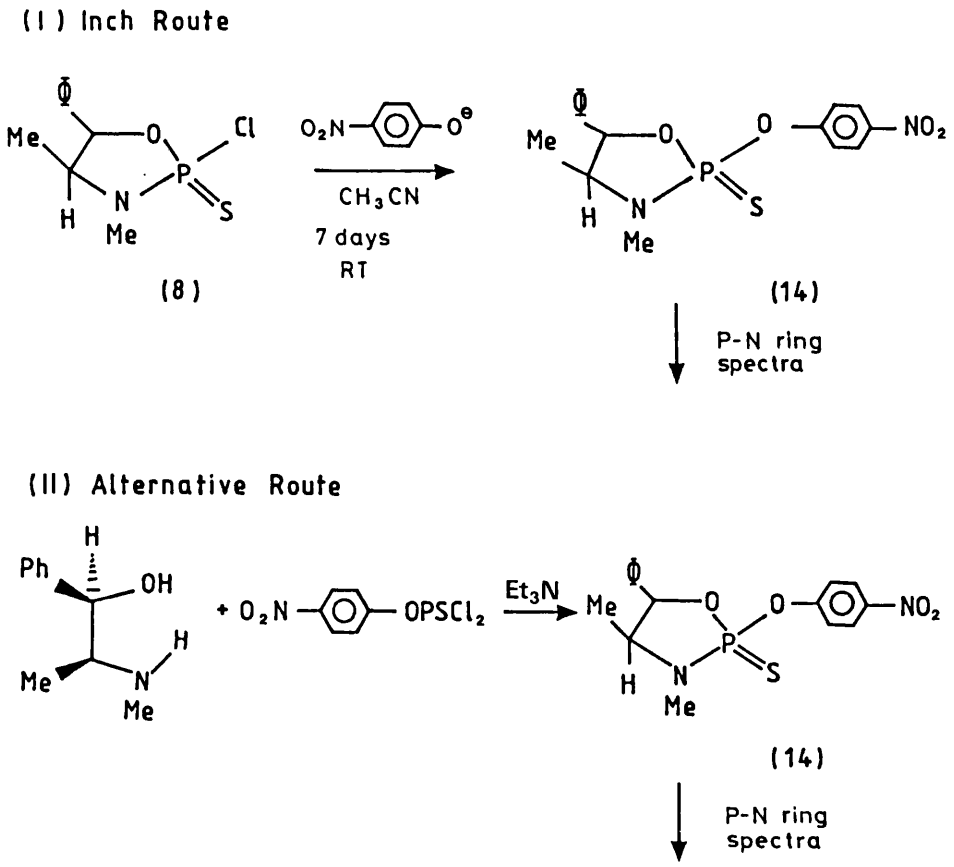


FIGURE 2.8 Routes to the synthesis of 2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione.

to assign the cis and trans on the basis of absolute chemical shift alone.

On P-N bond cleavage, however, the ^{31}P nmr showed two major resonances, illustrated in Figure 2.10, suggesting that both diastereoisomers of the cyclic triester (14a/14b) were present. Apparently ^{31}P nmr resonances of the cis and trans p-nitrophenyl cyclic esters (14a/14b) were not resolved although the diastereomeric P-N bond cleavage products were readily distinguished by ^{31}P nmr spectroscopy. Attempts to separate the cis and trans 2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thione (14a/14b) by thin layer chromatography or column chromatography were unsuccessful.

Despite the long reaction times for displacement of chloride from the

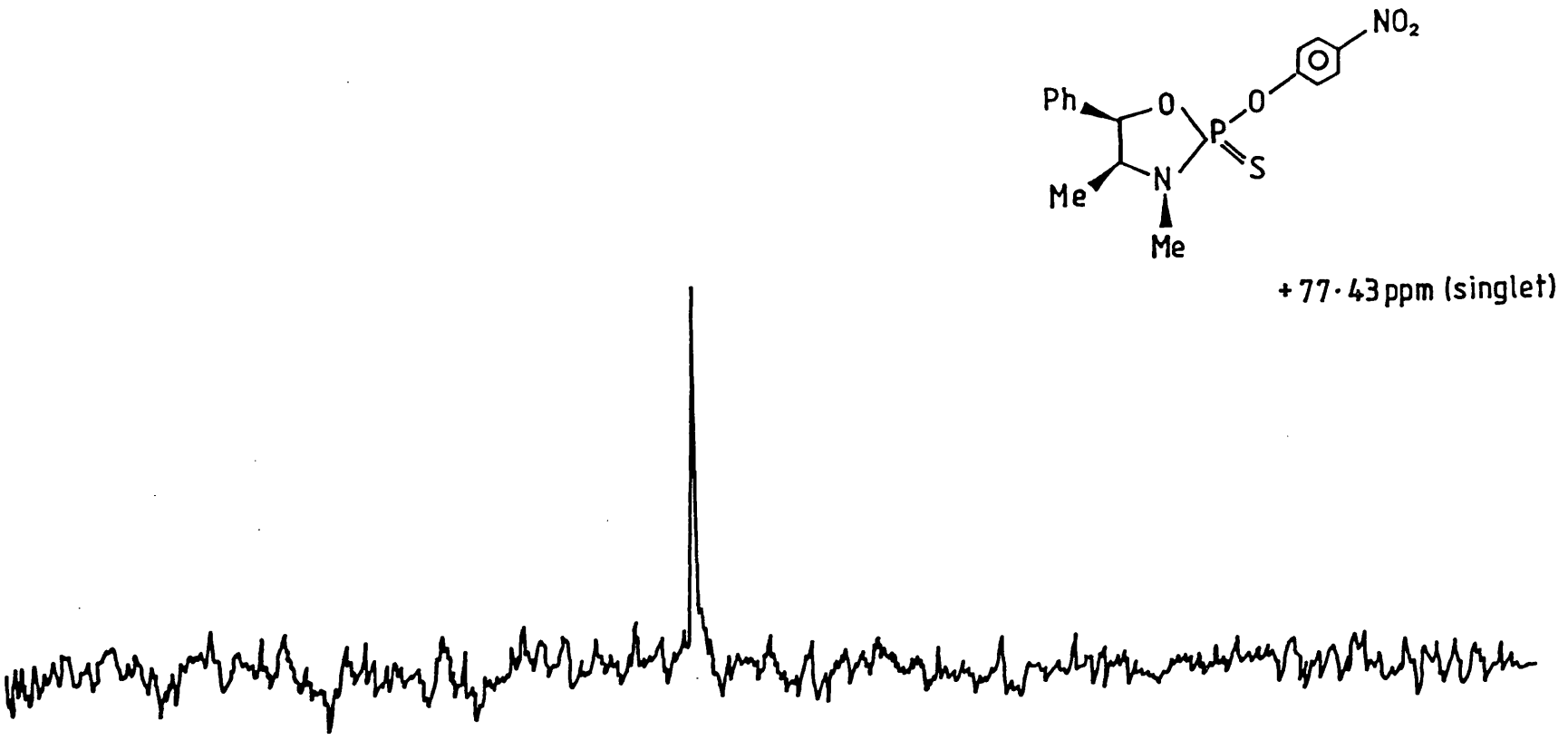


FIGURE 2.9 The ^{31}P nmr of the reaction of aryloxythiophosphoryldichloride with ephedrine in toluene in the presence of triethylamine.

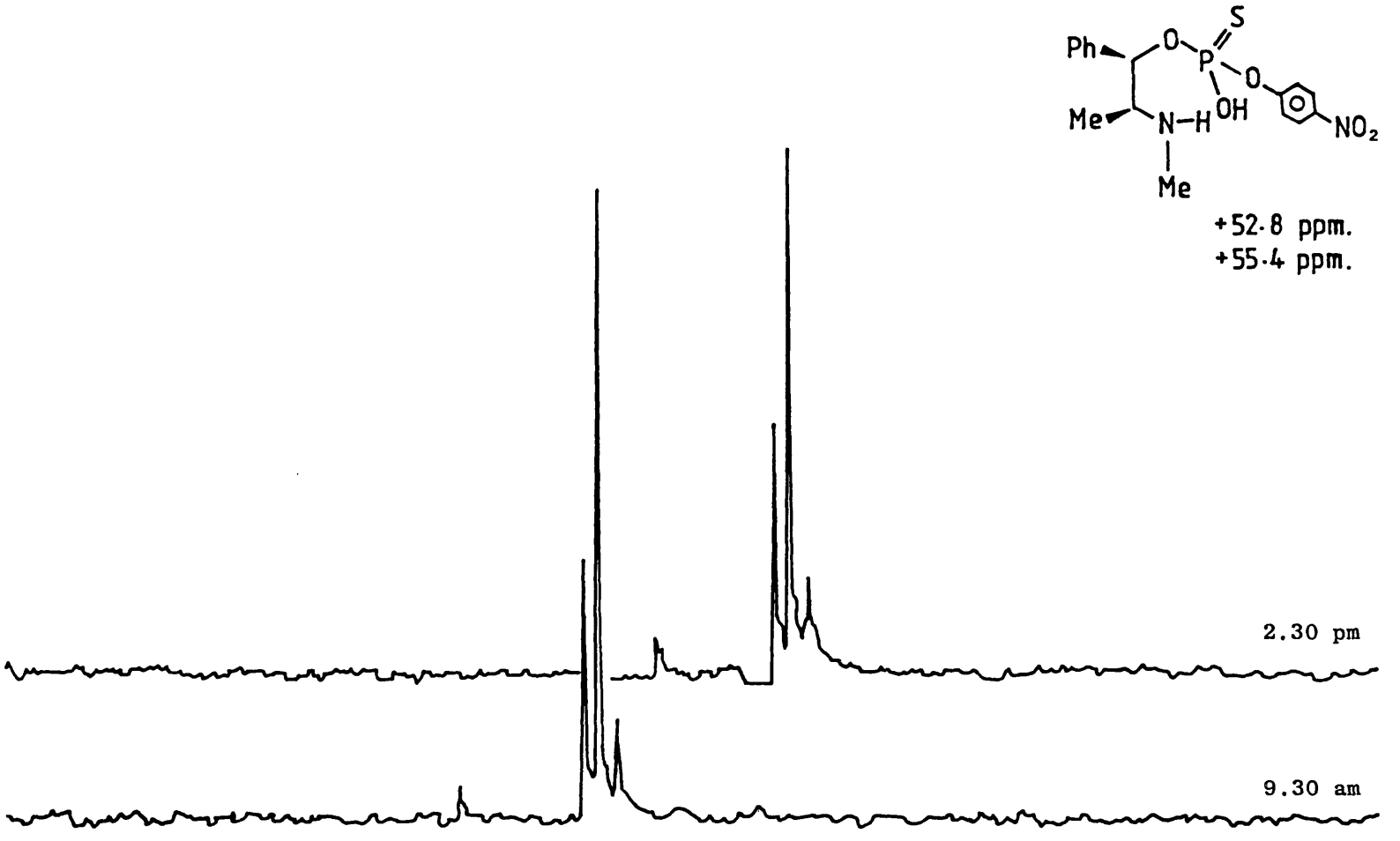


FIGURE 2.10 The ^{31}P nmr of the P-N bond cleavage of 2-p-nitrophenyl-3,4-dimethyl-1,5-phenyl-1,3,2-oxazaphospholidine-2-thione.

cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione by p-nitrophenoxide, the reaction proceeded stereospecifically to give the cis aryl triester (14) as a single diastereoisomer as judged by ^{1}H nmr and ^{31}P nmr spectroscopy on the product obtained on P-N bond cleavage. This route was, therefore, the method of choice for all subsequent work.

P-N bond cleavage (completion in one hour using trifluoroacetic acid in $H_2{}^{18}O$ and $H_2{}^{16}O$ in dioxan) gave only one peak in the ^{31}P nmr. P-N bond cleavage occurs with inversion of configuration, therefore the absolute configuration is expected[80] to be as shown in Figure 2.7, Route (1).

The next step involves C-O bond cleavage as shown in Figure 2.11. Routes (a) and (b) are ineffective as for phenyl phosphate. Route (c) using sodium in liquid ammonia cannot be employed since this would be expected to lead to a rapid Birch reduction[95] of the aromatic group

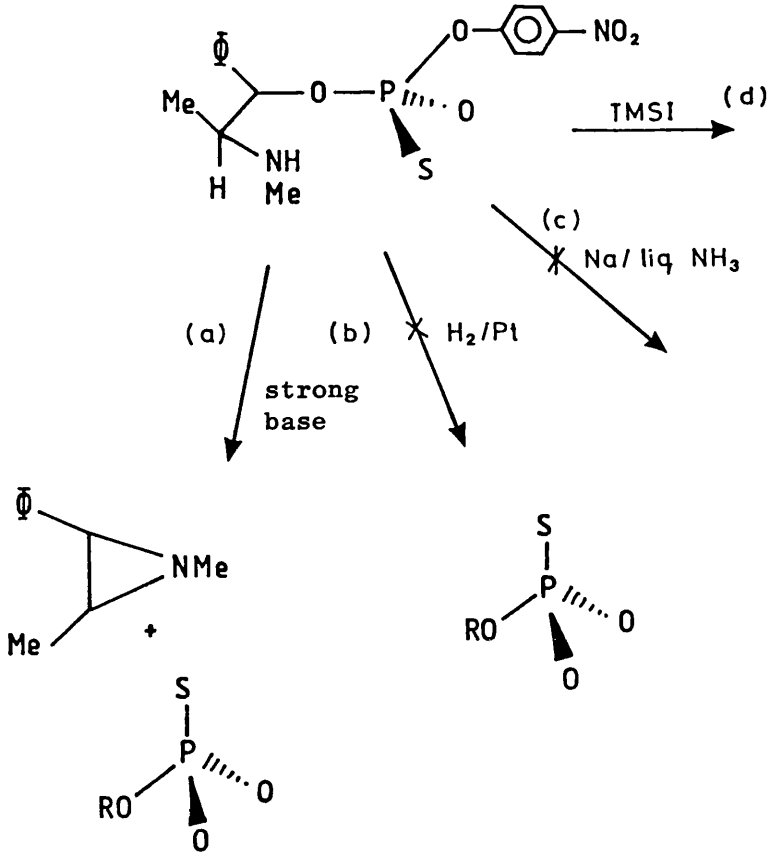
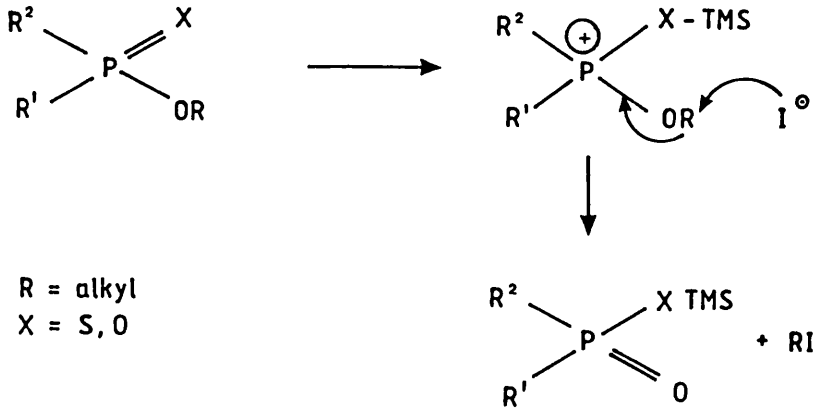


FIGURE 2.11 Routes to P-O bond cleavage.

enhanced by the electron-withdrawing effects of the nitro group.

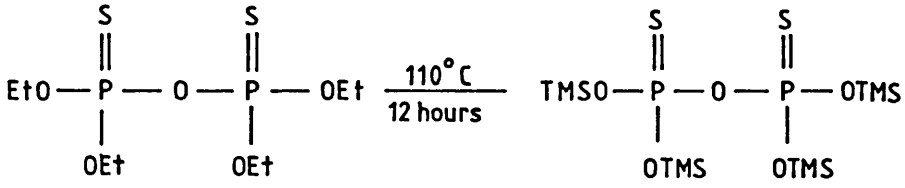
Therefore, the only other route was the use of trimethylsilyliodide, as demonstrated by Michalski on O-alkyl groups. The reaction of TMSI on this group is shown below.



Removal of the ephedrine moiety by TMSI however proved ineffective[93] as shown in the table below.

TMSI USED	TEMPERATURE	RESULT	TIME
4 equivalents	RT	incomplete reaction	over several days
Excess	RT	"	" " "
Excess	35°C	"	" " "
Excess	70°C	decomposed	" " "

It has been noted by Cullis[96] that the removal of O-ethyl groups from a pyrophosphate requires extreme conditions, as shown below.



The low reactivity of P=S compounds with trimethylsilyl halides is well documented in the literature.[93]

The problems encountered in removal of the ephedrine framework undoubtedly arise because the initial silylation occurs on oxygen as shown in Figure 2.12, pathway (e), which leads to the unreactive P=S

system. To get around this, prior methylation of sulphur leads to a P=O system [Figure 2.12, pathway (d)] which is now considerably more reactive with TMSI. The reason for this difference is that Si-O bonds are strong compared to Si-S (silicon sulphur) bonds and so silylation on sulphur is a slow process.

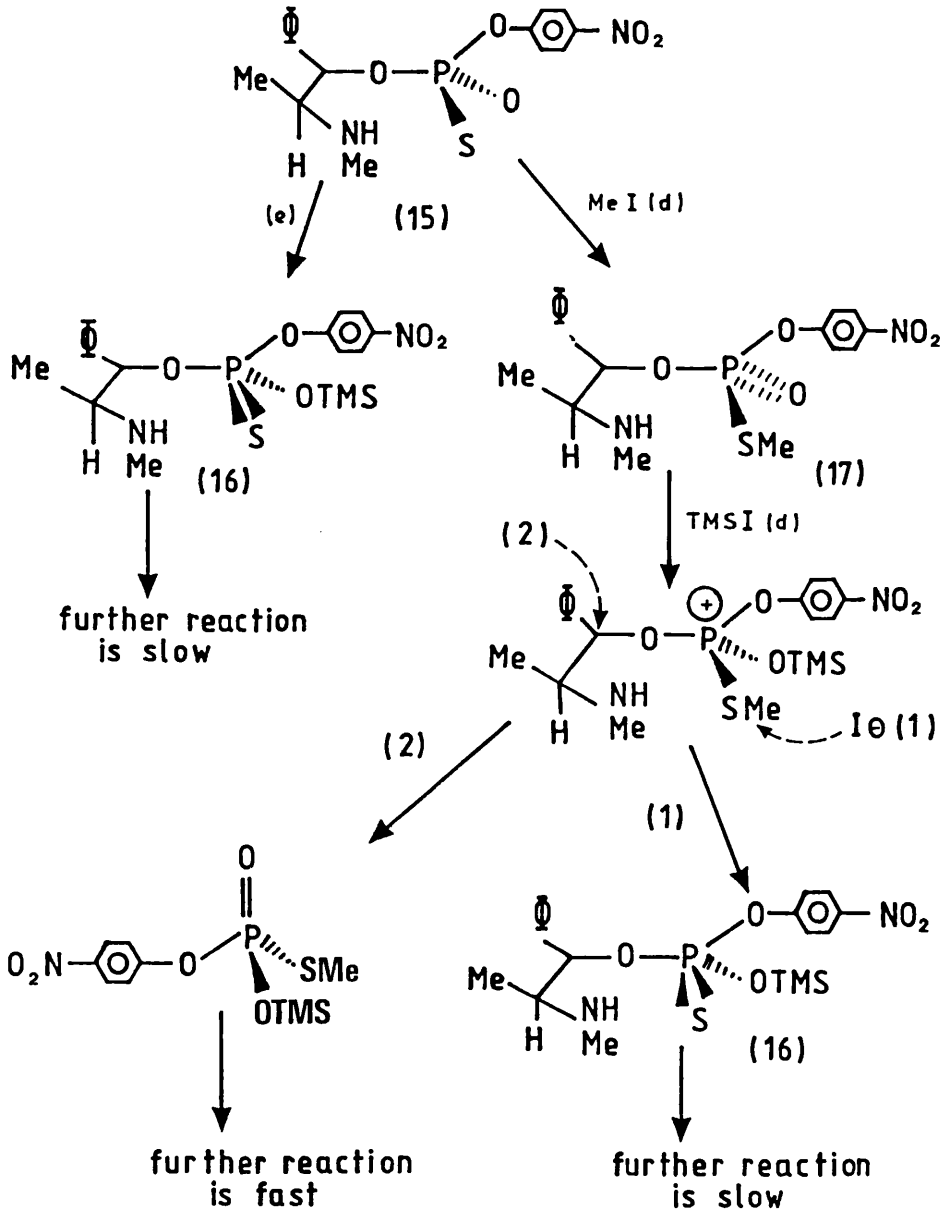


FIGURE 2.12 Methylation and silylation of 2-p-nitrophenyl-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thione.

Michalski[93] has shown the following order of reactivity towards silyl halides.

$$>P(=O)-OR \gg P(=O)-SeR \gg P(=O)-SR; \quad >P(=O)-OR \gg >P(=S)-OR, \quad >P(=O)-OR \gg >P(=Se)-OR.$$

Reactivity of analogous compounds in the silylation involving the substitution of the alkyl group at oxygen, sulphur and selenium decreases in the sequence.

$$Y = O > Se > S$$

The equilibria for the thiono, thio rearrangements lie over to opposite sides for alkyl and silyl esters.[97]

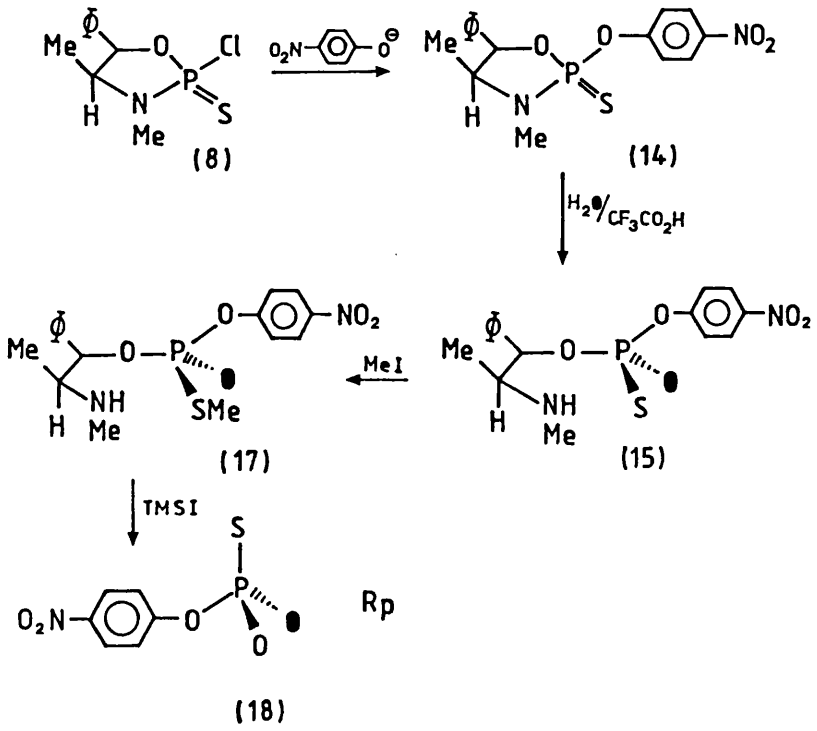
$$>P(=S)(OAlk) \rightleftharpoons >P(=O)-SAlk$$

Sulphur is more reactive with methyl iodide forming compound (17) shown in Figure 2.12. Addition of trimethylsilyliodide now silylates on oxygen. The second step of the reaction can now take two paths - d(1) or d(2).

Pathway d(1) is where iodide attacks the methyl group to restore a P=S; in an S_N2 reaction. Pathway d(2) is where iodide attacks the benzylic carbon centre to remove the ephedrine moiety and to restore a P=O.

If pathway d(1) predominated then there would be no advantage from methylation of sulphur and the reaction will stop at the intermediate (16) as in route (e). It was observed that further reaction was facile, leading to bis(trimethylsilyl) p-nitrophenyl thiophosphate in good yield, thus confirming that pathway d(2) dominates. Presumably the preferential removal of the benzylic centre suggests that the second step of the reaction with trimethylsilyliodide can proceed with a large degree of S_N1 character. The product with P=O is also presumably the more thermodynamically stable product.

The bis(trimethylsilyl) ester is spontaneously hydrolysed on stirring in aqueous buffer to give p-nitrophenyl thiophosphate (18) in good yield, which was readily isolated by ion-exchange chromatography. The hydrolysis involves attack at silicon and does not affect the stereochemistry at phosphorus, thus completing the synthesis of p-nitrophenyl thiophosphate. The route to isotopically chiral [^{16}O,^{18}O] p-nitrophenyl thiophosphate involving the use of $H_2{}^{18}O$ is shown in Scheme 2.13.

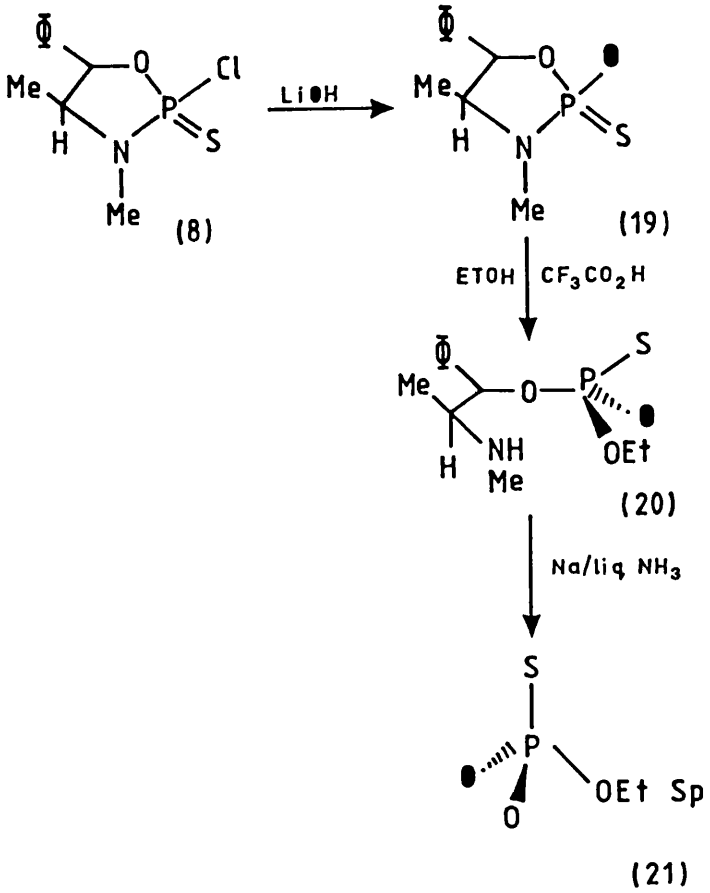


SCHEME 2.13 Synthesis of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate.

Synthesis of Sp-O-Ethyl [^{16}O,^{18}O] thiophosphate

Sp-O-Ethyl [^{16}O,^{18}O] thiophosphate was also synthesised. An alternative route was followed, as shown in the general Scheme 2.14. The isotope was introduced *via* the use of lithium hydroxide made from lithium in (1/1) $H_2{}^{16}O : H_2{}^{18}O$ which was added to the chloride (8) in dioxan. This gives a product with retention of configuration, as established by Inch *et al.*

P-N bond cleavage in ethanol/CF_3CO_2H occurs to give a single diastereoisomer (20) as shown in Scheme 2.14.



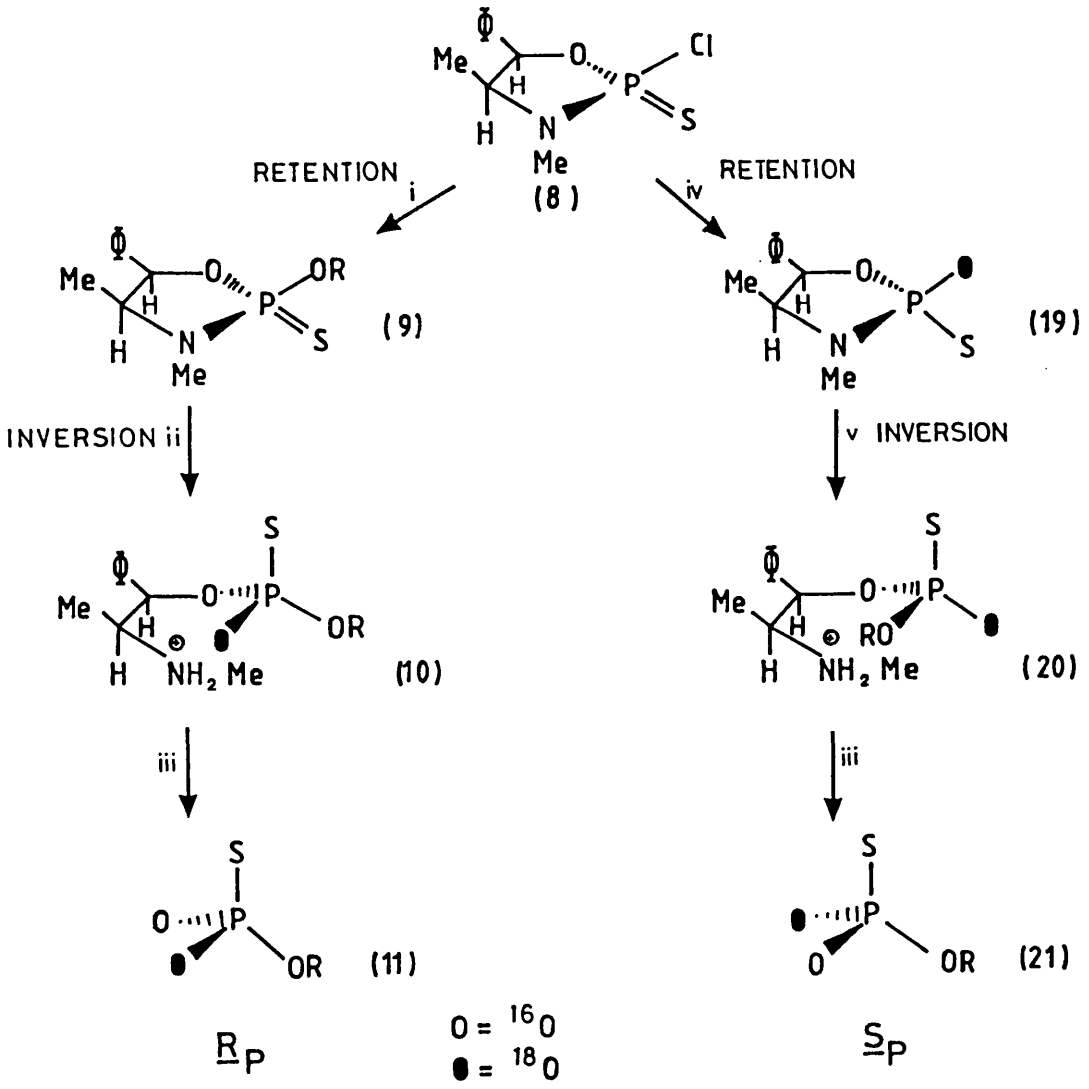
SCHEME 2.14 Synthesis of Sp-O-Ethyl [^{16}O,^{18}O] thiophosphate.

Removal of the ephedrine moiety could not be achieved by TMSI in an analogous fashion to the aryl esters because nucleophilic attack at the ethyl group would occur leading ultimately to inorganic thiophosphate. However, reductive cleavage of the benzylic group in sodium liquid ammonia proved successful in this case.

The two general syntheses of chiral [^{16}O,^{18}O] thiophosphate monoesters are summarised in Scheme 2.15.

Racemic ethyl thiophosphate

Racemic ethyl thiophosphate was required to establish the configura-

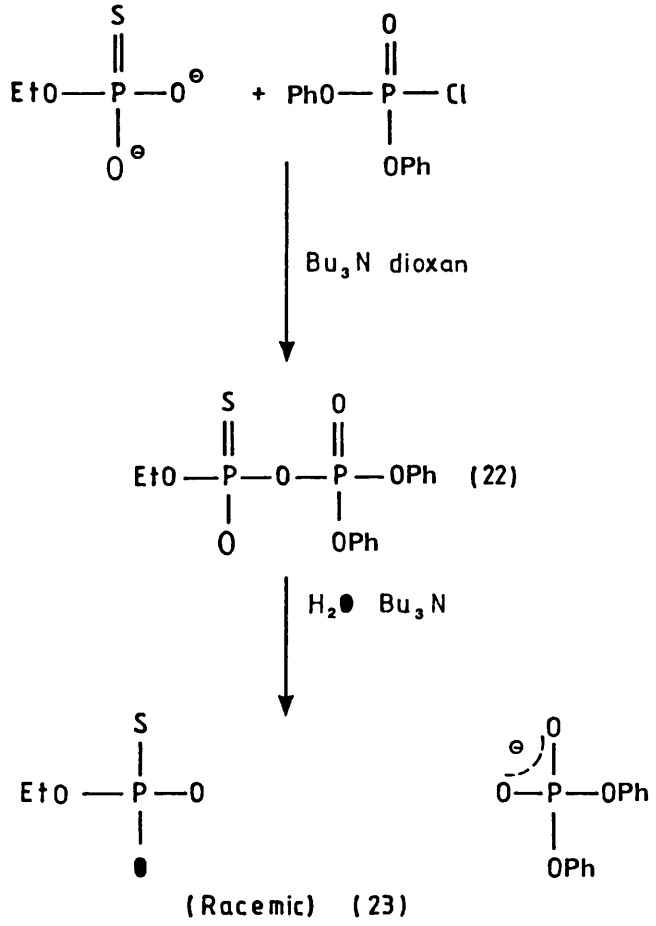


REAGENTS

i) $RO^{\ominus}/ROH$
ii) $H_2{}^{18}\bullet/CF_3CO_2H$
iii) Me_3SiI or Na liquid NH_3
iv) $Li{}^{18}\bullet H$
v) ROH/CF_3CO_2H

SCHEME 2.15 Two general syntheses of chiral [^{16}O,^{18}O] thiophosphate monoesters.

tional analysis. This was prepared by reaction of ethyl thiophosphate with diphenyl phosphorochloridate. The intermediate pyrothiophosphate was not isolated but was hydrolyzed <u>in situ</u> in $H_2{}^{18}O$ to give racemic ethyl [^{16}O,^{18}O] thiophosphate which was isolated by ion exchange chromatography as shown in Figure 2.16.



<u>FIGURE 2.16</u> Synthesis of racemic ethyl thiophosphate.

<u>CONCLUSION</u>

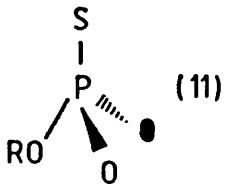
The stereospecific synthesis of isotopically chiral thiophosphate esters have thus been achieved by an extension of the work of Inch and coworkers. This constitutes the best general synthesis of such species to date. The independent configurational analysis was the next objective.

CHAPTER 3

Thiophosphoryl Transfer Reactions: Configurational Analysis of Isotopically Chiral Thiophosphate Esters

INTRODUCTION

In order to study the stereochemical course of solvolysis reactions of substituted thiophosphates a reliable configurational analysis of structures such as (11) is required.



(11)

Over the past years several general methods have been developed to effect the configurational analysis of oxygen isotopically chiral phosphoryl centres. These methods will be briefly reviewed. Three methods of configurational analysis of [^{16}O,^{17}O,^{18}O] phosphate monoesters have been developed:-

(1) Chiroptical

(2) Mass spectroscopy

(3) ^{31}P nmr spectroscopy.

(1) Chiroptical

Chiroptical methods of analysis would be the simplest and most direct configurational analysis. However, although methyl [^{16}O,^{17}O,^{18}O] phosphate was shown to possess a measurable circular dichroic spectrum it was too small to be of more than theoretical interest.[76,98]

Raman optical activity as a measure of chirality due to isotopic substitution initially appeared to be a more promising method of analysis,[99] however it has yet to prove its importance in the stereochemical analysis of such chiral esters.

(2) Mass spectral method

The first reliable method for the configurational analysis of isotopically chiral phosphate esters was based on metastable ion mass

spectroscopy.[100]

The principles of the method are illustrated in Figure 3.1. It requires that the [^{16}O,^{17}O,^{18}O] phosphoryl residue be attached to a diol with a conventional chiral centre. For example S-1,2-propane diol-1-(Rp)-[^{16}O,^{17}O,^{18}O] phosphate was cyclised to produce a mixture of three cyclic phosphodiesters which differ in their isotopic composition. Methylation of the isotopomers converts the diesters into a mixture of syn and anti triesters in which the exocyclic oxygens are chemically distinguished. The syn and anti isomers are diastereomeric and are separated chromatographically. These isomers are shown in Figure 3.1 that would arise from either the Rp or Sp [^{16}O,^{17}O,^{18}O] phosphate ester. The ring closure is achieved through activation of one of the peripheral oxygens with diphenyl phosphoimidazole and leads to loss of one of the three isotopic sites. The stereochemical course of this reaction has been shown to proceed with inversion of configuration[100,101] as shown in Figure 3.1.

The stereochemical information is contained in the identity of the oxygen isotope that is methylated as well as the isotope in the other site, e.g. in the ^{16}O,^{18}O labelled cyclic methyl ester the ester is methylated on ^{16}O from the R(syn) monoester and on ^{18}O from the S(syn) monoester. However, this alone is not sufficient to distinguish the Rp and Sp configurations in the parent molecule.

Normal mass spectral techniques cannot be used to determine directly the distribution of oxygen isotopes, because each mixture contains equal amounts of species that are methylated on each isotope of oxygen.

However, metastable ion mass spectroscopy of these derivatives have led to unambiguous assignment of the configuration of [^{16}O,^{17}O,^{18}O] phosphate group.

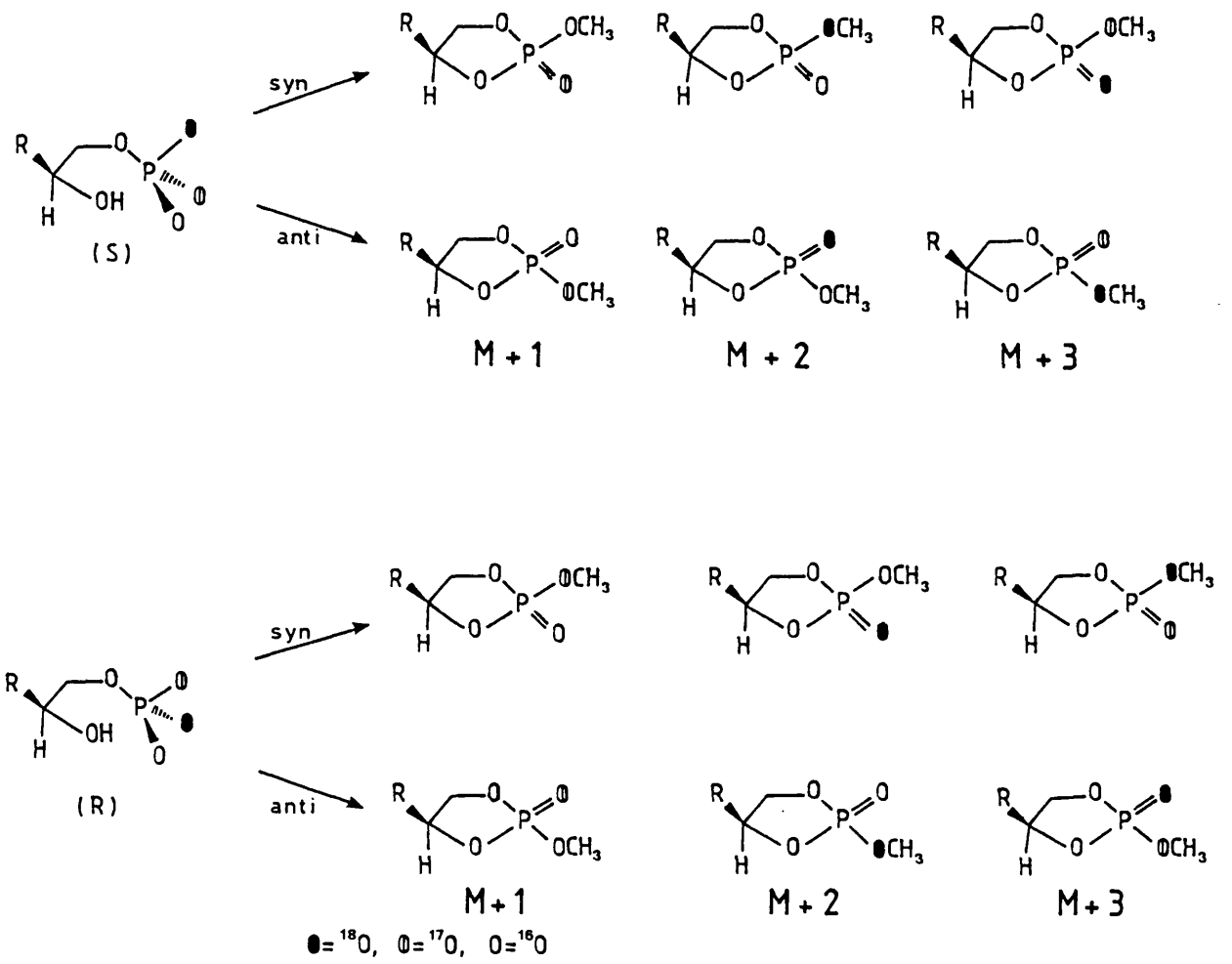


FIGURE 3.1 Mass spectral analysis of isotopically chiral [^{16}O,^{17}O,^{18}O] phosphate monoester by Knowles et al.

In brief, the technique allows the origin of the -OMe derived fragment to be determined by relating daughter and grandaughter peaks to the parent ions. For example, the -^{18}OMe derived fragment from the analysis of the syn Sp starting material would arise from the M+2 molecular ion whereas the corresponding ^{18}O-CH_3 derived from this analysis of the syn Rp isomer would originate from the M+3 molecular ion.

The analysis is technically quite difficult in that the five-ring cyclic triesters are hydrolytically labile and the method requires handling and separation of the syn and anti isomers. It is also restricted to systems that give suitable metastable peaks.

(3) ^{31}P nmr spectroscopy

^{31}P nmr spectroscopy has become the most important method for the configurational analysis of chiral [^{16}O,^{17}O,^{18}O] phosphates. The combined effects of ^{17}O and ^{18}O substitution on ^{31}P nmr are exploited in the stereochemical analysis of [^{16}O,^{17}O,^{18}O] phosphate monoester.

The configurational analysis was described by Lowe[102] and Knowles and Buchwald.[103] The analysis is conceptually related to the mass spectral method in that it requires the phosphate monoester to be cyclised onto an appropriate functionality to provide a conformationally locked system as shown in Figure 3.2.

The analysis of Knowles *et al.* requires the general [^{16}O,^{17}O,^{18}O] PO_3 unit to be attached to propane-1,2-diol (and latterly butane-1,3-diol). Analysis of a range of simple phosphate monoesters and pyrophosphates can be achieved using alkaline phosphatase to catalyse transphosphorylation reactions in which propanediol serves as the acceptor of a phosphate monoester. This has been shown to occur with retention of configuration.[100,67] The diastereomeric monoesters are converted into the corresponding phosphodiesters as with the mass spectroscopy method.

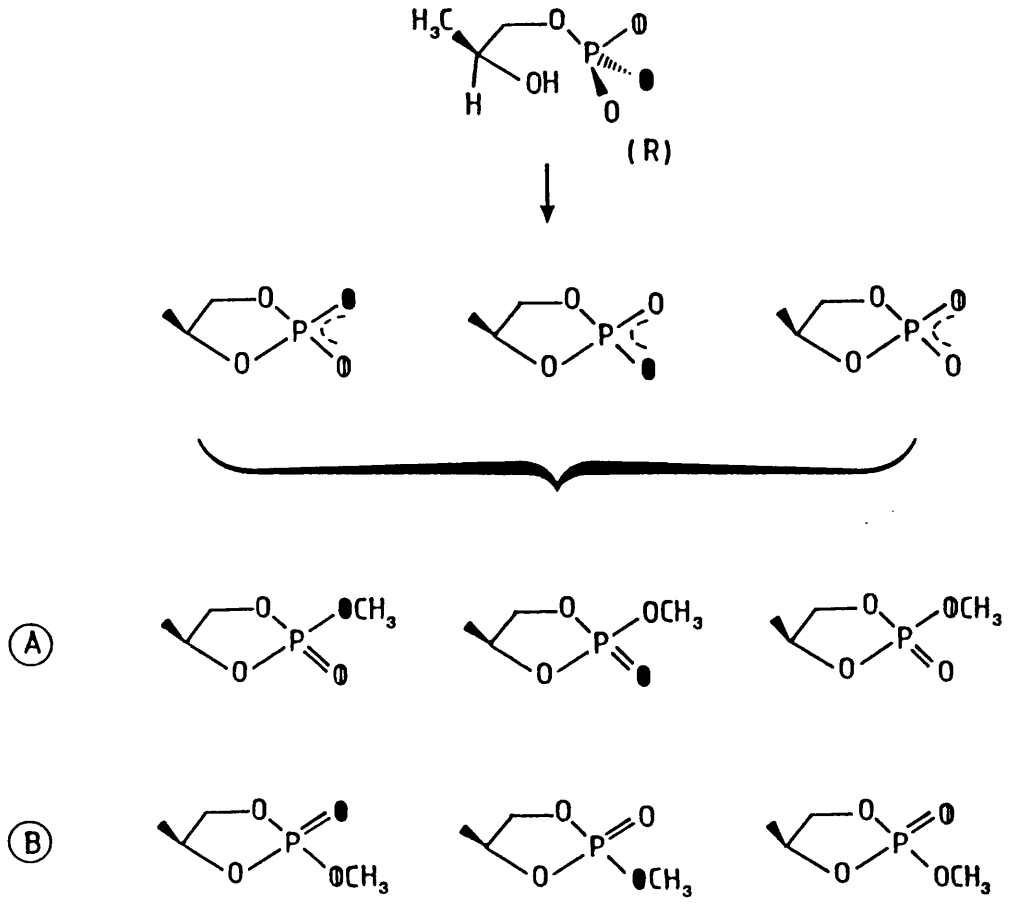


FIGURE 3.2 ^{31}P nmr configurational analysis of isotopical chiral [^{16}O,^{17}O,^{18}O] phosphate monoester.

As with the mass spectral method the phosphate esters are cyclised and methylated to give the syn and anti isotopomers of the cyclic phosphate diester. In contrast to the analysis based on metastable ion mass spectroscopy, the ^{31}P nmr spectral analysis does not require the separation of the syn and anti isomers since these are well separated in terms of the ^{31}P resonances.

The configurational analysis relies upon the observation that ^{18}O directly bonded to phosphorus exerts an upfield shift on the ^{31}P reson-

ance,[32,33,34] the magnitude of which depends on the bond order, a double bond shift being larger than a single bond. These shifts are also observed to be additive.[35,36,88] These ^{18}O isotope shifts in ^{31}P nmr are summarised in recent reviews.[104,105,106]

^{17}O has a nuclear spin of $5/2$ and therefore would be expected to couple to phosphorus. However, because of the permanent quadrupole moment in practise the ^{31}P nmr resonances of phosphorus centres are broadened to such an extent that they are undetectable.[35,107,108]

These two effects have been elaborated into a semiquantitative configurational analysis. The ^{31}P nmr spectra for the analysis of Sp and Rp [^{16}O,^{17}O,^{18}O] phosphate monoesters are shown in Figure 3.3. The 1st. and 5th. peaks are due to unlabelled product and the 4th. and 8th. peaks are due to doubly labelled material. The stereochemically informative peaks are the 2nd., 3rd. and the 6th., 7th. The absolute configuration is determined by the size of the intensity slope on these lines and the enantiomeric excess can be estimated from the relative intensities.[32,33,24] The independent report of Lowe et al. is conceptually similar, however glucose or adenosine are used in place of propane-1,2-diol as the chiral framework for the analysis.

Analyses of [^{16}O,^{18}O] thiophosphate esters

Hitherto, there have not been any general configurational analyses of [^{16}O,^{18}O] thiophosphate monoesters reported. However, two methods specific for nucleotides such as [^{16}O,^{18}O] adenosine-5'-thiophosphate have been developed as illustrated in Figure 3.4.

Frey[79] has shown that myokinase (≡ adenylate kinase) catalyses the phosphorylation of AMPS on oxygen to give the Sp isomer of ADPαS[78] which was established by snake venom phosphodiesterase digestion.[174] By the action of pyruvate kinase and phosphoenol pyruvate this is converted to the corresponding Sp ATPαS triphosphate. Applying this

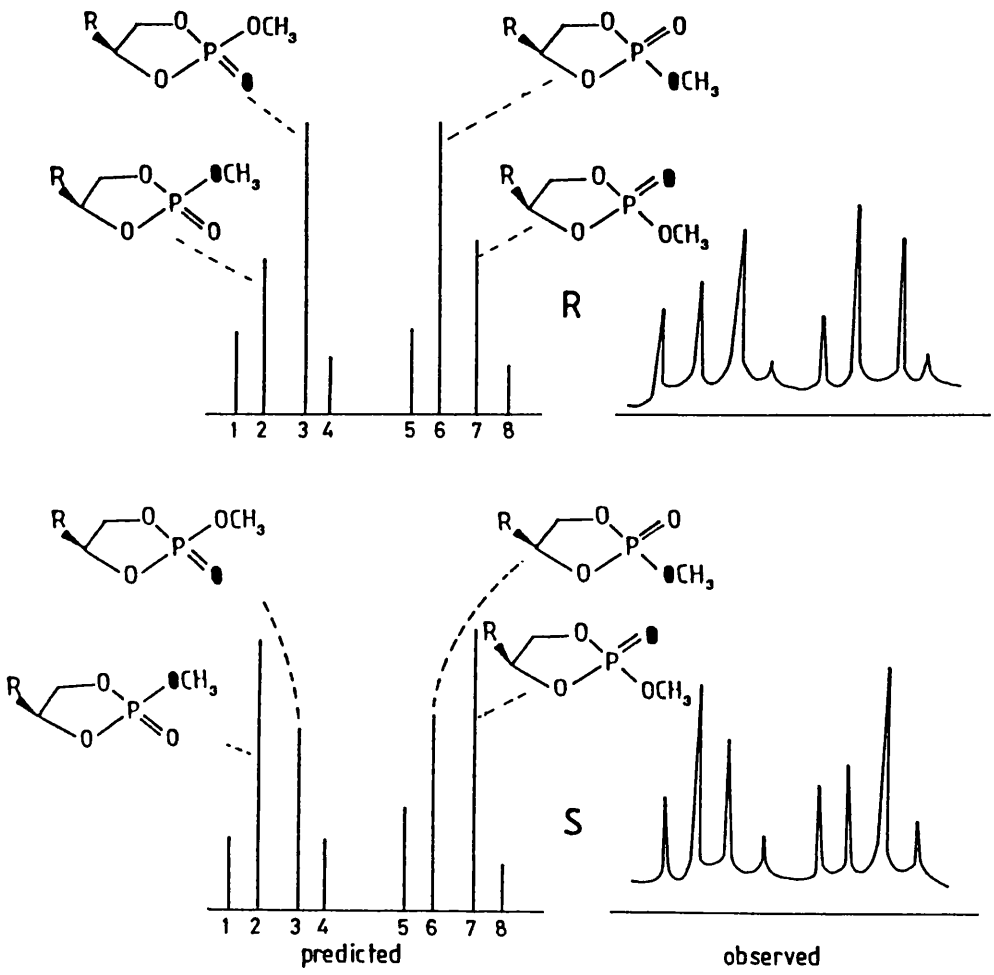
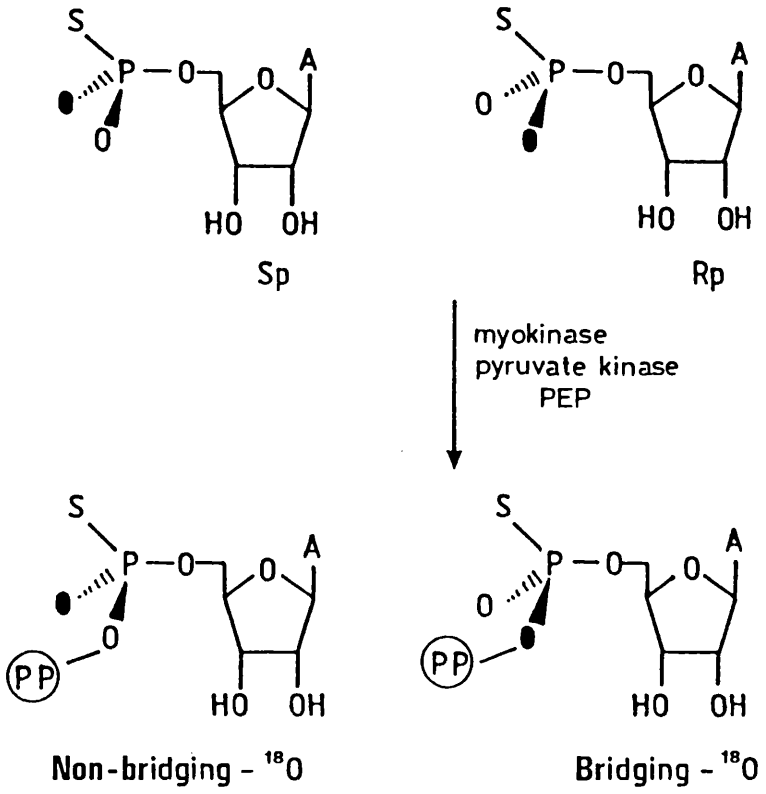


FIGURE 3.3 The predicted and observed ^{31}P nmr spectra of the mixture of methyl esters prepared from the diastereomers of [^{16}O,^{17}O,^{18}O]-phospho-(S)-1,2-propandiol (1:2:1 for ^{16}O,^{17}O,^{18}O).

sequence of reactions to Sp [^{16}O,^{18}O] AMPS leads to ^{18}O exclusively in the non-bridging position. These two can be distinguished by mass spectroscopy following degradation or directly on the Sp ATPαS by ^{31}P nmr spectroscopy.

More recently[175] the absolute configurations of O,S-dimethyl nucleoside thiophosphates have been assigned by relating them to the corresponding O-methyl nucleoside thiophosphate diester. The latter have been assigned on the basis of the known stereoselectivity of snake venom phosphodiesterase.[174]

1. Enzymatic Method



2. Chemical

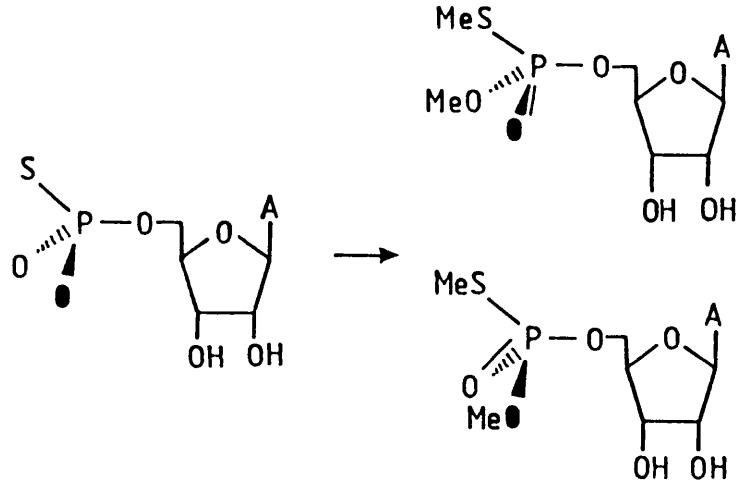
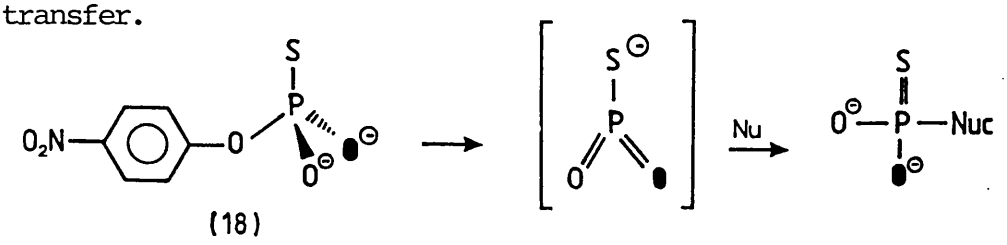


FIGURE 3.4 Enzymatic and chemical analysis of [^{16}O,^{18}O] adenosine-5'-thiophosphate.

The configurational analysis of [^{16}O,^{18}O] nucleoside thiophosphates can now be achieved by methylation to the O,S-triester level and determining the location of the ^{18}O in each of the diastereoisomers by ^{31}P nmr spectroscopy.

Development of a general configurational analysis

In order to study the stereochemical course of thiophosphoryl transfer reactions that may proceed by a monomeric thiometaphosphate intermediate, a stereochemical analysis needs to be able to determine the absolute configuration of both starting materials and product of thiophosphoryl transfer.



configurational analysis

configurational analysis

The existing methods used for the analysis of thiophosphate esters as described are not applicable to our study.

Conceptually, the analysis must distinguish between the pro R and pro S oxygens in a thiophosphate monoester. The analysis should ideally take account of the following:-

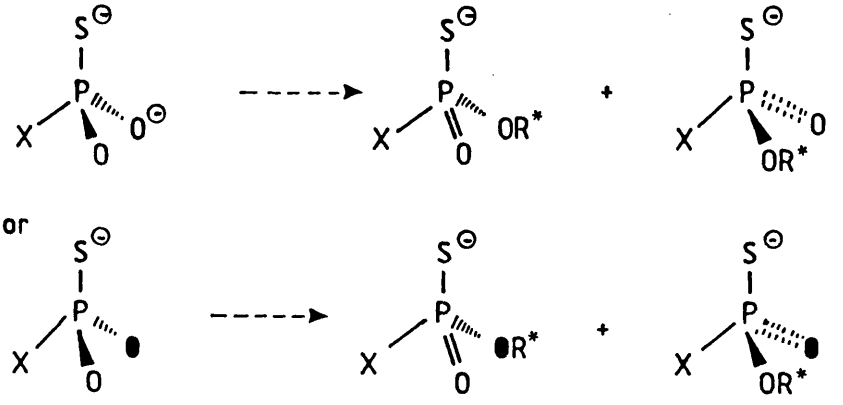
(i) It should be an efficient method;

(ii) It should not involve any displacement reactions at the isotopically chiral phosphorus since these would introduce some stereochemical ambiguity;

(iii) The diastereomeric derivatives should be stable and give sharp, well resolved ^{31}P nmr resonances;

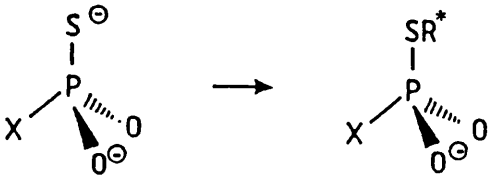
(iv) It should be applicable to both aryl and alkyl thiophosphate monoesters.

In order to distinguish between the pro R/S oxygens in an O-substituted thiophosphate ester, the simplest strategy would be to attach a chiral auxiliary to either of the terminal oxygens as shown.



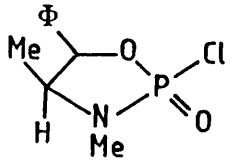
Determining whether the ^{18}O is in a bridging position or in a P=O of a given diastereoisomer establishes the absolute configuration of the original isotopically chiral thiophosphate monoester providing the absolute configuration of the diastereoisomers is known.

Unfortunately, chiral alkylating reagents would react at sulphur selectively which leaves the phosphorus centre prochiral in the conventional sense.



Even "hard" alkylating agents such as diazomethane and trimethyl oxonium tetrafluoroborate react preferentially at sulphur.[176]

During the course of work on the stereochemical course of phosphoryl transfer from P^1P^1-disubstituted pyrophosphates, Cullis and Rous showed that the 2-chloro-1,3,2-oxazaphospholidin-2-one (24) reacts with O-ethyl thiophosphate exclusively at oxygen to give principally two dia-



(24)

stereomeric pyrophosphates that were readily distinguished by ^{31}P nmr spectroscopy.

Figure 3.5 shows the proposed application of this reaction sequence to the analysis of Rp and Sp-O-ethyl [^{16}O,^{18}O] thiophosphate. No reaction on sulphur could be detected by ^{31}P nmr spectroscopy, however initial reaction on sulphur followed by rapid rearrangement onto oxygen could not be excluded. Pyrophosphates with sulphur in the bridging position rapidly isomerise to the P-O-P derivatives.[177]

The chloro compound (24) can be obtained as a mixture of diastereoisomers from the reaction of (-) ephedrine with phosphorus oxychloride. The major cis isomer can be obtained by either crystallisation or column chromatography.

Reactions of nucleophiles with (24) are expected to proceed stereospecifically with retention of configuration according to the work of Inch et al. Ethyl thiophosphate (21) was reacted with (24) under a variety of conditions. In pyridine the reaction proceeded smoothly to give the expected pyrophosphates. However, the product was shown to be a mixture of four diastereoisomers (25/26/27/28) that would arise if the displacement were non-stereospecific. Other experiments suggest that this proceeds by way of epimerisation of the chloro compound (24) prior to reaction with the thiophosphate rather than loss of stereospecificity in the displacement step [see Chapter 6]. Similar problems were experienced with other nucleophilic catalysts such as N-methylimidazole and N,N-dimethylaniline.

Ethyl thiophosphate reacted with (24) under anhydrous conditions in the presence of tertiary amines. The best conditions appeared to be reaction of the bis(t-butylammonium) salt of ethyl thiophosphate with (24) in dry dioxan in the presence of excess tributylamine. The product was

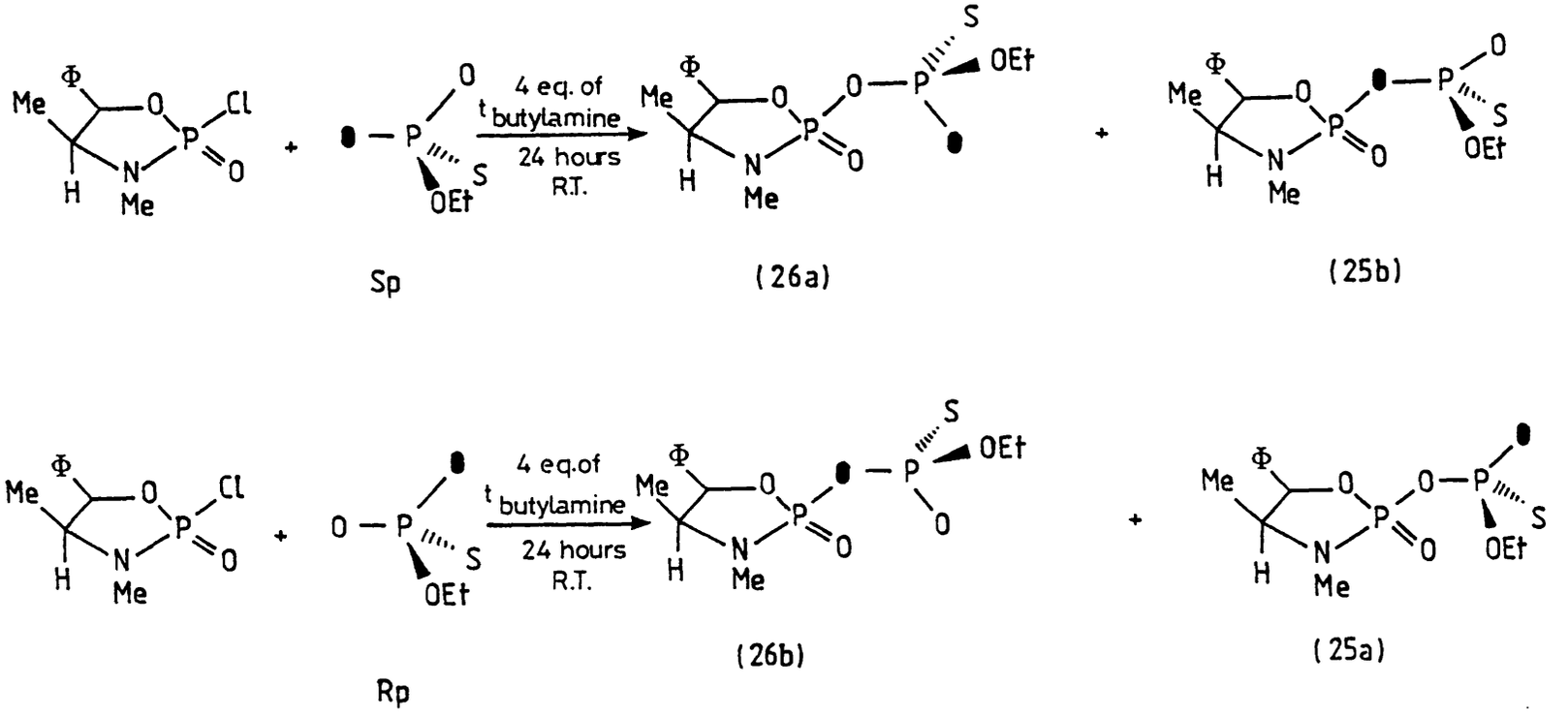
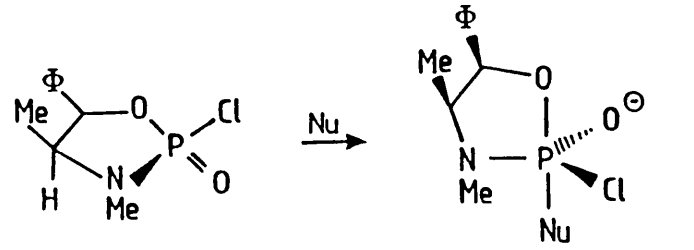


FIGURE 3.5 Proposed stereochemical analysis of Rp and Sp-O-ethyl [^{16}O,^{18}O] thiophosphate.

shown to be principally two diastereoisomers (25/26). Based on previous precedent, the major diastereoisomers are expected to have the cis geometry. This arises because of the pronounced preference for a five-membered ring to span apical-equatorial positions in the intermediate pentacoordinate trigonal bipyramid. The alternative pentacoordinate



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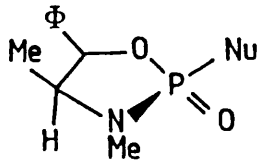
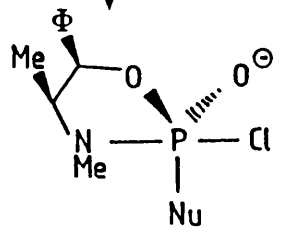
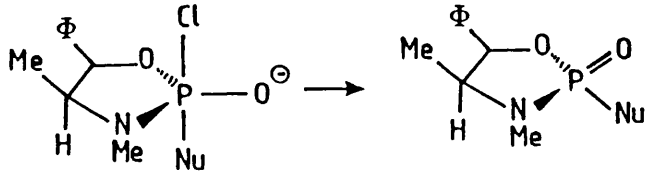


cis product



(31)

trigonal bipyramid (32) would be of ca. 20 kcal mol^{-1} higher energy.



(32)

trans product

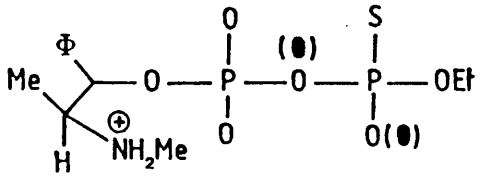
Two significant problems were encountered during this reaction. If care was not taken to render the solution totally anhydrous, a competing reaction became dominant. The product had the following characteristics:-

(i) ^{31}P nmr spectrum showed doublets at +47.00 and -13.00 ppm indicating a pyrophosphate.

(ii) The ^{31}P nmr shift at -13.00 ppm suggests that the oxazaphospholidine ring has been lost and that the P-N bond has been cleaved.

(iii) This product has a faster elution time than the desired product ethyl thiophosphate, it is collected between fractions 6-10.

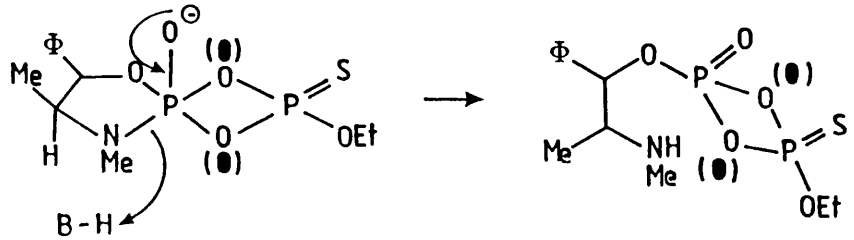
These observations would be consistent with the structure shown below.



(33)

It is somewhat surprising that this competing reaction becomes significant in view of the demonstrated stability of the oxazaphospholidine system (24) to isolation by chromatography.
Presumably in non-polar solvent the base hydrochloride ($Bu_3NH^+Cl^-$) is a sufficiently strong acid to promote P-N bond cleavage.

Although the ring-opened product is still a mixture of diastereoisomers, it was not of use in the chiral analysis because the chiral centres are now in a 1,4 relationship and the ^{31}P nmr shifts may not be sufficiently well resolved. It would also be necessary to establish the precise mechanism for the ring-opening reaction. For example, the neighbouring thiophosphoryl group could participate in this reaction.



Since this could lead to a displacement reaction at the thiophosphoryl centre which would have stereochemical consequences.

Recent work (M. Schilling, unpublished observations) have shown that care in rendering the reactants anhydrous and inclusion of molecular sieves (type 4A) in the reaction can almost completely eliminate this ring-opening reaction.

Occasionally reaction in dioxan gave none of the expected product but the ethyl thiophosphate ^{31}P nmr resonance (+42.58 ppm) moved significantly upfield to (+11.49 ppm) as shown in Figure 3.6. Although thiophosphates

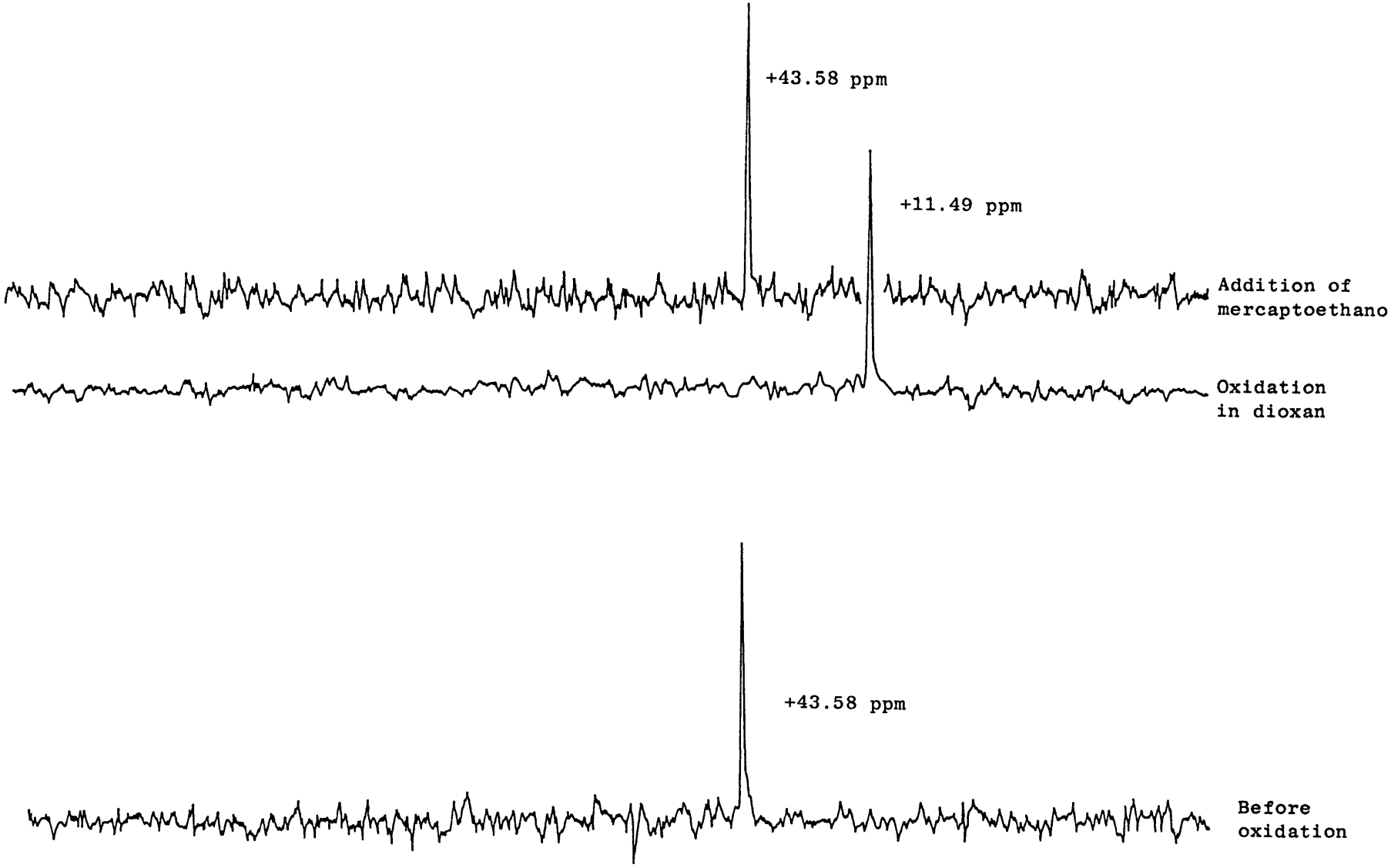
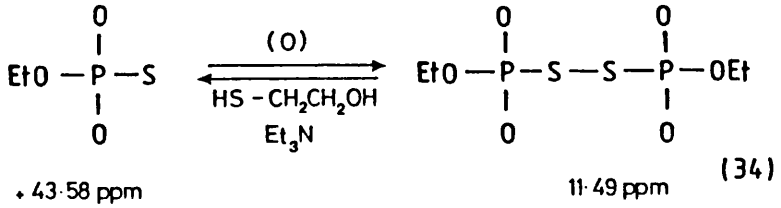


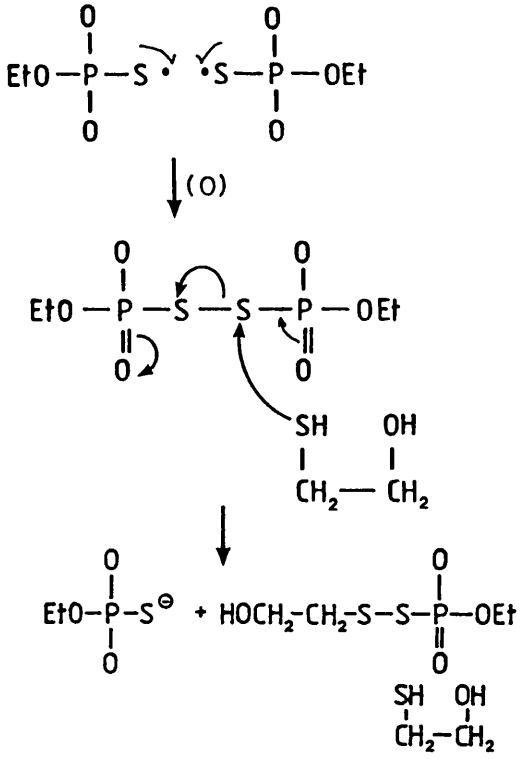
FIGURE 3.6 Spectra of ethyl thiophosphate in dioxan.

show considerable variations in ^{31}P nmr resonance with solvent and pH, this shift was beyond the range of such variations. An upfield shift is consistent with conversion from P=S to P-S-X. It was suspected that the reaction was, in fact, an oxidation to the disulphide (34). If the



(34)

species with ^{31}P nmr shift of +11.49 ppm is indeed the disulphide (34), it should be possible to reduce this back to ethyl thiophosphate. Addition off excess mercaptoethanol gave ethyl thiophosphate as indicated by a ^{31}P nmr shift of +42.58 ppm. The oxidant in the formation of the disulphide appears to be peroxides formed in the dioxan on exposure to air[178] as shown.



The presence of peroxides was confirmed using starch[179]/KI. With freshly distilled dioxan this problem did not arise.

Oxidation of thiophosphates to the corresponding disulphides has previously been reported using various oxidising agents but, in particular, hydrogen peroxide.[177]

Configurational analysis of Sp-O-ethyl-[^{16}O,^{18}O]-thiophosphate by high-field ^{31}P nmr spectroscopy

(Sp)-O-ethyl-[^{16}O,^{18}O]-thiophosphate (21) was synthesised as described in the previous chapter. The assignment of the absolute configuration is based on good precedent for the stereochemical courses of each of the key reactions as previously discussed.

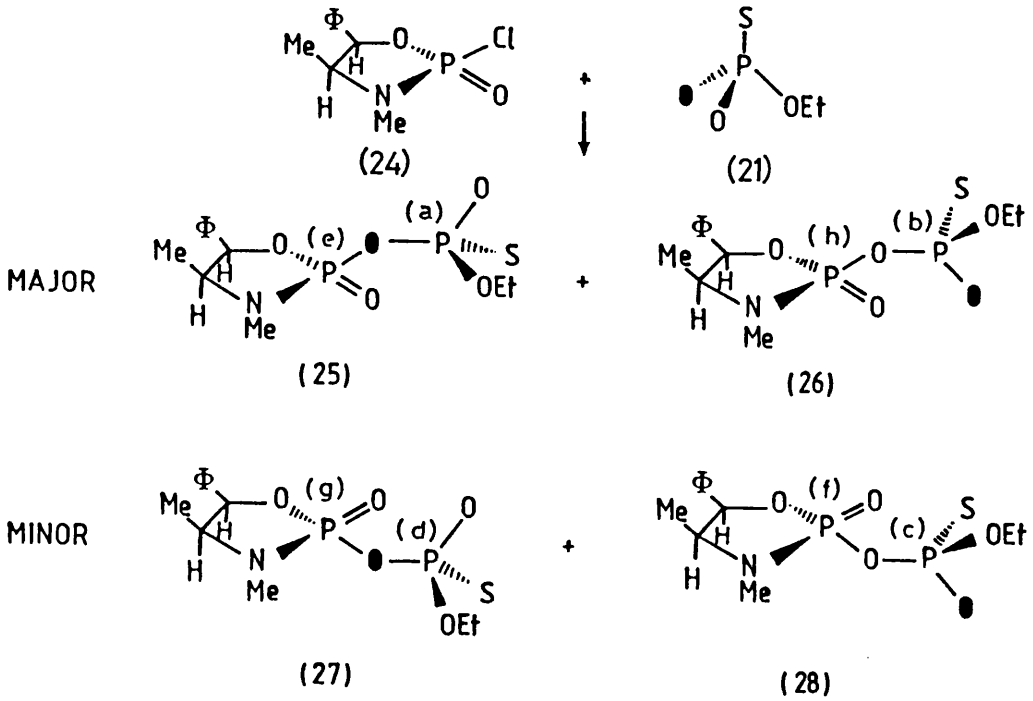
Reaction of Sp-O-ethyl-[^{16}O,^{18}O]-thiophosphate (21) (ca. 33% ^{18}O at the enriched site) with the chlorocompound (24) gave a mixture of diastereomeric pyrophosphates which were isolated and purified by ion-exchange chromatography. The position of the ^{18}O in each of these diastereoisomers was established by high-field ^{31}P nmr spectroscopy. As previously discussed ^{18}O gives rise to an upfield shift on the ^{31}P nmr resonance of the phosphorus to which it is directly attached. The magnitude of shift is bond-order dependent, P = ^{18}O shift being larger than P-^{18}O-R, and these shifts are approximately additive.

The high-field ^{31}P nmr is shown in Figure 3.7 and the resonances can be assigned to the expected products (25) and (26) as follows:-

(i) The resonances in the region of +7 ppm correspond to the oxazaphospholidine phosphorus and those at approximately +45 ppm are from the thiophosphoryl position.

(ii) Diastereoisomer (25) will show an ^{18}O shift on both phosphorus centres because the ^{18}O is located in the bridge whereas diastereoisomer (26) will exhibit an ^{18}O shift on the thiophosphoryl position only.

(iii) Diastereoisomer (25) will experience a smaller shift on the thiophosphoryl centre than the diastereoisomer (26).



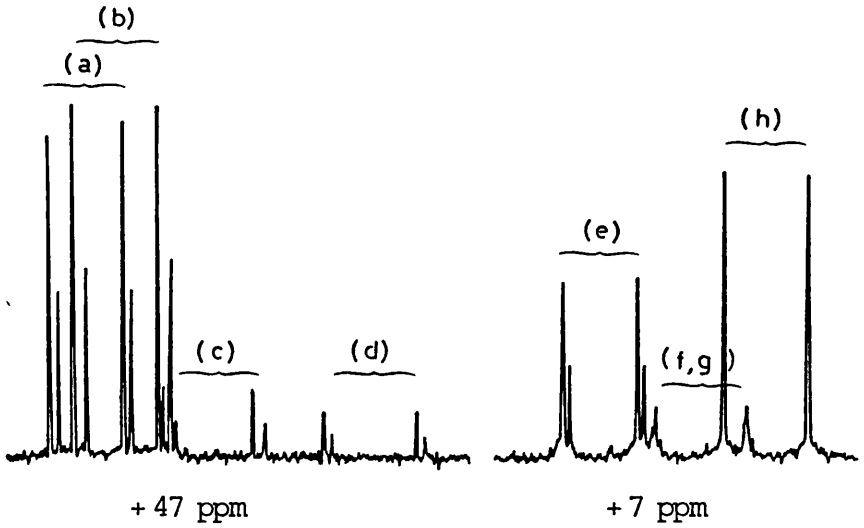


FIGURE 3.7 High-field ^{31}P nmr: assignment of the absolute configurations of the diastereomeric pyrophosphates from Sp-O-ethyl-[^{16}O,^{18}O]-thiophosphate. [33% $H_2{}^{18}O$]

These observations allow the assignments shown to be made. Diastereoisomer (25) can be assigned to resonances (a) and (e) while diastereoisomer (26) can be assigned to resonances (b) and (h). The fact that there is no ^{18}O shift on resonance (h) and that resonances (a) and (b) show only one ^{18}O line indicates that ethyl [^{16}O,^{18}O] thiophosphate was, indeed, a single enantiomer (ca. >90%).

The minor additional resonances are assigned to the diastereoisomers that are epimeric at the ring phosphoryl position (27/28). Based on the magnitude of the ^{18}O shift on the thiophosphoryl phosphorus, the assignments shown can be made. The oxazaphospholidine phosphorus resonances (f) and (g) are not resolved.

The ^{31}P nmr data from the spectrum are shown in the table below.

Phosphorus Centre	Chemical shift (ppm)	Hz	^{18}O shift
1,3,2-oxazaphospholidine-2-one	+7.14	d, J_{pp}, 25.9Hz	2.28Hz
Rp thiophosphoryl centre	+46.39	d, J_{pp}, 25.9Hz	2.84Hz
1,3,2-oxazaphospholidine-2-one	+6.65	d, J_{pp}, 29.7Hz	
Sp thiophosphoryl centre	+46.29	d, J_{pp}, 29.7Hz	4.46Hz

Configurational analysis of Rp-O-p-Nitrophenyl [^{16}O,^{18}O] thiophosphate by high-field ^{31}P nmr spectroscopy

The same procedure was applied to Rp-O-p-nitrophenyl [^{16}O,^{18}O] (18) thiophosphate as shown in Scheme 3.8. Comparable reaction conditions were required, however, because p-nitrophenyl thiophosphate proved to be a significantly poorer nucleophile longer reaction times were required.

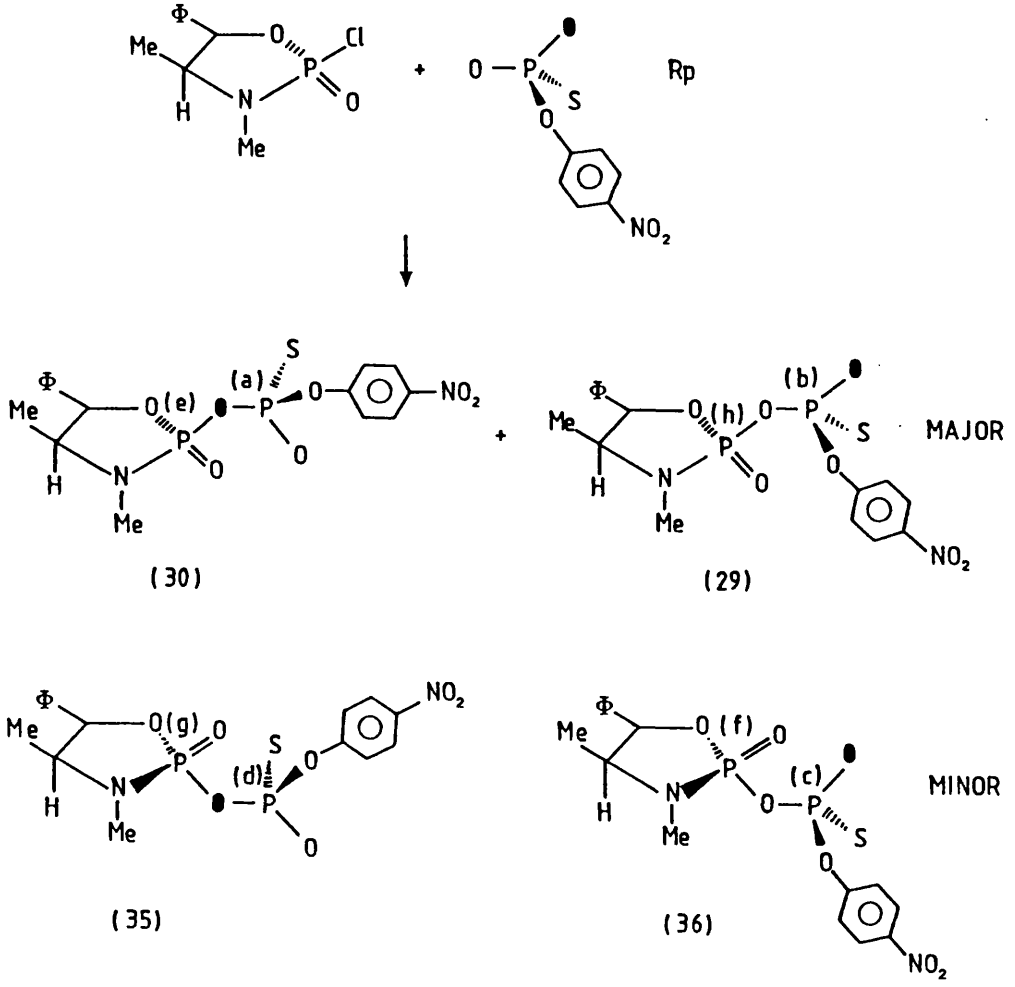


FIGURE 3.8 Proposed analysis of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate.

Attempts to isolate and purify the product by ion-exchange chromatography on DEAE Sephadex in aqueous triethylammonium bicarbonate buffer lead to complete hydrolysis to p-nitrophenyl thiophosphate and 2-hydroxy-3,4-(S)-dimethyl-5(S)-phenyl-1,3,2-oxazaphospholidin-2-one (19). The second pKa of p-nitrophenyl thiophosphate is ca. 3.6 which presumably

makes it a very good leaving group.

The configurational analysis could be achieved by high-field ^{31}P nmr of the crude reaction mixture (29/30/35/36). The assignments could be made analogously to the Sp ethyl [^{16}O,^{18}O] thiophosphate that was discussed in detail above. The resonances at the thiophosphoryl region are not well resolved. The spectrum details are given in Chapter 4.

For the diastereomeric pyrophosphates formed, both ethyl thiophosphate and p-nitrophenyl thiophosphate, the diastereoisomer with the Rp configuration at the thiophosphoryl centre is downfield and that with the Sp configuration is upfield. This may hold for a range of alkyl and aryl functions, however this has yet to be established.

CONCLUSIONS

The use of 2-chloro-3,4-(S)-dimethyl-5(S)-phenyl-1,3,2-oxazaphospholidin-2-one (24) as a chiral auxiliary for the analysis of the absolute configurations of O-substituted [^{16}O,^{18}O] thiophosphate *via* formation of diastereomeric pyrophosphates has been established. This represents the first simple chemical configurational analysis of such species, and allows the study of simple thiophosphoryl transfer reactions.

CHAPTER 4

Thiophosphoryl Transfer Reactions: The Stereochemical Course of Solvolysis p-Nitrophenyl Rp [^{16}O,^{18}O] Thiophosphate

INTRODUCTION

In order to study the stereochemical course of thiophosphoryl transfer reactions that may proceed by thiometaphosphate, it is necessary to propose mechanisms by which this transfer occurs.

Ever since monomeric metaphosphate was first postulated as an intermediate of the many hydrolysis reactions of phosphoric esters, much evidence has accumulated in support of its existence. This evidence will be briefly reviewed.

Evidence for the existence of monomeric metaphosphate in:-

(a) protic solvents

It has been found that for phosphate monoesters that have a leaving group of pKa >5.5 the monoanion is the most reactive species which is thought to react by a pre-equilibrium proton transfer followed by heterolysis to give metaphosphate and the neutral phenol, as shown in Figure 4.1.

Dianions react faster if the leaving group has a pKa less than 5.5. This is also shown in Figure 4.1. This is rationalised in terms of a dissociative reaction since it is hard to imagine how the introduction of a second negative charge on the phosphate ester would accelerate an associative reaction.

Also Kirby[109] and Jencks[110] have found the $\Delta S^{\ddagger}$ (entropy) around 0 entropy units for phosphate monoesters. This result is more consistent with a unimolecular reaction. A typical bimolecular reaction shows $\Delta S^{\ddagger}$ -20 entropy units.

The linear free energy relationship for the hydrolysis of phosphate monoester monoanions shows a β value for the leaving group of -0.3,[109,111] consistent with the departure of a neutral leaving group. For the dianion the β^{lg} is considerably larger at -1.2 indicating that the reaction is

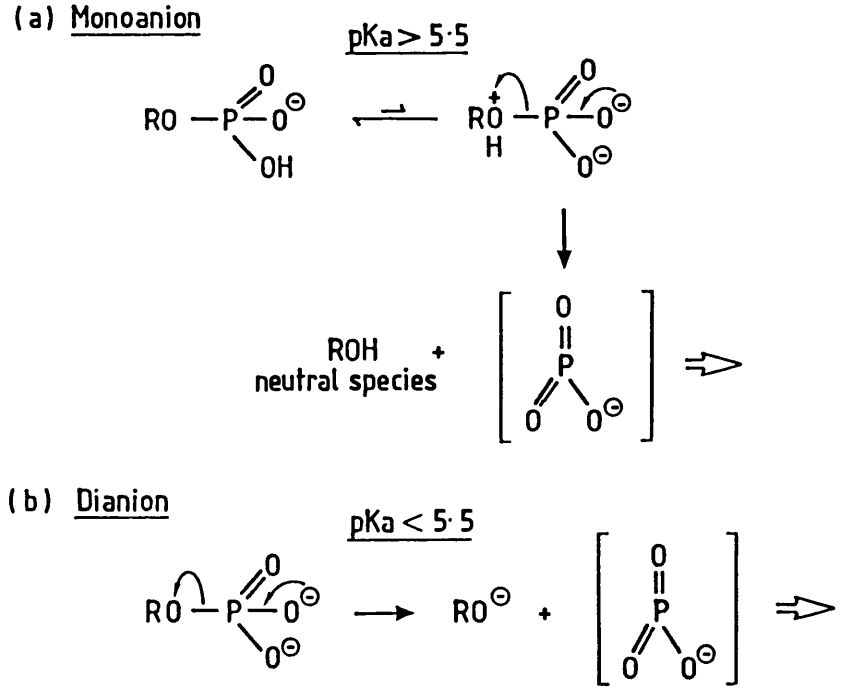


FIGURE 4.1 Pathway for the solvolysis of:-
(a) monoester monoanion of a leaving group of pKa >5.5;
(b) monoester dianion of a leaving group of pKa <5.5.

sensitive to the nature of the leaving group.

For the reaction of aryl phosphate monoesters with amines the linear free energy relationship yields a β value for the nucleophile of ca. 0-0.1 demonstrating an insensitivity to the nature of the nucleophile suggesting little bond formation at the transition state.

The report of significant kinetic ^{18}O isotope effect in the hydrolysis of p-nitrophenyl phosphate is indicative of a large degree of P-O bond breakage in the transition state.[112,115] This is consistent with an S_N1 mechanism but would also be observed for a loose exploded S_N2 transition state.

A reactive electrophilic phosphorylating agent has been inferred from the correlation of the ratio of alkyl phosphate to inorganic phosphate with the molar ratio of alcohol and water in solvolysis reactions of

phosphate monoesters in mixed solvents.[113,115]

Also solvent deuterium isotope effects were found to be small for monoanions and negligible for dianions, these results are consistent with a dissociative mechanism.[113,114]

All the evidence cited above is consistent with a dissociative mechanism involving a monomeric metaphosphate. The above evidence relates to studies carried out in protic solvents. The existence of monomeric metaphosphate in aprotic solvents is arguably more substantial.

(b) aprotic solvents

Monomeric metaphosphate was detected in the "three-phase test" as devised by Rebek.[116-119] This is illustrated in Figure 4.2.

In the three-phase test an insoluble polymer P_1, carrying acyl phosphate as a functional group, and a polymer P_2 with a primary amine as functional

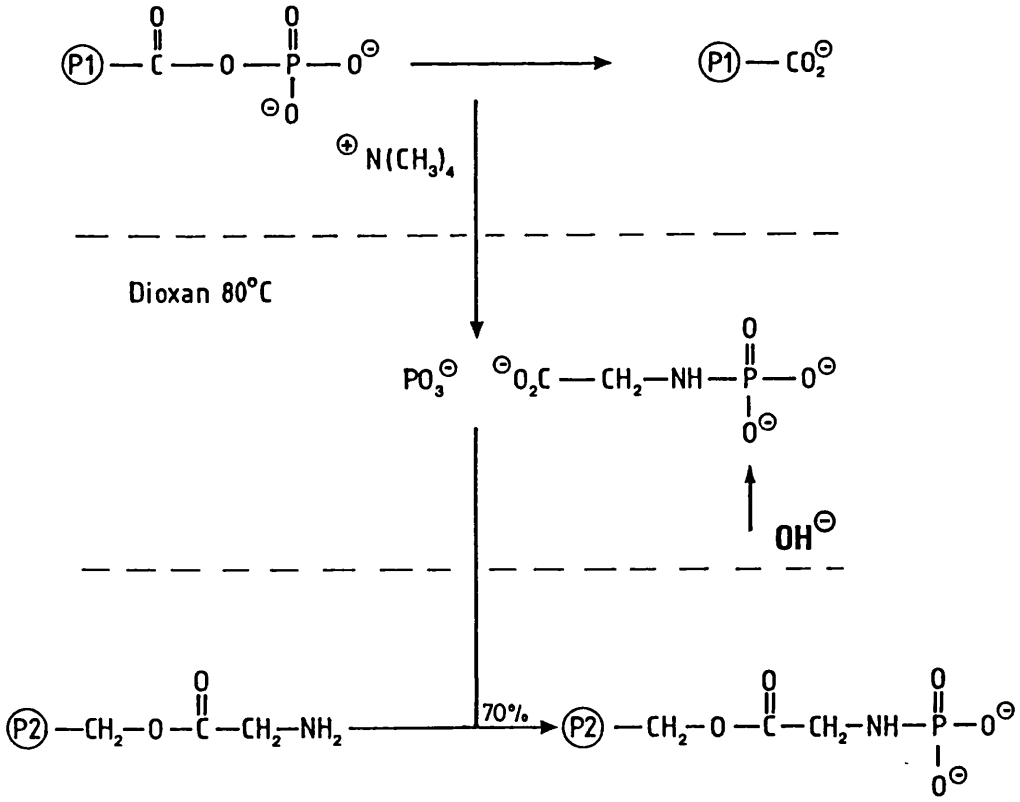
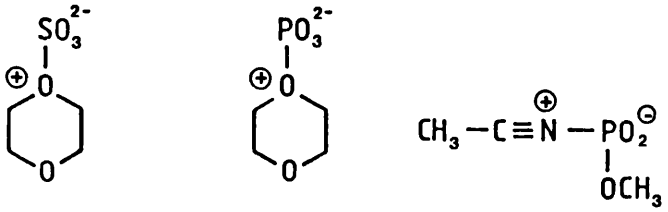


FIGURE 4.2 The three-phase test by Rebek et al.

group are suspended in a solvent. Direct reaction between the functional groups bound to the polymers is not possible such that any detectable phosphoryl transfer to the amine is due to a diffusing reactive intermediate. It is not known whether the intermediate is a monomeric PO_3^- ion or PO_3^- solvent complex.

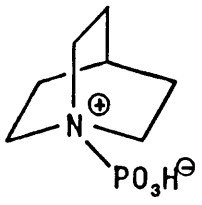
Monomeric metaphosphate ion is isoelectronic with SO_3 and would be expected to add to an oxygen atom of dioxan or to the nitrogen atom of acetonitrile as with SO_3. The solvent complex adducts expected would be as shown.



There is evidence in favour of such solvent complexes as follows.

Satterthwait[120] has investigated the aromatic substitution of N-methylaniline by monomeric methyl metaphosphate in a range of solvents. He has found that the yield of aromatic substitution is reduced significantly when acetonitrile or dioxan are used. This suggests that the monomeric metaphosphate is coordinated to the heteroatom in these solvents to produce a less active phosphorylating agent.

Ramirez and Maracek[121] have observed the ^{31}P nmr spectrum of the adduct of monomeric metaphosphate and quinuclidine. The results showed that an unstable intermediate was formed consistent with a zwitterionic species as shown below.



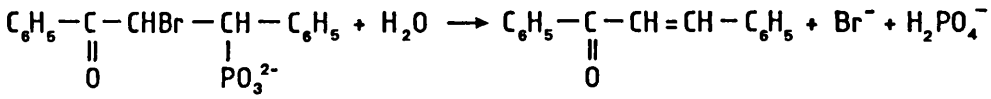
These results suggest that in the presence of dioxan, the adduct rather than free metaphosphate is the phosphorylating agent.

However, whether phosphorylation of the polymer bound amine in the presence of dioxan or similar solvents occurs by way of a rapid reaction with a small amount of monomeric metaphosphate that is free in solution or <u>via</u> a slower reaction with the zwitterionic product is not yet known.

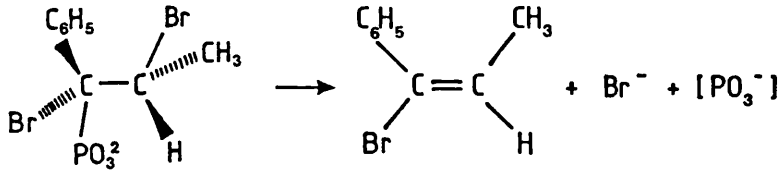
Further evidence for monomeric metaphosphate in aprotic solvents comes from the Conant-Swan fragmentation reactions, as extensively studied by Maynard, Kenyon and coworkers.

<u>Conant-Swan Fragmentation</u>

In the 1920's Conant and coworkers[122-125] synthesised several β-halophosphonates and phosphinates and found that their anions decomposed in aqueous solutions. Kenyon[126] much later examined the stereochemistry of

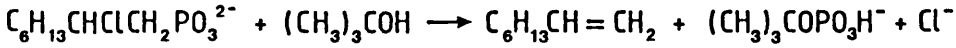


the Conant-Swan fragmentation. Fragmentation of both the threo and erythro isomers of 1,2-dibromo-1-phenyl propane-phosphonic acid was carried out in water or acetonitrile as solvent.[126] The fragmentation shown below is of trans stereochemistry.



The phosphorus containing intermediate formed from this reaction reacts with acetophenone to yield enol phosphate which has been taken as support for monomeric metaphosphate.

In 1963, Maynard and Swan[127,128] found that when β-halophosphonates, e.g. 2-chlorooctyl phosphonate is fragmented as shown below in the presence of tertiary-butyl alcohol, it yields tertiary-butyl phosphate.



It is assumed that tertiary alcohols have reduced nucleophilicity due

to steric constraints and that the production of $^{t}BuOPO_3^{2-}$ indicates an extremely reactive electrophile.

Knowles has recently carried out stereochemical studies to seek direct evidence for the participation of monomeric metaphosphate. If metaphosphate is a discrete intermediate, we would expect it to be a planar intermediate; highly reactive and indiscriminate towards nucleophiles. Attack by nucleophiles on a freely solvated intermediate should lead to complete racemisation.

Stereochemical investigation of monomeric metaphosphate in protic solvents

Knowles et al. investigated the role of monomeric metaphosphate and the nature of the transition states in the alcoholysis of phosphoric monoesters.[30] He carried out studies on the following:-

[1] The monoanion of phenyl phosphate which has a leaving group of pKa >5.5.

[2] The dianion of dinitrophenyl phosphate which has a leaving group of pKa <5.5.

Phenyl (R[^{16}O, ^{17}O, ^{18}O]) phosphate was reacted with a 1:1 mixture of water and methanol at pH 4 where the monoanion predominates while the dianion of 2,4-dinitrophenyl phosphate (R[^{16}O, ^{17}O, ^{18}O]) was reacted with a mixture of water and methanol at pH 10.2. The products were separated by ion-exchange chromatography and subject to the analysis developed for phosphate monoesters.

High field ^{31}P nmr configurational analysis showed that the phosphoryl transfer in both cases proceeded with inversion of configuration (i.e. ~87% ±(S) configuration).

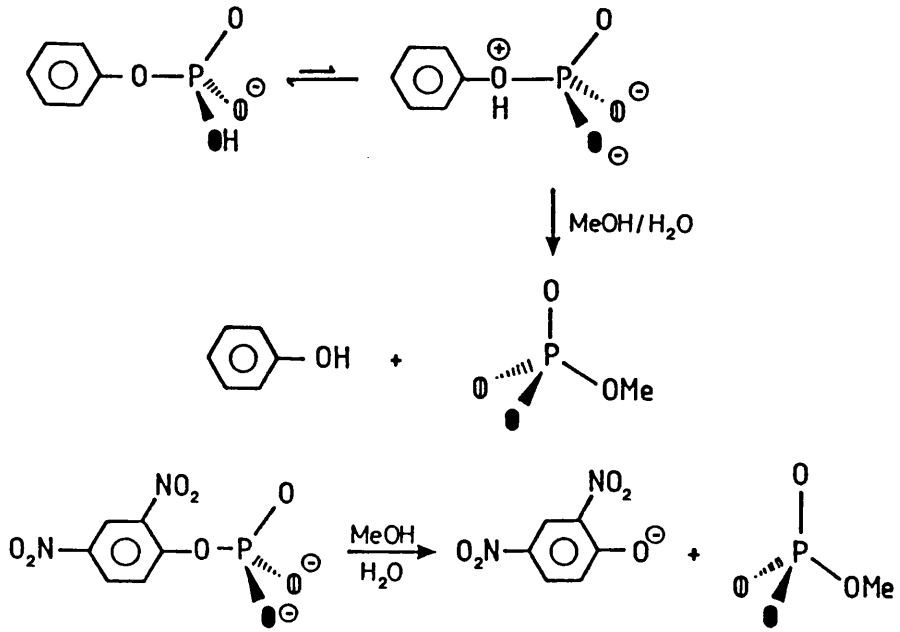
This stereochemical evidence seems to conflict the earlier kinetic data and is inconsistent with a mechanism involving a free liberated

symmetrically solvated monomeric metaphosphate intermediate. The stereochemical course is more consistent with an S_N2-like transition state. These apparent conflicts can be reconciled in terms of a preassociative reaction mechanism as defined by Jencks.

Jencks[129] has considered reaction mechanisms in the borderline region between S_N1 and S_N2. A reaction is preassociative if either the reaction intermediate does not exist or if it is so unstable that it collapses back to starting materials or on to products faster than a nucleophilic acceptor can diffuse away from the complex. Furthermore, there are two extremes of preassociative mechanisms, the first is a concerted preassociative mechanism in which the postulated monomeric metaphosphate has no significant lifetime whatsoever (i.e. like a S_N2 transition state). The second is a stepwise preassociative reaction. This involves bond breaking before nucleophilic attack. In this mechanism the putative monomeric metaphosphate has a significant lifetime (but less than a molecular vibration of 10^{-13} s). A species with a significant lifetime longer than this is defined as an intermediate as long as it has barriers for its breakdown to both reactants and products.

In both mechanisms bond breaking dominates the rate limiting process and inversion is the stereochemical outcome. Although it is difficult to distinguish between the two pathways, it would appear likely that a preassociative concerted mechanism occurs in protic solvent and that the monomeric metaphosphate will not have a significant lifetime. This preassociative mechanism is shown in Figure 4.3.

This mechanism accommodates not only the stereochemical data but also the previous kinetic data. Further stereochemical evidence came from the study of the phosphoryl transfer reactions of N-phosphoguanidine.[130] This species is among the most rapidly solvolysed phosphorylated deriv-



J.R.Knowles et al J.A.C.S. 1984, 106, 4911

Stereochemistry : INVERSION

Preassociation Mechanism

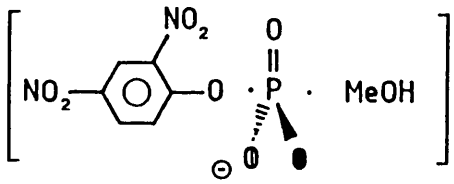
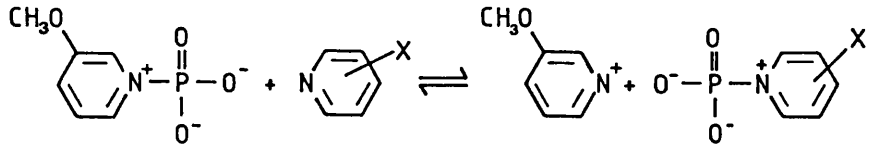


FIGURE 4.3 Stereochemical studies on [^{16}O, ^{17}O, ^{18}O] phosphate esters by Knowles et al.

ates[131] and the most reactive precursor of "metaphosphate".[132] Of all the phospho groups donor species the product composition correlation in solvolysis reactions in aqueous alcohols is the most convincing for N-phosphoguanidines. The stereochemical outcome of the phosphoryl transfer from N-phosphoguanidine to methanol was that of inversion of configuration which suggests that if a metaphosphate intermediate is formed it is captured before any rotation about a P-O bond can occur, i.e. the metaphosphate is certainly not a liberated intermediate, nor is it even long-

lived enough to tumble within the cage in which it was generated.

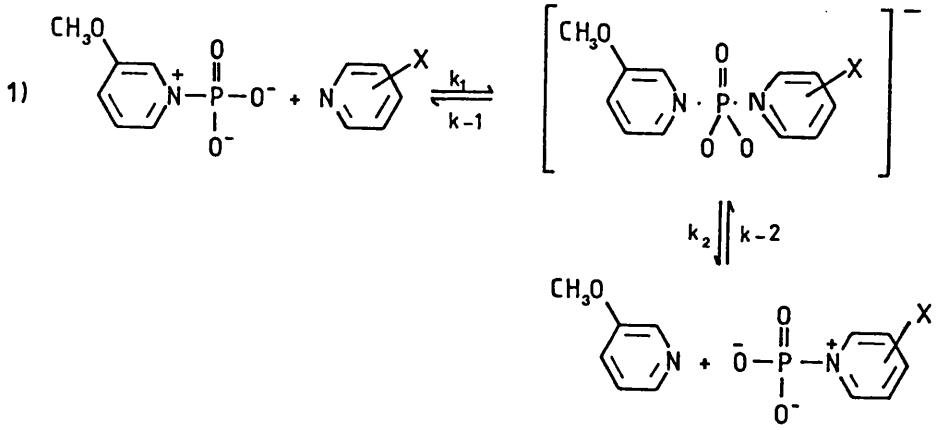
Two groups have investigated the transfer of a phospho group between N-phosphorylated pyridines. Jencks et al.[133] have studied the phosphoryl transfer from 3-methoxypyridine to pyridines of varying basicity and Williams et al.[134] have studied the phosphoryl group transfer from isoquinoline-N-phosphonate to pyridine. These studies helped to distinguish between a concerted preassociation mechanism and a stepwise preassociation mechanism shown in Figure 4.4.

1) 

2) $^{+}isq - PO_3^{-} + xpyr \rightleftharpoons [^{+}isq - PO_3^{-} \cdot xpyr] \longrightarrow$

$$[isq \ldots PO_3 \ldots xpyr]^{\ddagger} \longrightarrow [isq \cdot^{+} xpyr - PO_3^{-}] \rightleftharpoons isq + {}^{+}xpyr - PO_3^{-}$$

concerted preassociation mechanism for:-
(1) 3-methoxypyridine
(2) isoquinoline-N-phosphonate

1) 

2) $isq - PO_3^{-} + xpyr \rightleftharpoons [^{+}isq - PO_3^{-} \cdot xpyr] \rightleftharpoons [isq \cdot xpyr \cdot PO_3] \rightleftharpoons$

$$[isq \cdot^{+} xpyr - PO_3^{-}] \rightleftharpoons isq + {}^{+}xpyr - PO_3^{-}$$

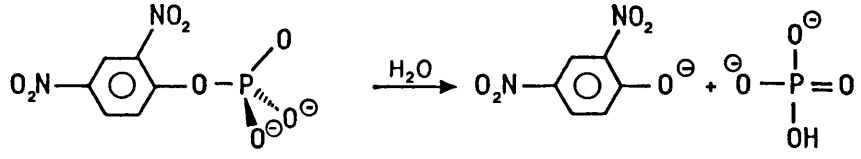
stepwise preassociation mechanism for:-
(1) 3-methoxypyridine
(2) isoquinoline-N-phosphonate

FIGURE 4.4 Concerted and stepwise preassociation mechanisms.

In both cases a linear free energy relationship between the rate of the phosphoryl transfer against the pKa of the attacking pyridine was observed. The absence of any "break" in the Brønsted plots is indicative of a fully concerted reaction. There is no evidence for any change in the rate-limiting step as the reactivity of the nucleophile is varied, as would be expected for a preassociative stepwise reaction.

These results are consistent with a concerted reaction mechanism in which a single, symmetrical transition state involves weak bonding to both the entering and leaving groups.

Recently le Noble *et al.*[135] have investigated the rate of hydrolysis of 2,4-dinitrophenyl phosphate dianion as a function of pressure. These studies have produced conflicting evidence as to the existence of monomeric metaphosphate. The liberation of 2,4-dinitrophenoxide from the phosphate dianion was found to be accelerated by pressure with $\Delta V^{\ddagger}$ = -4.8 cm^3/mol (see Figure 4.5).



$\Delta V^{\ddagger} = -4.8\ cm^3/mol$

Typical S_N2 displacement reactions in water lead to activation volumes of -5 to -10 cm^3/mol. This result is, therefore, inconsistent with a totally free metaphosphate ion.

An examination of the temperature dependence of the rate of hydrolysis of the dianion, as shown in Figure 4.5(b), produces a linear plot over a range of temperatures. This implies that an associative reaction is unlikely.

These results show that there is no definite proof that monomeric metaphosphate exists in protic solvents.

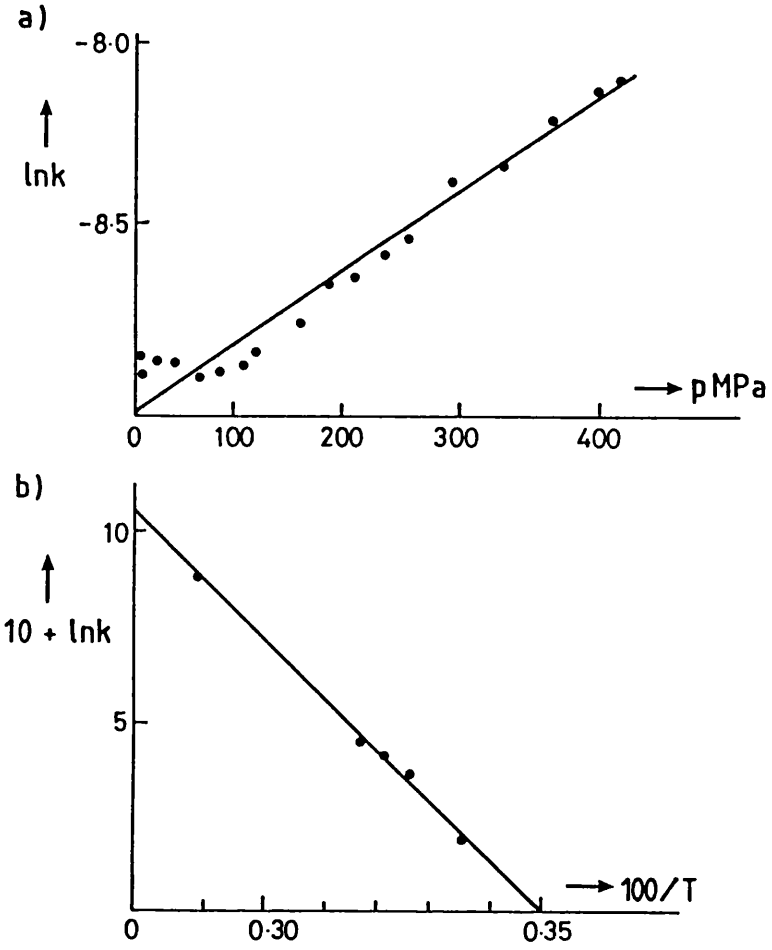
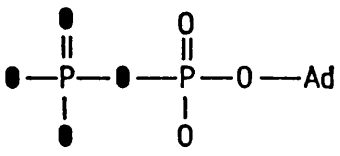


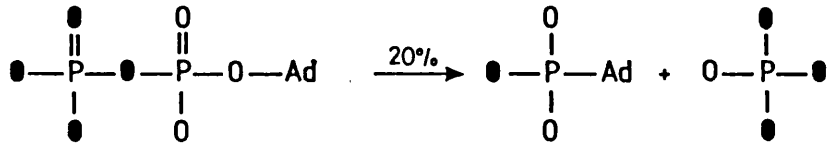
FIGURE 4.5 Work by le Noble et al.
(a) Pseudo-first order rate constants as a function of pressure (1 MPa = 10 bar) for the hydrolysis of 2,4-dinitrophenyl phosphate at pH 12 (43.2°C);
(b) Arrhenius plot for the hydrolysis at atmospheric pressure.

Lowe and Tuck[136] have undertaken positional isotope exchange experiments with adenosine 5'-[β-$^{18}O_4$] diphosphate[137] (shown below) in order to resolve the type of preassociative reaction which takes place within the solvent cage during phosphoryl transfer reactions.



When [β-$^{18}O_4$] ADP was incubated in tris HCl aqueous buffer in the

presence of $MgCl_2$ and EDTA for three weeks at 20°C for a series of pH values spanning pH 5-9, 20% of the [β-$^{18}O_4$] ADP had been hydrolysed to [^{18}O] AMP and [$^{18}O_3$] P_i as shown below.



The high resolution spectrum of the recovered 80% [β-$^{18}O_4$] ADP showed no evidence of positional ^{18}O exchange between Pβ-O-Pα bridge and the non-bridging sites.

However, when [β-$^{18}O_4$] ADP tris (tetra-n-butyl ammonium) salt was incubated in dry acetonitrile at 70°C, the products formed after two days were AMP, ADP, adenosine 2',5'-bisphosphate (pAp) and adenosine-2'-phospho-5'-diphosphate (ppAp).

The high resolution spectrum of the recovered ADP (and ppAp) showed that extensive ^{18}O exchange from the Pβ-O-Pα bridge to the non-bridging site at Pα had occurred.

The PIX results with [β-$^{18}O_4$] ADP in protic solution is consistent with a preassociative concerted mechanism and this would be expected to occur with inversion of configuration of the transferred phosphoryl group (although the possibility of a stepwise mechanism occurring if the leaving group has a low pKa has not been excluded).

A stepwise preassociative mechanism is consistent with the results that show positional isotope exchange of [β-$^{18}O_4$] ADP in aprotic solution (acetonitrile), this would be expected to be accompanied by racemisation of the transferred phosphoryl group if the lifetime of the intermediate is sufficient.

Stereochemical investigation of monomeric metaphosphate in aprotic solvents

Recent stereochemical studies in aprotic solvents were undertaken by Cullis and Rous[138] on the phosphoryl transfer from isotopically chiral [β(s)^{16}O, ^{17}O, ^{18}O] adenosine 5'-diphosphate in acetonitrile to 2-O-benzyl-(S)-propane-1,2-diol shown in Figure 4.6. The phosphorylated products were separated by ion-exchange and subjected to the analysis developed for phosphate monoesters. The phosphoryl transfer was shown to proceed with extensive racemisation consistent with monomeric metaphosphate. An alternative explanation without invoking a free metaphosphate, would be the involvement of a transient phosphoryl transfer to an alternative acceptor, i.e. acetonitrile. This adduct between acetonitrile and metaphosphate must undergo multiple phosphoryl transfer reactions with other solvent molecules, in order to account for the racemic product, before being trapped by the alcohol. The experiment cannot distinguish between the alternatives.

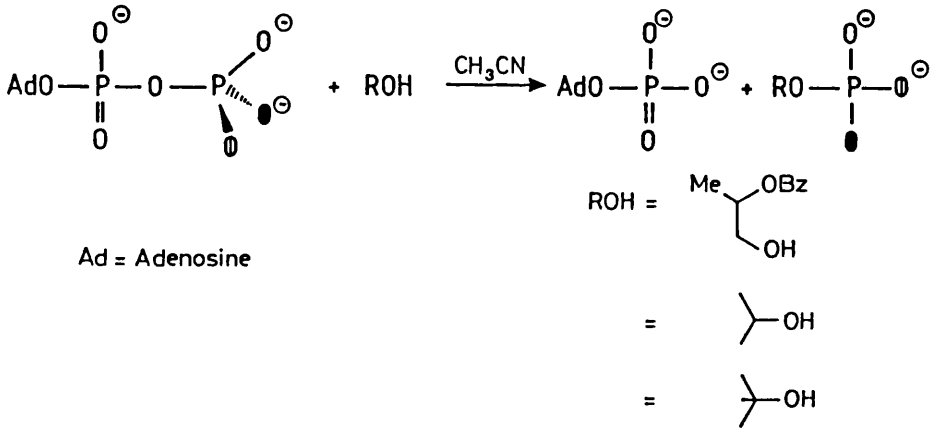


FIGURE 4.6 Phosphorylation reactions of ADP in acetonitrile by Cullis and Rous.

Other studies by Cullis and Rous[139] on the stereochemical course of phosphoryl transfer from a P^1-P^1-disubstituted pyrophosphate derivative to 2-O-benzyl-(S)-propane-1,2-diol in dichloromethane also showed extensive

racemisation. Dichloromethane is less likely to participate in the phosphoryl transfer in an analogous manner. Therefore this latter study provides the most convincing support for a pathway involving a relatively free metaphosphate. Also a similar result was obtained by Knowles et al.[140] during the stereochemical course of phosphoryl transfer from phenyl phosphate to tertiary butyl alcohol in acetonitrile.

These studies leave open the possibility of an intermediate with an appreciable lifetime.

Development and analysis of the stereochemical course of solvolysis of p-nitrophenyl [^{16}O, ^{18}O] thiophosphate

The objective of this work has been to develop methods to determine the stereochemical course of thiophosphoryl transfer reactions and to probe the participation of monomeric thiometaphosphate.

Unlabelled p-nitrophenyl thiophosphate required to establish the conditions of the thiophosphoryl transfer was prepared from the corresponding dichloride (37) as shown in Scheme 4.7. The sodium salt of p-nitrophenylthiophosphate was stored as an aqueous solution and was used without purification.

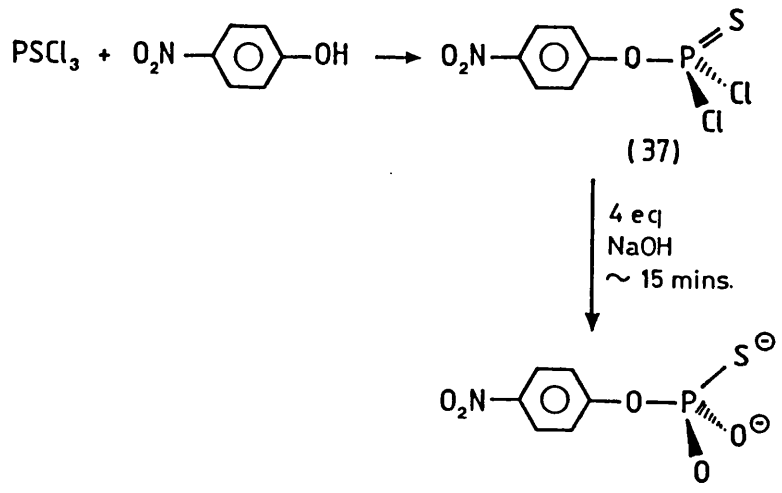


FIGURE 4.7 The synthesis of unlabelled p-nitrophenyl thiophosphate.

A brief study of the solvolysis of p-nitrophenyl thiophosphate in mixed solvents was conducted. A close correspondence between the ratio of alkylphosphate to inorganic phosphate and the ratio of alcohol to water has been noted in the solvolysis of monosubstituted phosphates.[113,114] This has been taken as evidence of a highly reactive and, therefore, indiscriminant electrophile, i.e. metaphosphate. Similar evidence was sought in the case of the corresponding thiophosphoryl transfer reactions.

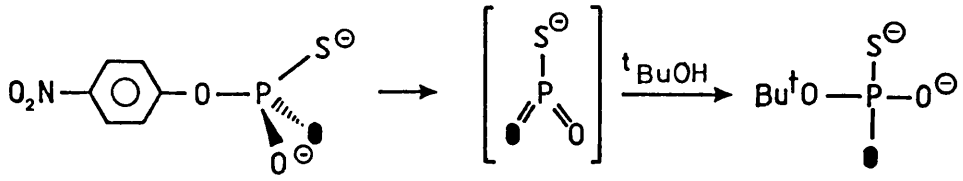
The experiment on the thiophosphate ester was undertaken as follows. Equal molar quantities of ethanol and water were added to p-nitrophenyl thiophosphate. The reaction was monitored by ^{31}P nmr and the products formed were identified. A 50/50 ratio of the products ethyl thiophosphate (38) and inorganic thiophosphate (39) would indicate that a highly indiscriminate phosphorylating agent exists.

The % total of products formed when the results from eight separate experiments were averaged are 55:45%, inorganic thiophosphate to ethyl thiophosphate.

The results infer that a highly indiscriminate phosphorylating agent is formed which reacts with both nucleophiles in approximately equal amounts.

Ramirez[141] has suggested that phosphorylation of tertiary butyl alcohol is a criterion for involvement of a PO_3^- species (monomeric metaphosphate). Therefore, it would be pertinent to carry out the analogous experiment on thiophosphorylation of tertiary butanol thus providing evidence for the involvement of a thiometaphosphate species and to study the stereochemical course of such a reaction.

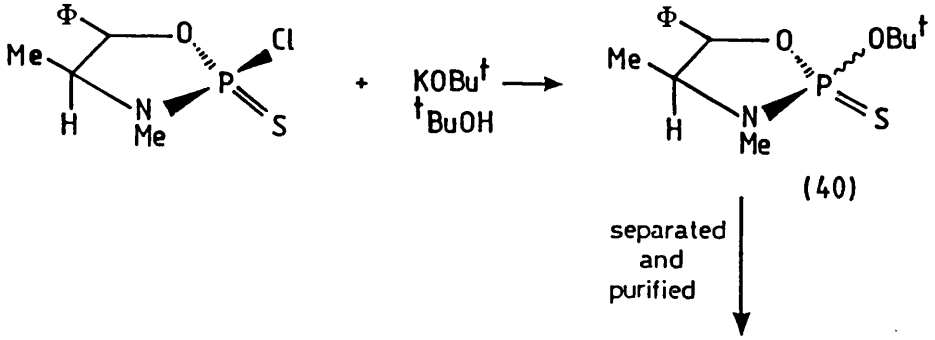
Preliminary experiments suggested that solvolysis of p-nitrophenyl thiophosphate in $^tBuOH/CH_3CN$ gave t-butyl thiophosphate analogous to the corresponding phosphates.



An essential control would be the demonstration of the configurational stability of the t-butyl thiophosphate.

The synthesis of tertiary butyl thiophosphate was to be carried out as shown in the general method of synthesis of thiophosphate esters using potassium butoxide as the nucleophile.

The synthesis of (40) was shown to be successful such that it would be possible to undertake such a study. However in the first instance the ethanolysis was studied since the absolute configuration of the isotopically labelled product had already been established.



(40)

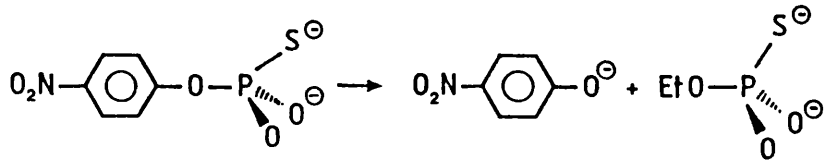
separated and purified

Stereochemistry

In seeking to demonstrate the existence of a relatively long-lived monomeric thiometaphosphate intermediate the solvolysis of p-nitrophenyl thiophosphate (18) in ethanol was studied. The product, ethyl thiophosphate (21) had already been synthesised in isotopically chiral form and an independent configurational analysis developed.

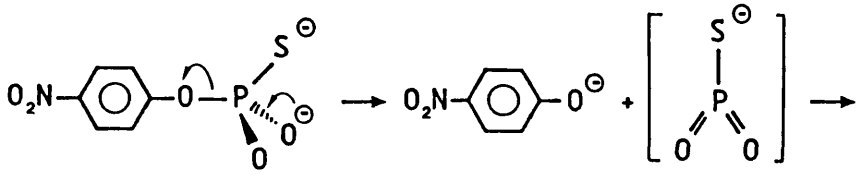
The mechanistic alternatives are illustrated in Figure 4.8. Racemisation or partial racemisation would be expected for a reaction involving a long-lived monomeric thiometaphosphate intermediate. Inversion of

Reaction



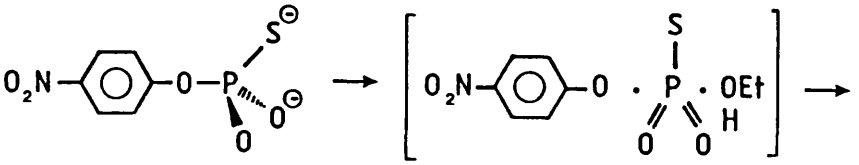
Mechanisms:

1. Dissociative mechanism via thiometaphosphate



stereochemical course: Racemisation

2. Preassociative mechanism (and at the limit associative)



stereochemical course: Inversion

FIGURE 4.8 The proposed mechanism for the solvolysis of p-nitrophenyl thiophosphate in ethanol.

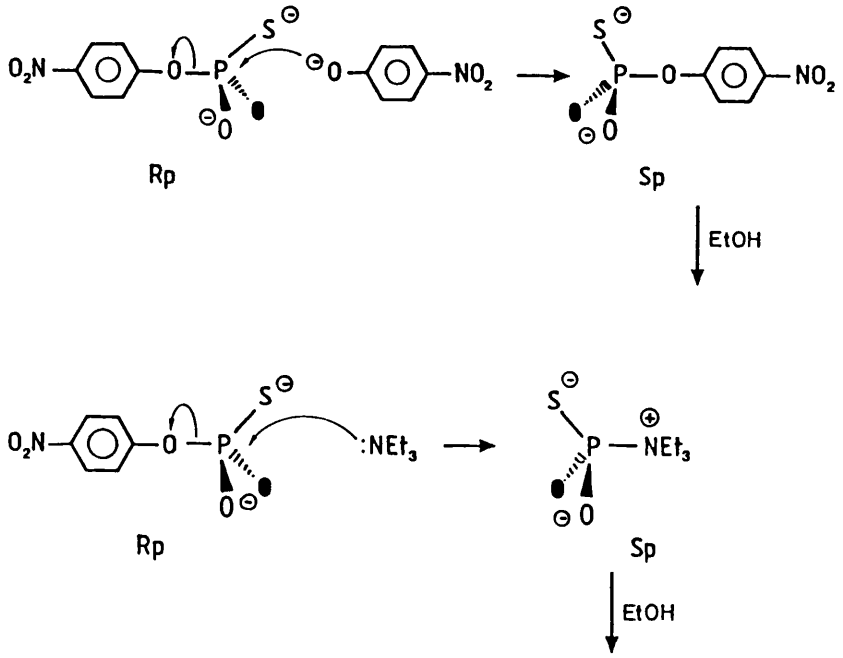
configuration could indicate a preassociative reaction (stepwise or concerted) or at the limit an associative reaction.

The stereochemical course of solvolysis of p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate

The solvolysis of p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate (18) was undertaken in ethanol containing a small amount of potassium bicarbonate to give an apparent pH ~10. The presence of the dianion was inferred from the ^{31}P nmr chemical shift (EtOH) [dianion + 40 ppm monoanion, +46.7 ppm]. The temperature of the reaction was maintained at 50°C over 1½ hours. After this time two resonances were shown in the ^{31}P nmr, one at +43 ppm and the other at 40 ppm. ^{31}P nmr proton coupled spectra gave a

triplet at +43 ppm indicative of the product ethyl thiophosphate, while a singlet at +40 ppm was consistent with the starting material. After this length of time the extent of reaction was ca. 50%. These products were separated by ion-exchange and subjected to the analysis developed for thiophosphate esters as described in Chapter 3 [see Figure 4.9].

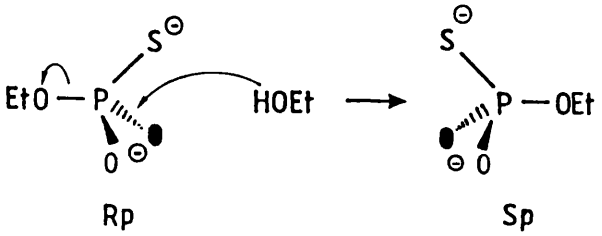
It is possible that any racemisation observed during the solvolysis may have arisen from the configurational instability of either the initial starting material p-nitrophenyl thiophosphate, or of the product ethyl thiophosphate under the solvolysis conditions. For example, in the case of the configurational instability of p-nitrophenyl thiophosphate, the starting material may racemise prior to thiophosphoryl transfer to ethanol.



The p-nitrophenyl thiophosphate could have suffered racemisation as a result of attack by a p-nitrophenoxide anion or by the triethylamine arising from the counter ion. The latter seems unlikely, as other counter ions such as sodium and tetrabutylammonium react at the same rate under solvolysis conditions.

In order to discount either of these possibilities, the starting material p-nitrophenyl thiophosphate was re-isolated in ~50% yield under solvolysis conditions and subjected to analysis.

The product from the solvolysis of p-nitrophenyl thiophosphate, by ethanol, ethyl thiophosphate, may undergo racemisation itself by further attack of ethanol as shown.



As a control experiment to exclude this, ethyl Sp [^{16}O, ^{18}O] thiophosphate was made via the pathway shown in Chapter 2 and subjected to the conditions of solvolysis for three hours, i.e. the conditions for the full conversion of reactants to products and then analysed.

The solvolysis of p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate along with the control experiments are shown in Figure 4.9.

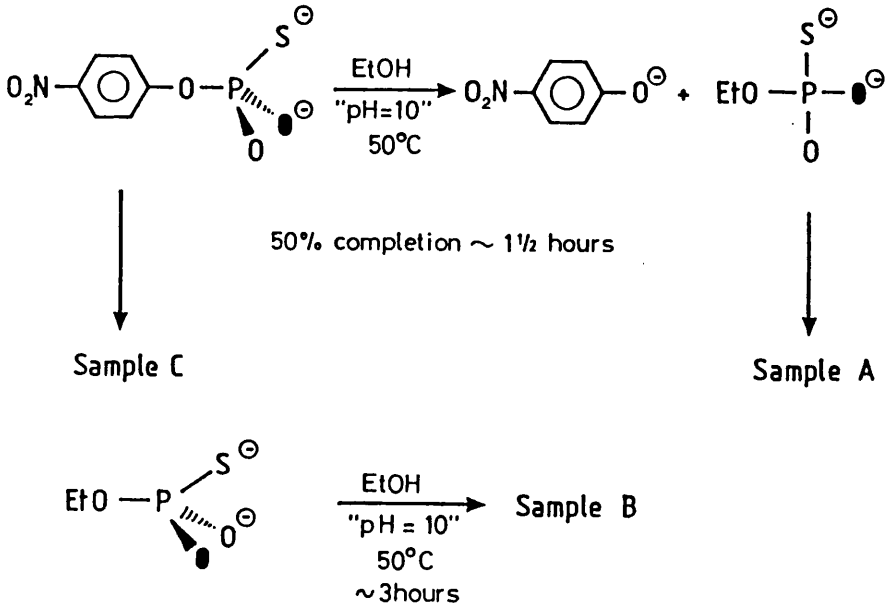


FIGURE 4.9 Experiments to investigate the stereochemical course of solvolysis of p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate.

The high-field ^{31}P nmr spectra of the pyrophosphate derived from ethyl Sp [^{16}O,^{18}O] thiophosphate (sample B), re-isolated Rp [^{16}O,^{18}O] p-nitrophenyl thiophosphate (sample C) and isolated product ethyl [^{16}O,^{18}O] thiophosphate (sample A) are shown in Figures 4.10, 4.11 and 4.12. These samples are assigned by the criterion established in the analysis of ethyl Sp [^{16}O,^{18}O] thiophosphate (Chapter 3). These are briefly the following:-

(i) The magnitude of ^{18}O shift is bond order dependence, $P = {}^{18}O > P-{}^{18}O-R$.
(ii) An ^{18}O shift occurs on both phosphoryl centres when ^{18}O is located in the bridge of pyrophosphate derivatives (25) and (26).

High-field ^{31}P nmr of ethyl Sp [^{16}O, ^{18}O] thiophosphate (sample B)

This spectrum is shown in Figure 4.10. The resonance in the region of +7.0 ppm corresponds to the oxazaphospholidine end and those at +45 ppm are from the thiophosphoryl region.

The ^{31}P nmr data from the spectrum are as follows:-

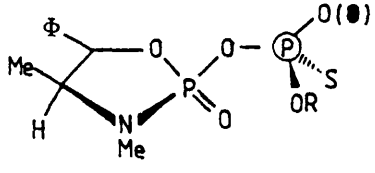
Phosphorus centre	Chemical shift (ppm)	Hz	^{18}O shift
1,3,2-oxazaphospholidin-2-one	+7.14	d, Jpp, 25.9Hz	2.28Hz
Rp thiophosphoryl centre	+46.39	d, Jpp, 25.9Hz	2.84Hz
1,3,2-oxazaphospholidin-2-one	+6.65	d, Jpp, 29.7Hz	-
Sp thiophosphoryl centre	+46.29	d, Jpp, 29.7Hz	4.46Hz

High-field ^{31}P nmr of p-nitrophenyl [^{16}O, ^{18}O] thiophosphate (sample C)

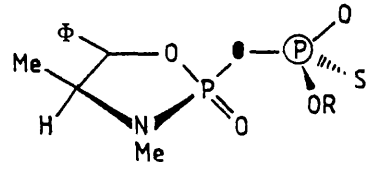
This spectrum is shown in Figure 4.11. The resonances in the region of +6.0 ppm correspond to the oxazaphospholidine end whilst the resonances at +39 ppm arise due to the thiophosphoryl moiety. The resonances at +39 ppm are absent as they are not well defined. This is because only crude material could be used (see Chapter 3). However the resonances in the region of +6.00 ppm are well defined and are shown in Figure 4.11.

The ^{31}P nmr data from the spectrum are as follows:-

thiophosphoryl region

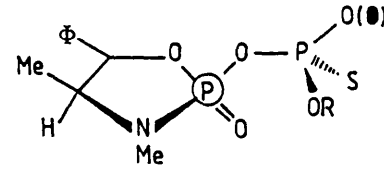


(25a)
(29a)

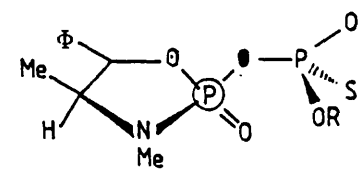


(25b)
(29b)

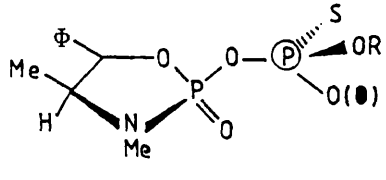
oxazaphospholidine region



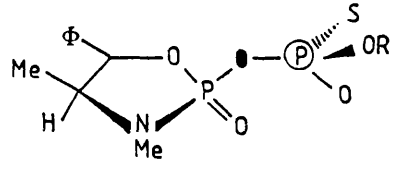
(25a)
(29a)



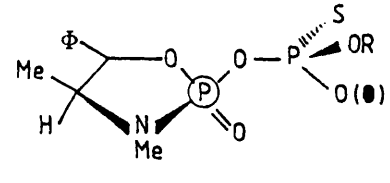
(25b)
(29b)



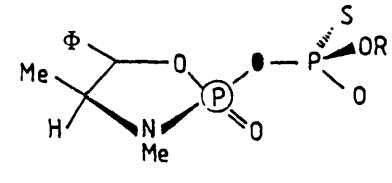
(26a)
(30a)



(26b)
(30b)



(26a)
(30a)



(26b)
(30b)

25 : 26=R = Ethyl
29 : 30=R = p-Nitrophenyl

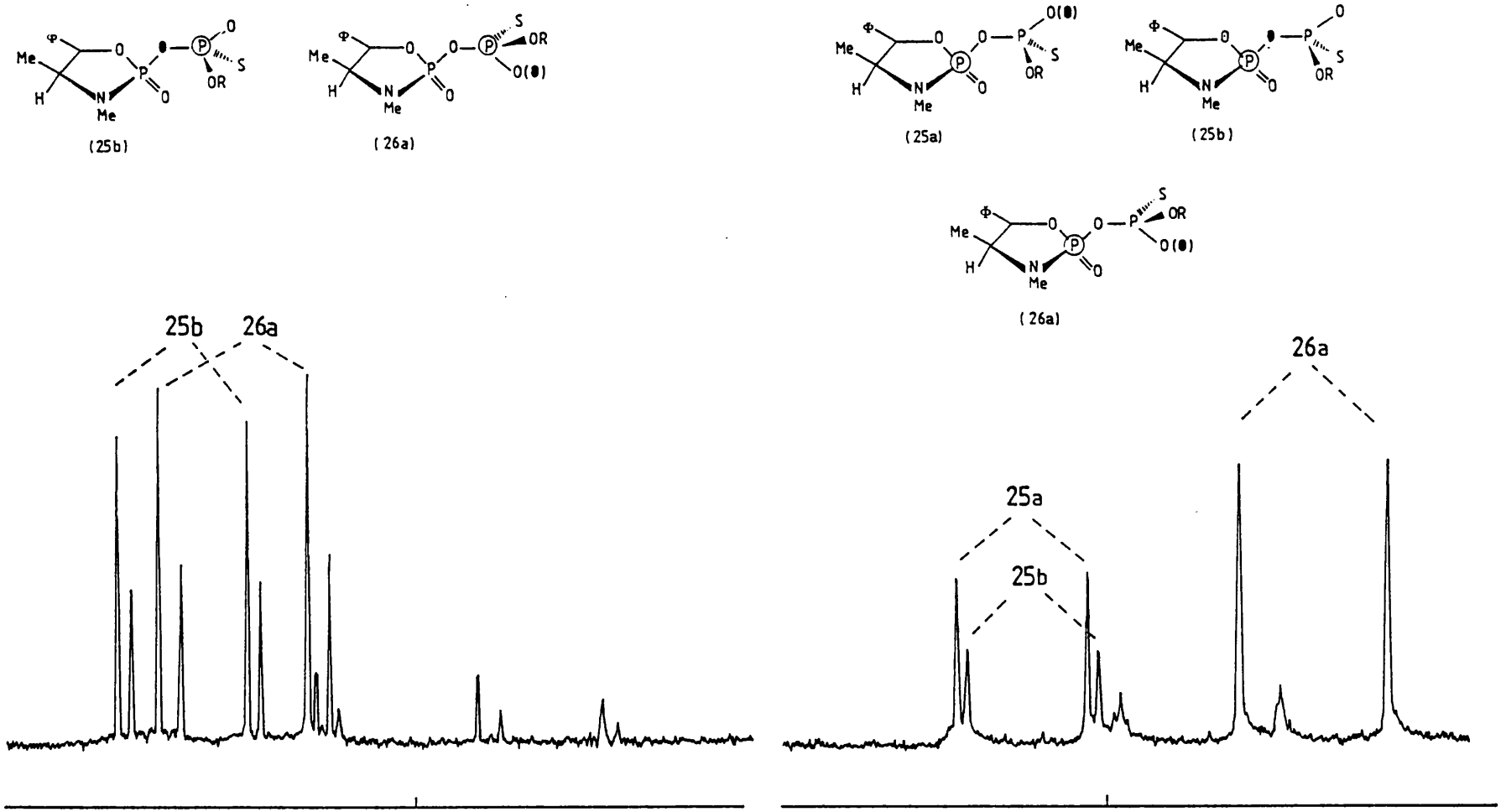
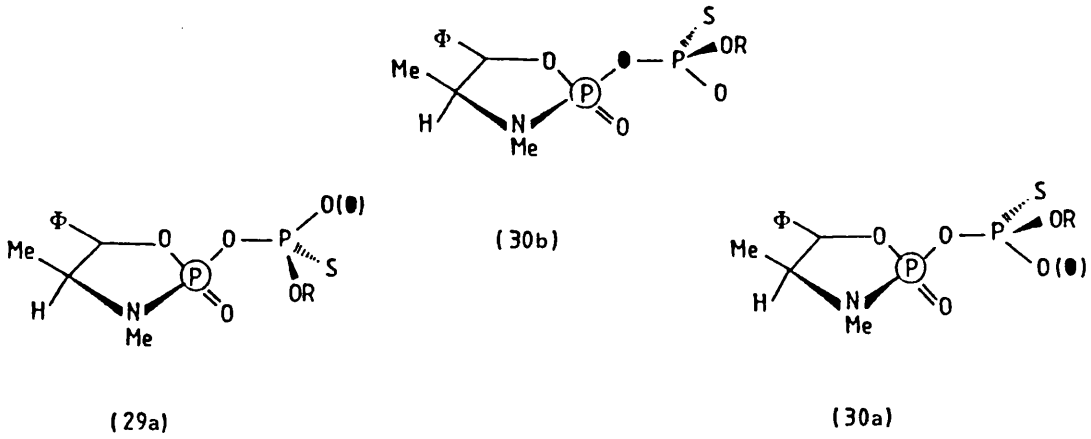


FIGURE 4.10

High-field ^{31}P nmr. Assignment of the absolute configurations of the diastereomeric pyrophosphate from Sp Ethyl [^{16}O,^{18}O] thiophosphate [subjected to transfer conditions]. [33% $H_2{}^{18}O$]



(29a) (30b) (30a)

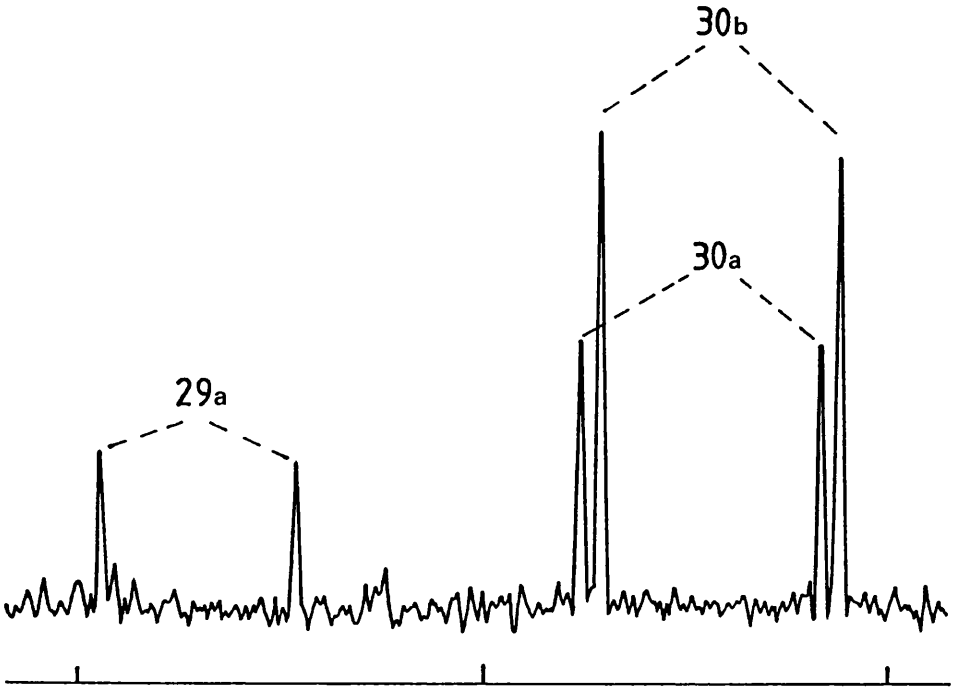


FIGURE 4.11

High-field ^{31}P nmr. Assignment of the absolute configurations of the diastereomeric pyrophosphate from Rp-p-nitrophenyl [^{16}O,^{18}O] thiophosphate.

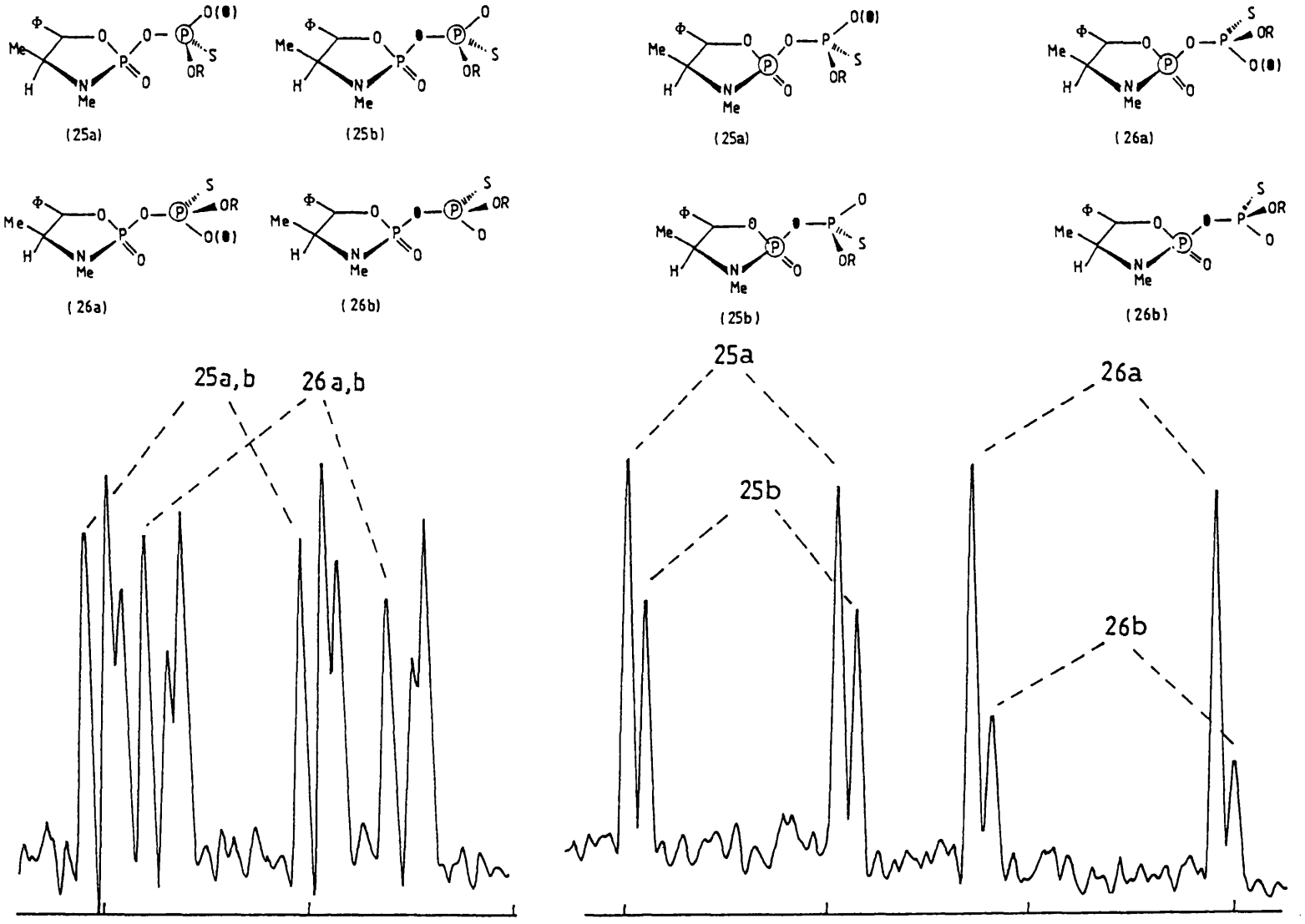


FIGURE 4.12

Phosphorus centre	Chemical shift (ppm)	Hz	^{18}O shift
1,3,2-oxazaphospholidin—2-one	+6.5	d, Jpp, 25.9Hz	-
Rp thiophosphoryl centre	+39.48	d, Jpp, 25.9Hz	4.46Hz
1,3,2-oxazaphospholidin—2-one	+6.0	d, Jpp, 29.7Hz	2.28Hz
Sp thiophosphoryl centre	+39.38	d, Jpp, 29.7Hz	2.84Hz

High-field ^{31}P nmr of ethyl [^{16}O, ^{18}O] thiophosphate (unknown configuration) (sample A)

This spectrum is shown in Figure 4.12. The resonances in the region of +6.5 ppm correspond to the oxazaphospholidine region and that at +43 ppm to the thiophosphoryl end.

The ^{31}P nmr data from the spectrum are as follows:-

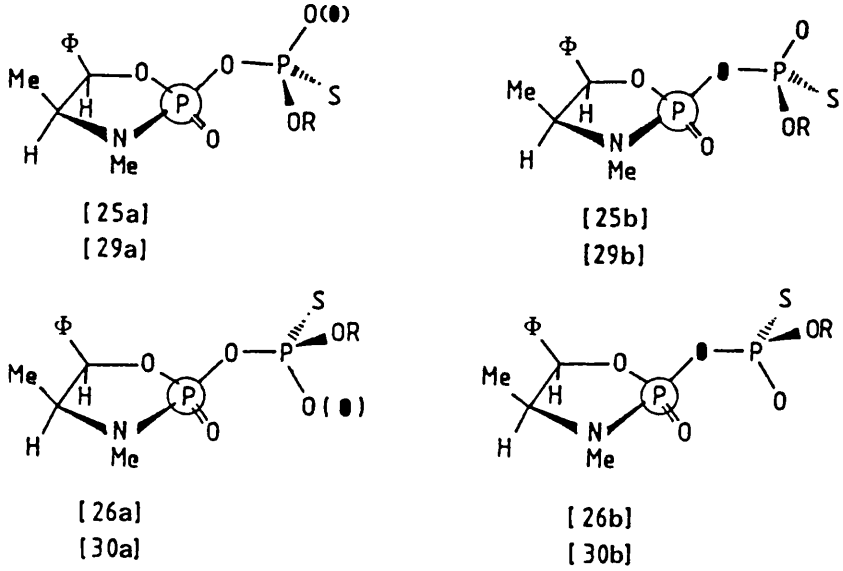
Phosphorus centre	Chemical shift (ppm)	Hz	^{18}O shift
1,3,2-oxazaphospholidin—2-one	+7.00	d, Jpp, 25.9Hz	2.28Hz
Rp thiophosphoryl centre	+46.29	d, Jpp, 25.9Hz	2.84Hz 4.46Hz
1,3,2-oxazaphospholidin—2-one	+6.65	d, Jpp, 29.7Hz	2.28Hz
Sp thiophosphoryl centre	+46.19	d, Jpp, 29.7Hz	2.84Hz 4.46Hz

From the spectra, full stereochemical information is defined in both ring and thiophosphoryl end. The ring phosphorus resonances are clearly defined and comparisons between the samples A, B and C are more pronounced at this region.

The spectra of the oxazaphospholidine region of samples A, B and C are shown in Figure 4.13.

Ethyl Sp [^{16}O, ^{18}O] thiophosphate (sample B)

From the spectrum it can be seen that there are ^{18}O lines on the downfield



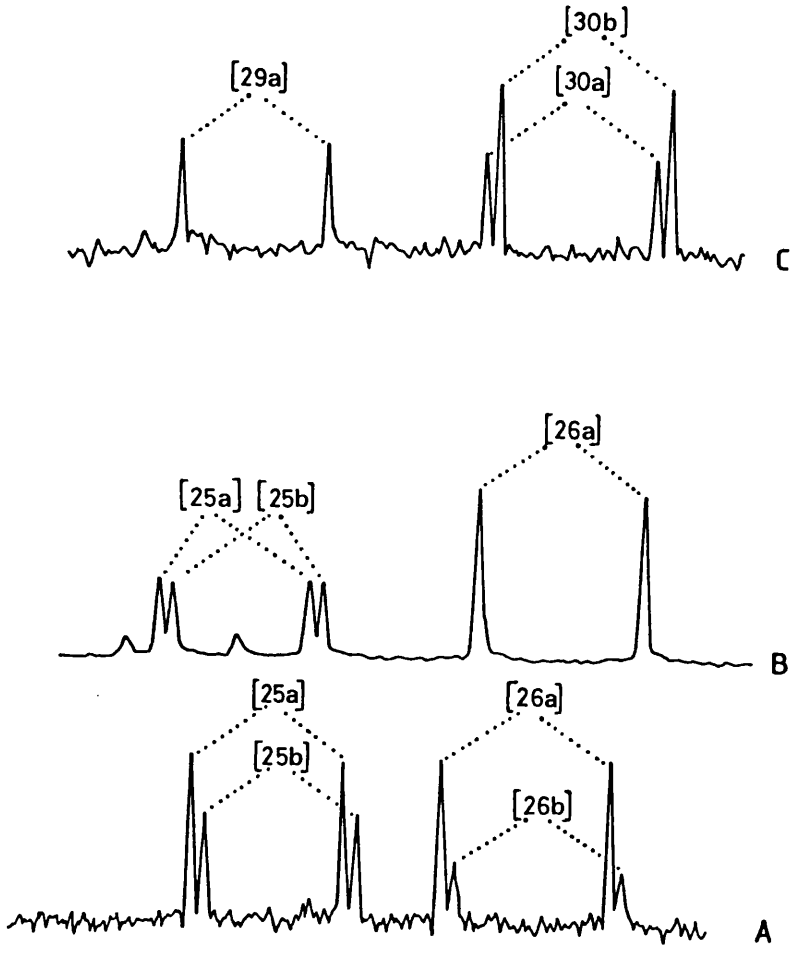


FIGURE 4.13

Spectra of the oxazaphospholidine region of samples A, B and C. [33% $H_2{}^{18}O$]

spectrum. These lines correspond to the Sp configuration of ethyl [^{16}O, ^{18}O] thiophosphate. Hence the product has not undergone any stereochemical changes. This spectrum is identical to that established in the analysis of Sp [^{16}O, ^{18}O] ethyl thiophosphate.

p-Nitrophenyl [^{16}O,^{18}O] thiophosphate (sample C)

In this case the ^{18}O resonances are shown on the upfield spectra. This implies that the starting material, p-nitrophenyl [^{16}O, ^{18}O] thiophosphate is of the Rp configuration, and so has not undergone any stereochemical changes.

Both the starting material p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate and the ethyl Sp [^{16}O, ^{18}O] thiophosphate have not undergone any stereochemical changes under the conditions of thiophosphoryl transfer in protic solvent. Therefore any stereochemical chemical changes which occur when a thiophosphoryl group is transferred from p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate to ethanol must have occurred solely during this thiophosphoryl transfer step. This is confirmed by the following spectrum.

Ethyl [^{16}O, ^{18}O] thiophosphate: Product of thiophosphoryl transfer step (sample A)

This region of the spectrum shows ^{18}O resonance both on the upfield and downfield spectrum corresponding to both Rp and Sp configurations. Thus the product ethyl [^{16}O, ^{18}O] thiophosphate formed from the thiophosphoryl transfer of p-nitrophenyl [^{16}O, ^{18}O] thiophosphate in ethanol has occurred with extensive racemisation.

From spectrum A (Figure 4.13), the ratios of the intensities of the resonances for labelled and unlabelled diastereoisomers, in the downfield region, correspond to 60% inversion. In the upfield region the same comparison of resonances gives a value of 40% retention. These results

correspond to thiophosphoryl transfer occurring with ca. 80% racemisation and a 20% excess of the Sp configuration which would arise from thiophosphoryl transfer occurring with inversion of configuration.

CONCLUSIONS

The result that ethanolysis of p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate proceeds with 80% racemisation implies that thiometaphosphate is indeed a longer-lived intermediate than metaphosphate. This apparent increased stability of thiometaphosphate over metaphosphate is in accord with the work of Breslow, Katz[49] and Roesky[50] (see Chapter 1). Breslow found that the (non-enzymatic) hydrolysis of the monoester of p-nitrophenyl thiophosphate was more rapid and rationalised this in terms of a dissociative mechanism. Such dissociative reactions of thiophosphate and phosphate monoesters occur through an elimination addition mechanism.

The isolation of PS_3^- by Roesky[50] as the tetraphenylarsonium salt implies that substitution of sulphur for oxygen produces a more kinetically stable species with a relatively long lifetime. Our findings obviously add weight to such an argument.

The stereochemical differences between chemical thiophosphoryl and phosphoryl transfers leads us to speculate whether any differences occur between enzyme-catalysed phosphoryl and thiophosphoryl transfer.

Thiophosphate analogues of natural substrates have been used for many years to probe enzyme-catalysed phosphoryl reactions. In Chapter 1 it was noted that the thiophosphate analogues of natural substrates follow the same stereochemical course as the natural substrate in enzyme-catalysed reactions that have been tested.

These results provide convincing evidence that the use of thiophosphate analogues as stereochemical probes was a valid approach for determining the stereochemical course of enzyme-catalysed phosphoryl transfer

reactions. This is perhaps to be expected since an enzyme-active site is unlikely to be able to provide more than one catalytic path for a given reaction.

Although many enzymatic reactions proceed much more slowly with thiophosphates than phosphates and, indeed, Breslow[49] has shown that p-nitrophenyl thiophosphates undergo enzymatic hydrolysis by alkaline phosphatase much more slowly than p-nitrophenyl phosphates, there are examples where there is little difference in reaction rates, for example DNA and RNA polymerase and myosin. Some enzymes such as phosphomutases do not accept thiophosphates as substrates, while others do but only at extremely slow rates, e.g. alkaline phosphatase has been reported to catalyse the hydrolysis of adenosine-5'-thiophosphate at 1/2000th the rate for AMP.

The stereochemical outcome of a non-enzymatic reaction suggest that differences in enzyme-catalysed phosphoryl and enzyme-catalysed thiophosphoryl reactions may exist.

The greater stability of the thiometaphosphate as shown by the stereochemical outcome of 80% racemisation, implies that the transfer of a thiophosphoryl group occurs <u>via</u> a different reaction mechanism than that of a phosphoryl group transfer.

A scheme for distinguishing between reaction mechanisms has been proposed by Jencks[113] and is illustrated in Figure 4.14 (see beginning of Chapter).

This scheme provides borderline mechanisms that depend upon the lifetime of the intermediate. There are three alternatives: preassociative concerted, preassociative stepwise and a liberated intermediate.

A preassociative concerted mechanism contains only one stage in which the two reacting molecules undergo a covalency change. A preassociative stepwise mechanism proceeds in two stages and through an intermediate (I)

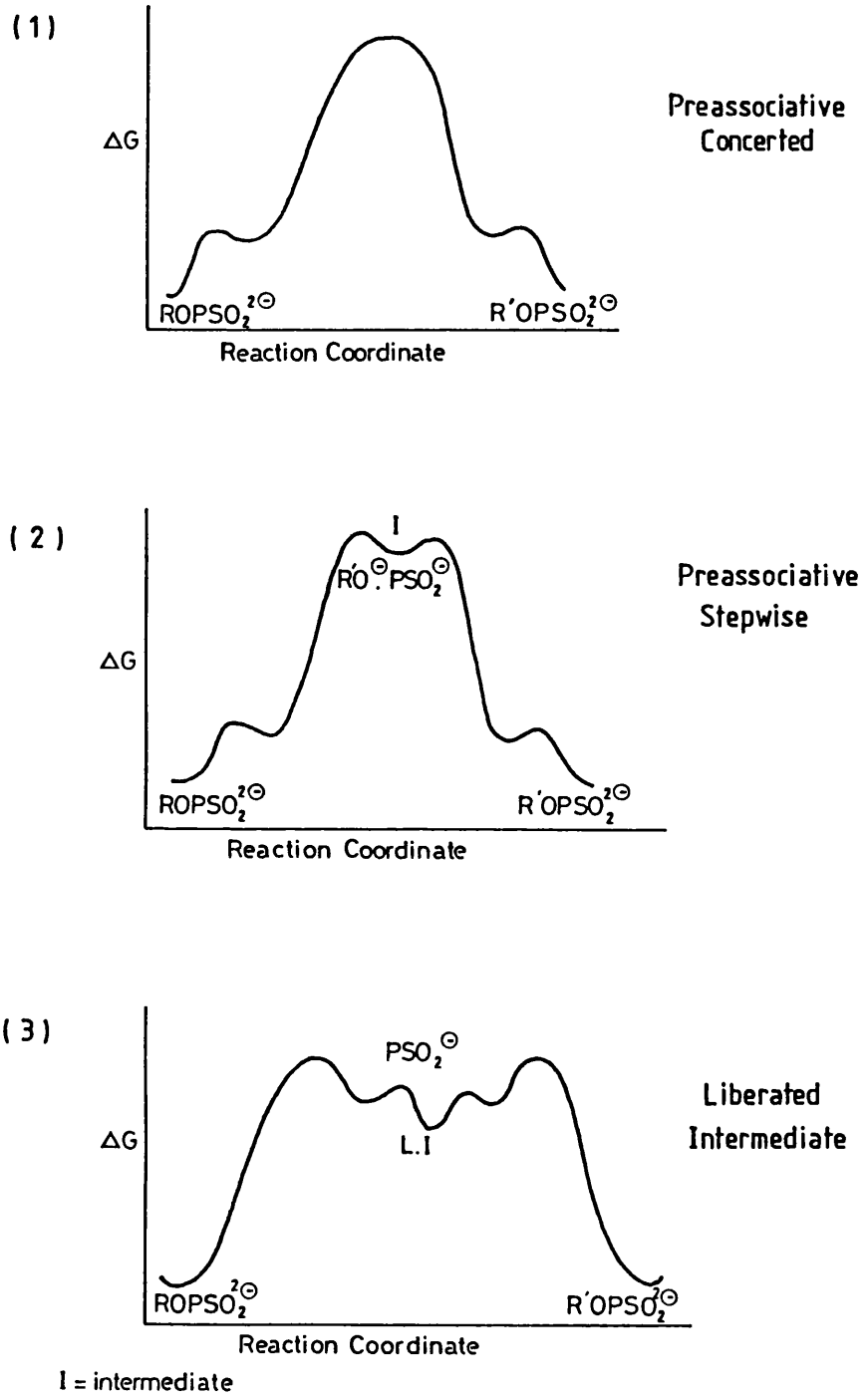


FIGURE 4.14 Classification of reaction mechanisms.

in a potential well as shown. Both preassociative mechanisms occur when the lifetime of the intermediate is short, i.e. below that of a molecular vibration of 10^{-13} s.

On the other hand, a liberated intermediate mechanism occurs when an intermediate has a sufficient lifetime (greater than the molecular vibration of 10^{-13} s) to diffuse through the solvent towards the nucleophile and it is then free to react with some degree of selectivity.

The stereochemical outcome of such extremes is inversion of configuration for preassociative mechanisms and racemisation for the liberated intermediate.

It has already been shown that the stereochemical consequence of methanolysis of 2,4-dinitrophenyl [^{16}O, ^{17}O, ^{18}O] phosphate proceeds with complete inversion at phosphorus.[30]

These results then provide evidence that the transfer of the phosphoryl group is best viewed as a preassociative-concerted mechanism where the single transition state is a loose one in which neither acceptor nor donor nucleophile is closely associated to phosphorus.

The stereochemical evidence obtained that the solvolysis of p-nitrophenyl [^{16}O, ^{18}O] thiophosphate proceeds with 80% racemisation implies that thiometaphosphate is a substantially liberated intermediate; reaction pathway (3), Figure 4.14. The reaction occurs with 80% racemisation and not 100%, 20% of the reaction has occurred with inversion of configuration for which a preassociative stepwise mechanism would seem to be reasonable.

In conclusion, differences between the thiometaphosphate and metaphosphate exist and these are reflected in the stereochemical outcome of the chemical reaction of ethanolysis of p-nitrophenyl Rp [^{16}O, ^{18}O] thiophosphate which proceeds with 80% racemisation.

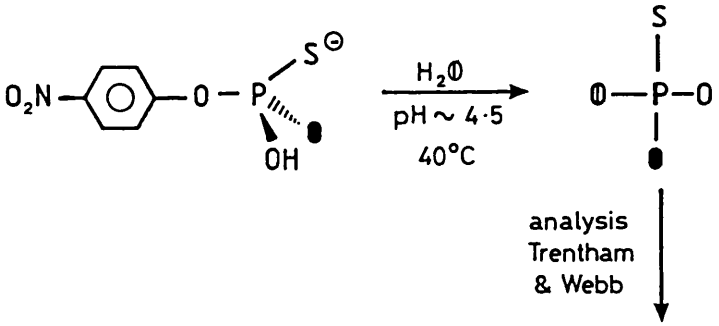
This study provides the first direct evidence for a monomeric liberated thiometaphosphate intermediate in protic solvent. The observation of extensive racemisation accompanying thiophosphoryl transfer from p-nitrophenyl [^{16}O,^{18}O] thiophosphate to ethanol is in marked contrast to the stereo-

specific phosphoryl transfer from phenyl [^{16}O, ^{17}O, ^{18}O] phosphate to methanol reported by Knowles et al. This has established significant mechanistic differences between phosphoryl and thiophosphoryl transfer reactions that may be relevant to the use of thiophosphate esters as substrate analogues in enzymatic reactions.

ADDENDUM

During the course of our work, two groups have also been working on the existence and lifetime of the thiometaphosphate.

Benkovic et al.[143] studied the reaction shown below.

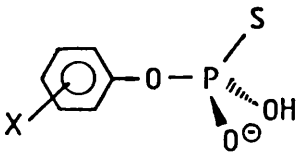


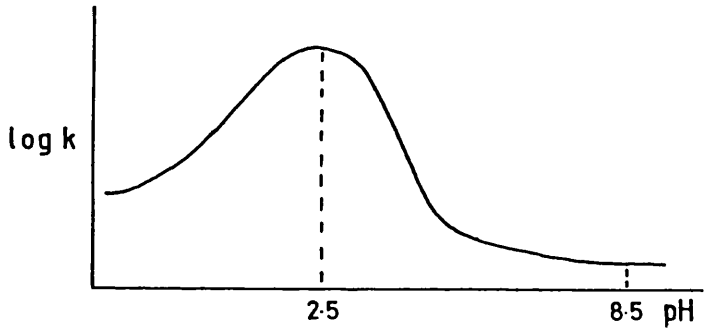
They studied the reaction of the monoanion of p-nitrophenyl thiophosphate. Since the reaction studied by them was the hydrolysis, a third isotope in the form of $H_2{}^{17}O$ was used. The product [^{16}O, ^{17}O, ^{18}O] thiophosphate is then analysed according to the method of Trentham and Webb (see Chapter 3). The analysis showed 30-40% racemisation of product. This result is consistent with our findings. However, the analysis used in this work has many shortcomings, especially in its use of enzymes (see Chapter 3). Therefore, this method is not an effective method of analysis.

Benkovic has also carried out kinetic studies on the hydrolysis of p-nitrophenylthiophosphate. He has found that using a whole range of nucleophiles the Brønsted coefficient for leaving groups is $\beta_{lg} \sim -1.2$ and the Brønsted coefficient for the nucleophile of the monoanion is $\beta^{nucl} \sim$ 0.1 units. These indicate that the monoanion is insensitive to the nature

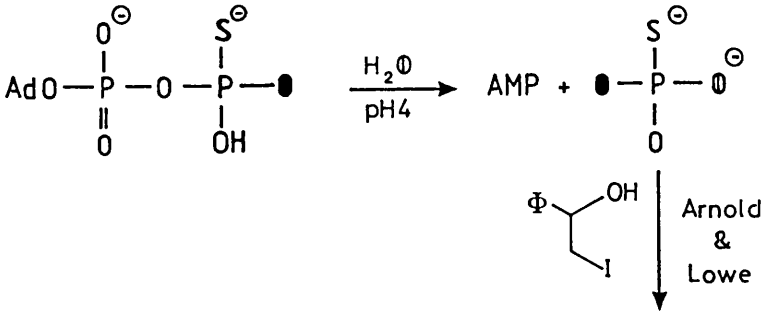
of the nucleophile but sensitive to leaving groups. This correlates well with results obtained implying a dissociative pathway. He has also found that the rate of thiophosphates compared to phosphates is $\sim 10^3$ times as great.

The pH/rate profile shows a maximum at pH 2.5 for the monoanion. These are shown below.





The second group interested in thiometaphosphate is G. Lowe *et al.*[144] This group has also concentrated on the monoanion but, in this case, adenosine-5'-β-thiodiphosphate was studied, as shown below.



This method is also carried out in protic solvent, this being $H_2{}^{17}O$. The incorporation of a third isotope produces inorganic thiophosphate containing three isotopes. This is then subjected to the analysis developed by Arnold and Lowe (discussed in Chapter 3). The results obtained from this experiment was ~25-30% racemisation. [Note the

results obtained are in early stages of development.]

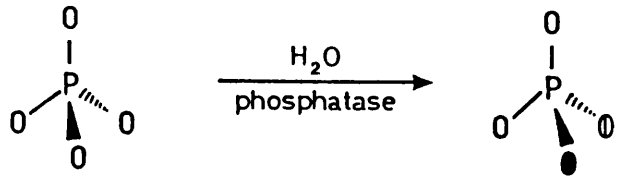
The results obtained so far add weight to the existence of a thiometaphosphate as a discrete intermediate, even in protic media including aqueous solutions.

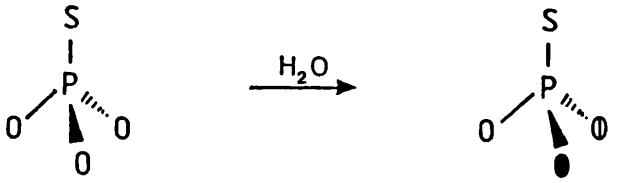
CHAPTER 5

Configurational Synthesis and Analysis of Isotopically Chiral Inorganic Thiophosphate

INTRODUCTION

For enzyme catalysed phosphoryl transfer to water, as in the phosphatases and synthetases, the phosphoryl is released either directly or indirectly as inorganic phosphate. The stereochemical course of this reaction cannot be determined purely by isotopic substitution, since there are only three stable oxygen isotopes. A solution to this problem has been found in the use of thiophosphates in which the final product is made chiral by ^{16}O, ^{17}O, ^{18}O and sulphur substitution. It is assumed

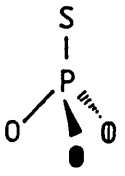




O = O^{16}, ⓪ = O^{17}, ● = O^{18}

that the enzyme catalysed thiophosphoryl transfer will proceed with the same stereochemical course as the corresponding phosphoryl transfer, although differences in pKa, metal ion coordination, charge distribution, etc., are almost certain to be important.

In order to study the stereochemical course of enzyme catalysed thiophosphoryl transfer and solvolysis reactions of thiophosphate monoesters, a reliable method for the synthesis and configurational analysis of isotopically chiral inorganic thiophosphate (41) is required together with a convenient and accurate configurational analysis.



(41)

Some synthetic and analytical methods have recently been reported.

The synthesis of isotopically chiral inorganic thiophosphate

The first method to be reported by Webb and Trentham[145] and independently by Tsai[146] harnessed the stereospecificity of several enzymes to generate the chirality and an enzymic step was used to liberate inorganic thiopyrophosphate. The method is illustrated in Figure 5.1.

AMPS [$^{18}O_2$] is chemically phosphorylated with [^{17}O] phosphate to give a 1:1 mixture of Rp ADP αs and Sp ADP αs diastereomers. The Sp diastereomer was enzymatically converted to ATP αs by use of pyruvate kinase and phosphoenol pyruvate.[78] ATP αs and ADP αs are readily separated chromatographically.

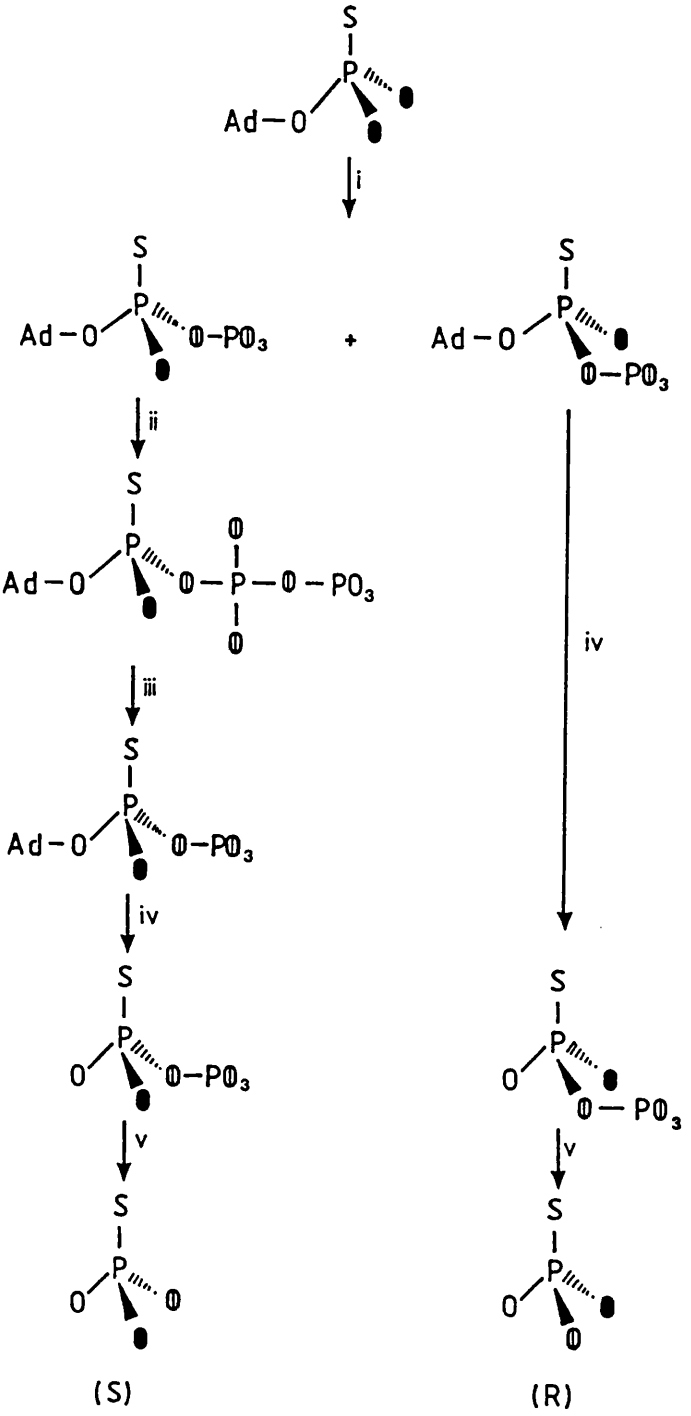
The ATP αs was converted with hexokinase to Sp ADP αs.

The diastereomers of ADP αs were separately oxidised with periodate followed by base to cleave the ribose ring which is then readily eliminated to form the thiopyrophosphate. These are then cleaved by pyrophosphatase to give the separate enantiomers of inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate.

This synthesis has limitations due to the incomplete stereospecificity of pyruvate kinase.

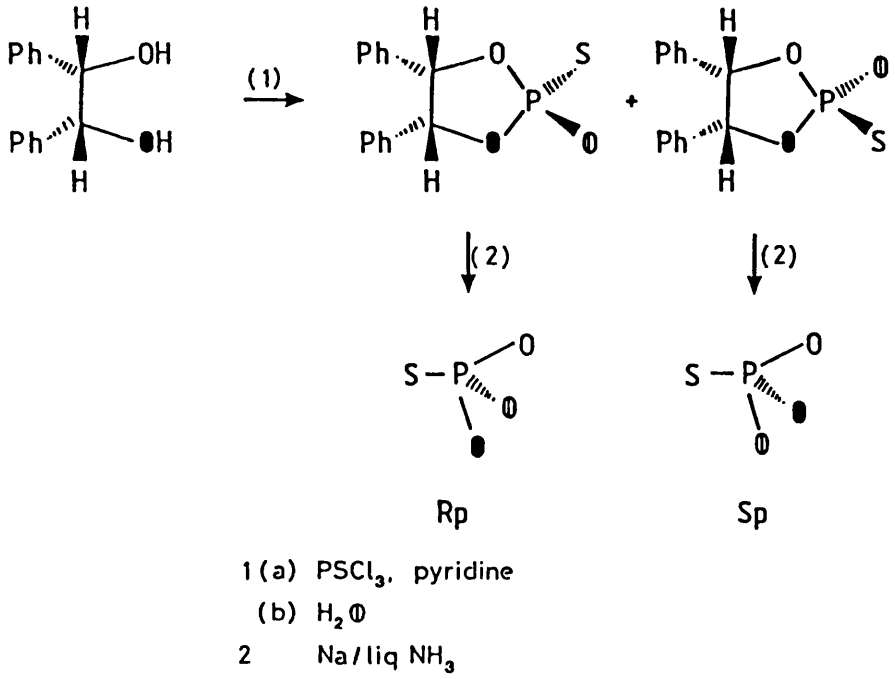
A chemical method was developed by Arnold and Lowe.[147] This synthesis is shown in Figure 5.2.

This synthetic method has close parallels with the synthesis of [^{16}O, ^{17}O, ^{18}O] phosphate monoesters. The difficulty surrounds the separation of the diastereoisomers Rp [^{16}O, ^{17}O, ^{18}O] AMPS thiophosphate and Sp [^{16}O, ^{17}O, ^{18}O] AMPS thiophosphate.



(i) $(PhO)_2POCl$, $P^{17}O_4^{2-}$
(ii) Pyruvate kinase, Mg^{2+}, PEP
(iii) Hexokinase, glucose, Mg^{2+}
(iv) $NaIO_4$ then base
(v) Pyrophosphatase, Mg^{2+}.

FIGURE 5.1 Synthesis of R and S inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate by Webb and Trentham.



1(a) $PSCl_3$, pyridine
(b) H_2O
2 Na/liq NH_3

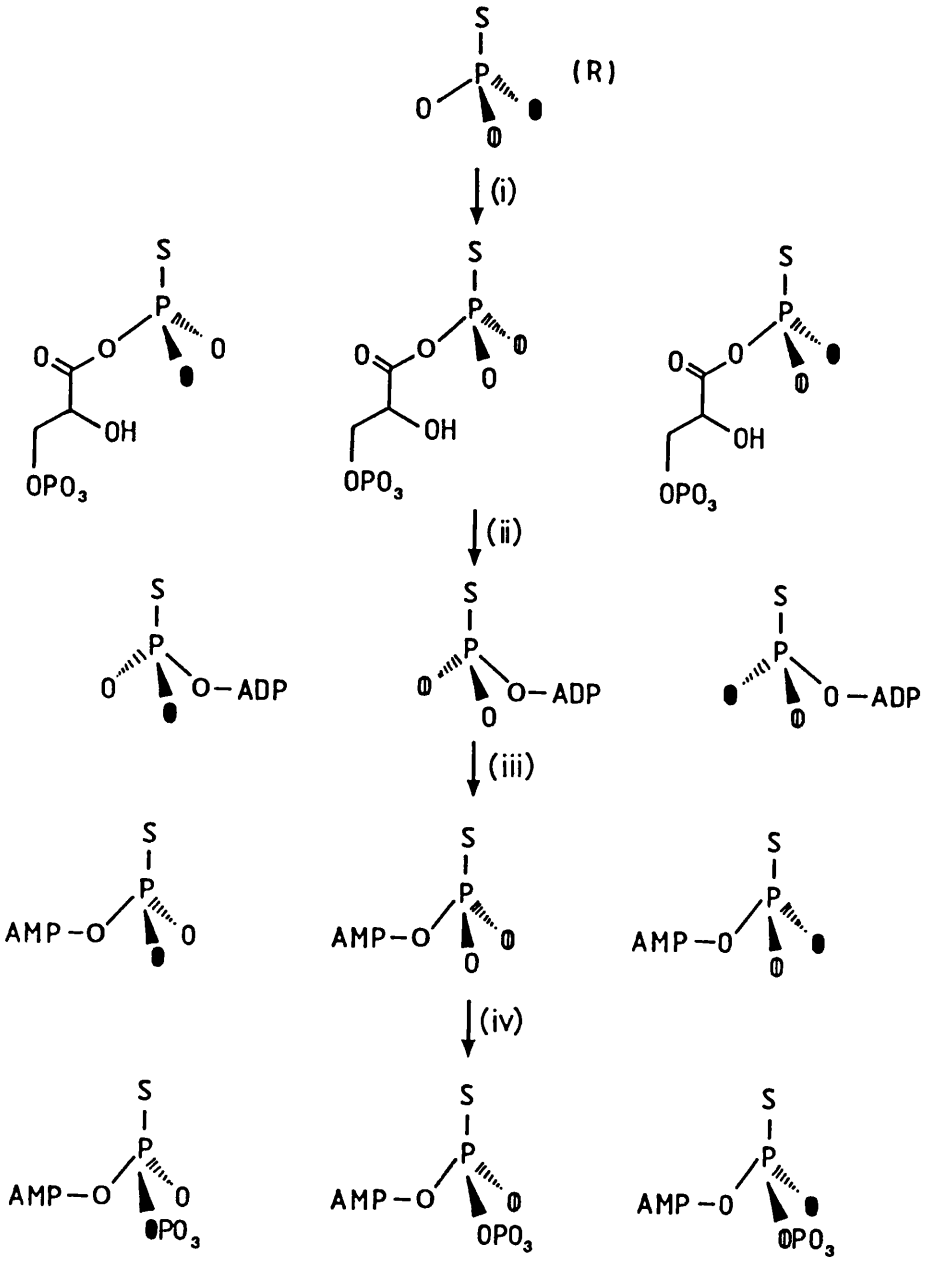
FIGURE 5.2 Synthesis of inorganic thiophosphate by Arnold and Lowe.

The configurational analysis of isotopically chiral inorganic thiophosphate

A ^{31}P nmr method has been developed by Webb and Trentham[148] and by Tsai.[73]

This method is achieved through the incorporation of the chiral [^{16}O, ^{17}O, ^{18}O] thiophosphate into the Sp diastereoisomer of ATP βs, via a series of enzyme catalysed reactions that proceed with known stereochemical course. This scheme is shown in Figure 5.3.

[^{16}O, ^{17}O, ^{18}O] thiophosphate is first incorporated into glycerate 1-phosphorothioate-3-phosphate by the action of glyceraldehyde phosphate dehydrogenase. Phosphoglycerate kinase transfers the thiophosphoryl group to ADP giving ATP γs then adenylate kinase transfers the thiophosphoryl group to AMP to form ADP βs. These transfers are known to proceed with inversion of configuration at phosphorus.[149] ADP βs is then phosphorylated on the pro (S) oxygen of the thiophosphoryl group of



(i) Glyceraldehyde phosphate dehydrogenase, glyceraldehyde phosphate, NAD^+;
(ii) MgADP, phosphoglycerate kinase;
(iii) Adenylate kinase MgAMP;
(iv) Phosphoglycerate kinase Mg^{2+} glycerate, 1,3-biphosphate.

FIGURE 5.3 The configurational analysis of isotopically chiral inorganic thiophosphate.

ADP βs to give (Sp) ATP βs.[150] This is then subjected to high field ^{31}P nmr spectroscopy.

The resonance for ^{17}O will effectively be absent from ^{31}P nmr detection, as the quadrupole nature of ^{17}O ($I = 5/2$) may be expected to produce resonances which are so broad that little, if any, quantitative data can be obtained[35,37] (see Chapter 1). The analysis therefore depends on whether ^{18}O is in a bridging or non-bridging position. ^{18}O in a non-bridging position is a greater shift than a bridging ^{18}O bond. The isotopic identity of the oxygens can now be determined by virtue of their chemical nonequivalence as was described for the configurational analysis of [^{16}O,^{18}O] chiral phospho diesters.

A shortcoming of this method is the observed significant loss of label from the inorganic thiophosphate during incorporation into ATP βs as well as the quality of the ^{31}P nmr spectra.

During the course of our own work a chemical analysis was developed by Arnold and Lowe shown in Figure 5.4.

The key reagent for their analysis is (S)-2-iodo-1-phenyl ethanol. This reagent reacts with Rp and Sp [^{16}O, ^{17}O, ^{18}O] thiophosphate at low temperatures to give [^{16}O, ^{17}O, ^{18}O] thiophosphates S-esters of Sp and Rp configuration. The reaction occurs on the S-esters due to the presence of both a soft electrophile and soft nucleophile.

Diphenylphosphoryl chloride was used to cyclise the esters. Upon cyclisation the products were methylated using diazomethane. The analysis is closely analogous to that developed for [^{16}O, ^{17}O, ^{18}O] phosphate mono-esters. The ^{31}P nmr spectrum reveals only ^{16}O, ^{18}O species (^{17}O species are not observable in the ^{31}P nmr since ^{17}O directly bonded to phosphorus causes broadening of the ^{31}P nmr resonances.[35,37,39,40,82] This analysis is based upon the greater ^{18}O perturbation when in a P=O bond compared

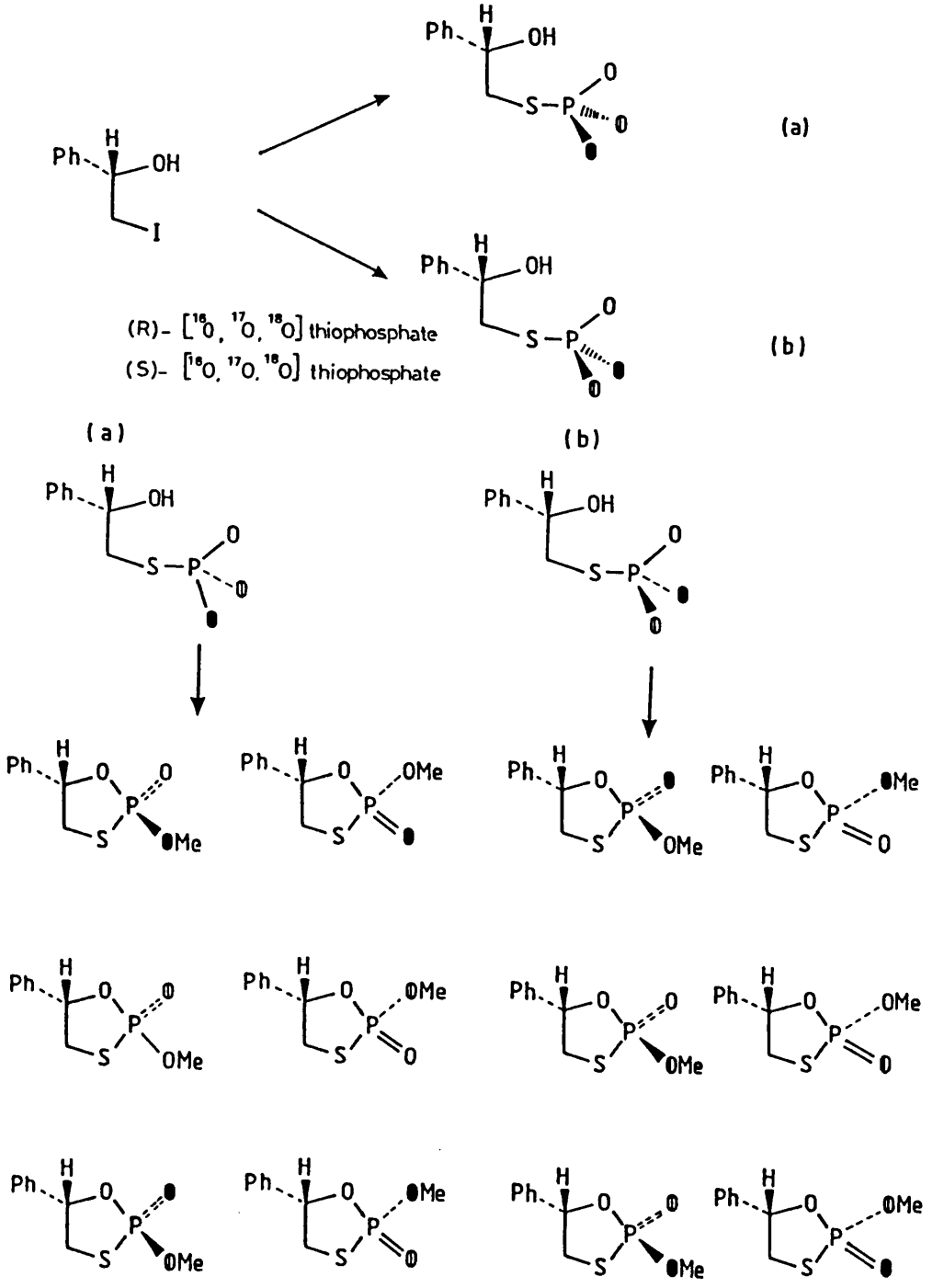


FIGURE 5.4 Configurational analysis of isotopically chiral inorganic thiophosphate by Arnold and Lowe based upon the analysis developed for [^{16}O, ^{17}O, ^{18}O] phosphate monoesters.

to a P-O-R bond. By comparing the ratio of the peak intensities with those calculated from the known isotopic compositions of the Rp and Sp [^{16}O, ^{17}O, ^{18}O] thiophosphate the precise enantiomeric excess can be determined.

Development of an alternative synthesis and configurational analysis of isotopically chiral inorganic thiophosphate

In this part of our study we want to develop an independent synthesis and potential novel configuration analysis of [^{16}O, ^{17}O, ^{18}O] inorganic thiophosphate. In order to exploit the pathway developed for the synthesis of isotopically chiral thiophosphate esters, a method of synthesis for isotopically chiral inorganic thiophosphate was sought based on this pathway. Also, the development of a general configurational analysis of inorganic thiophosphate was investigated.

The synthesis of isotopically chiral inorganic thiophosphate

This synthesis again exploits the stereochemical route developed for isotopically chiral phosphate monoesters. The synthesis uses the 1,3,2-oxazaphospholidin—2-thione ring system to generate chirality. Hydrolysis of the cis chlorocompound (8) in Li^{18}OH introduces the first isotope. Then P-N bond cleavage in $CF_3CO_2H/H_2{}^{17}O$ introduces the second isotope. Removal of the ephedrine moiety was achieved using trimethylsilyliodide.

The product formed Sp [^{16}O, ^{17}O, ^{18}O] inorganic thiophosphate has been derived from the cis chlorocompound (8). The synthesis is shown in Figure 5.5.

The configurational analysis of isotopically chiral [^{16}O, ^{17}O, ^{18}O] inorganic thiophosphate

The proposed analysis is shown in Figure 5.6. This method is concept-

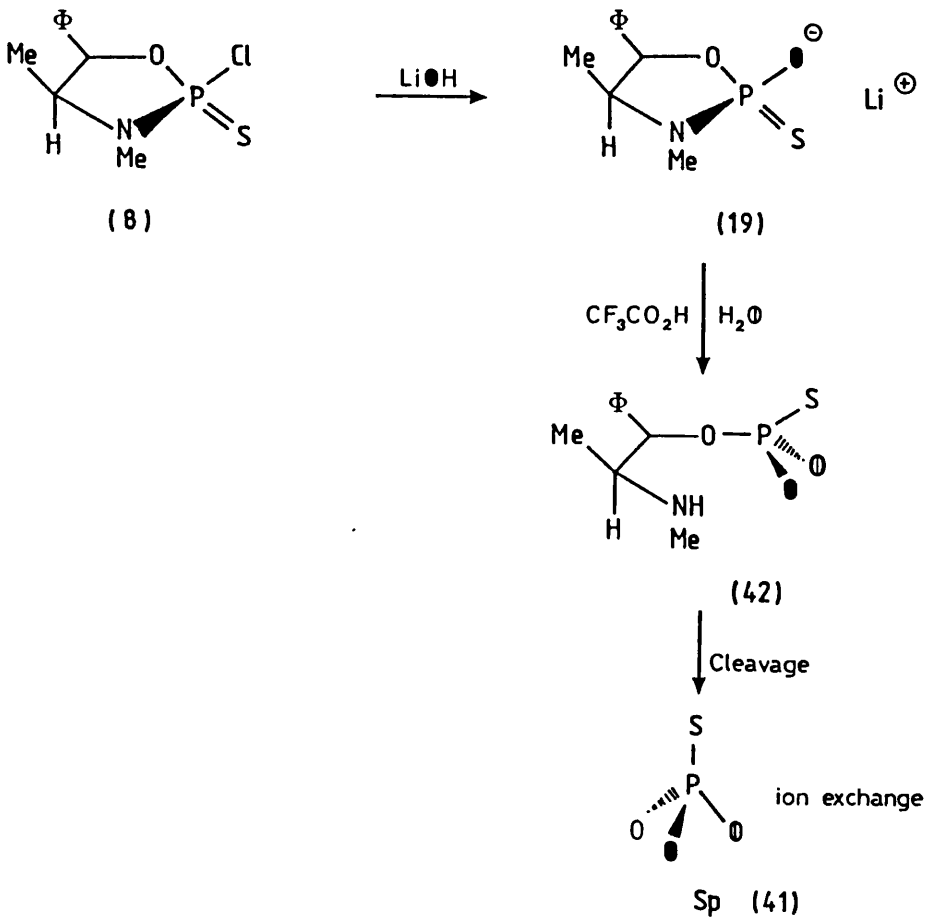


FIGURE 5.5 The proposed synthesis of inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate.

ually similar to that developed for phosphate monoesters in that it requires a phosphoryl residue to be attached to a conventional chiral centre as part of a diol then cyclised to an appropriate conformationally locked system. This system is methylated to give syn and anti isotopmers, which are analysed on the magnitude of bond order shift of ^{18}O via the use of ^{31}P nmr.

However, in this case, a different strategy is required to transfer the phosphate group to a suitable chiral alcohol. The phosphoryl group is to be transferred from inorganic thiophosphate. This then requires the elimination of sulphur. Sulphur can be activated by reaction with a

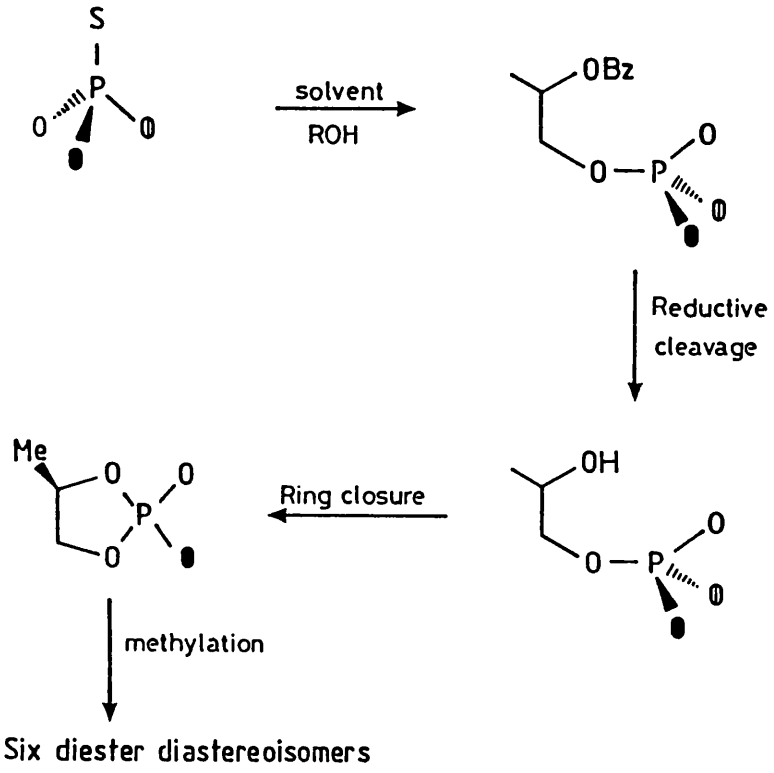


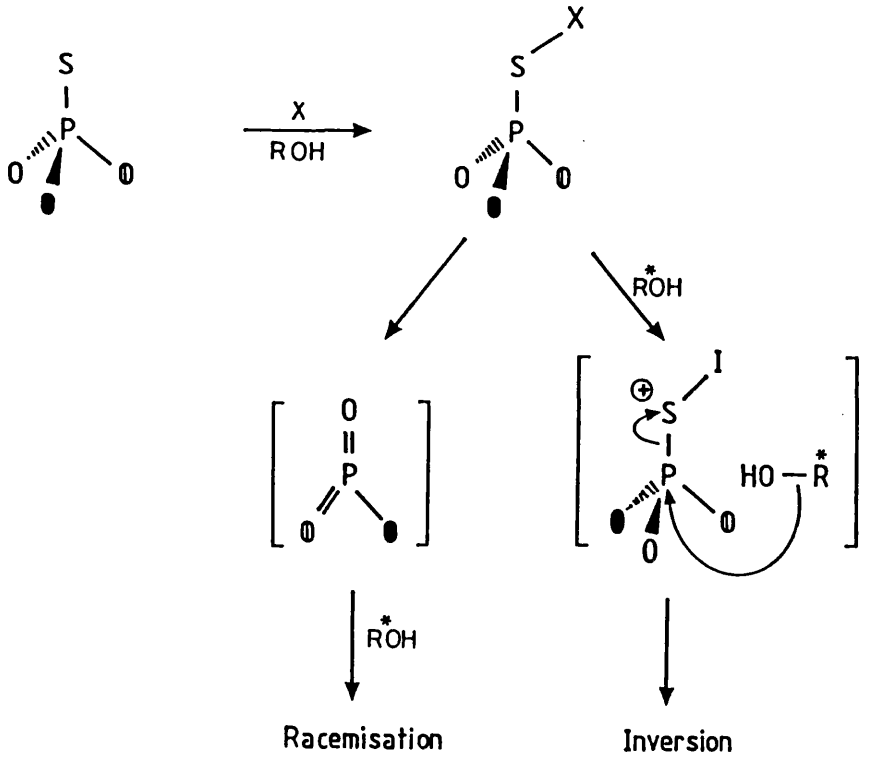
FIGURE 5.6 The proposed analysis of isotopically chiral inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate.

halogen which in the presence of alcohol leads to phosphate monoesters.[151]

The potential problem with this reaction as a configurational analysis of [^{16}O, ^{17}O, ^{18}O] thiophosphate relates to the mechanism of the S-displacement step. If the reaction is associative then the reaction may be expected to proceed stereospecifically, most probably with inversion of configuration. Alternatively, a dissociative reaction could arise if the activated sulphur were a particularly good leaving group and it is unclear what the precise nature of the leaving group is in such reactions.

If a stereospecific transfer could be achieved (i.e. inversion or, less likely, retention) this would then be adapted as a general method of analysis for the absolute configuration of inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate of unknown chirality. If, on the other hand, a non-stereospecific result occurred (racemisation), this would be evidence of the

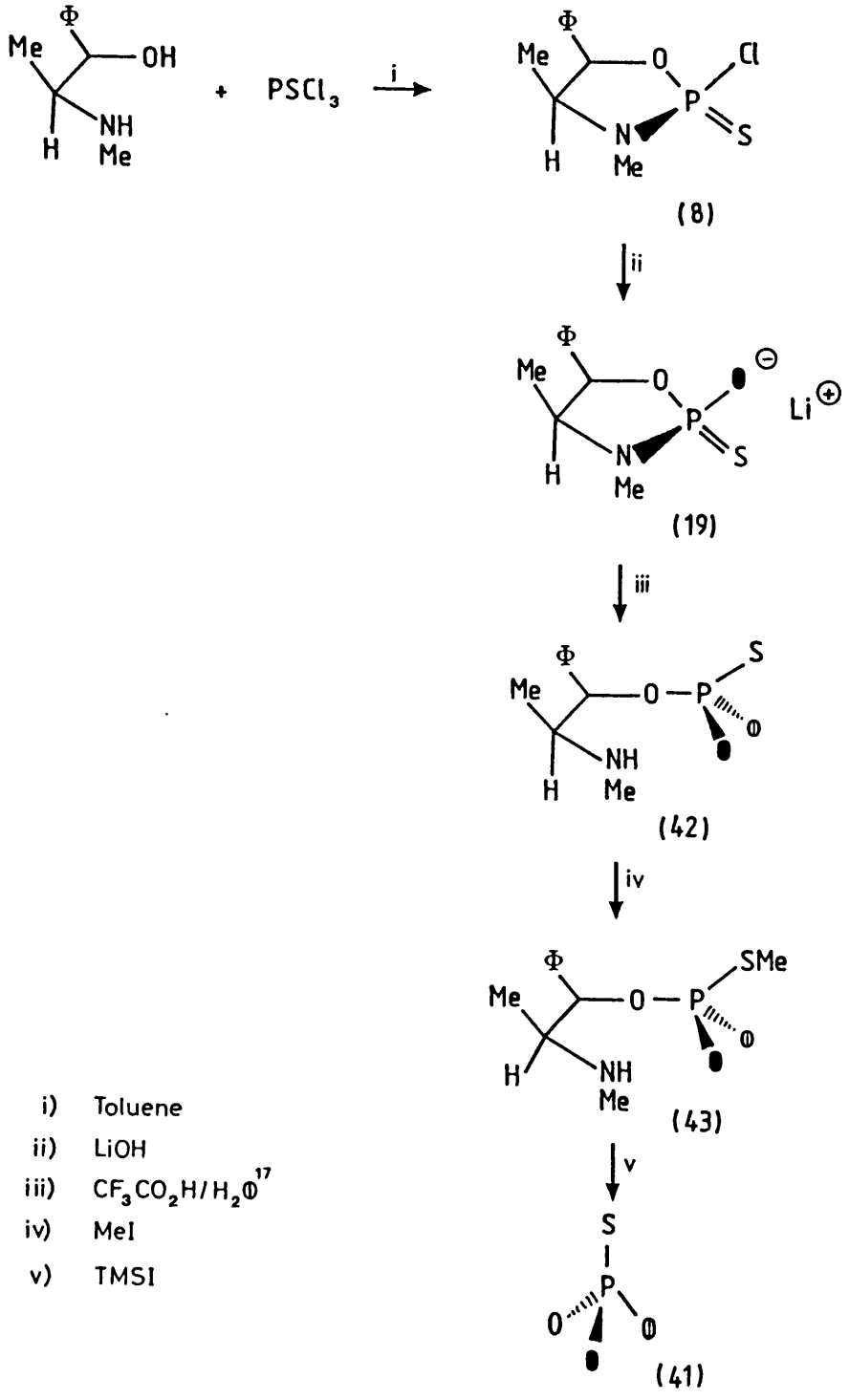
existence of a monomeric metaphosphate in protic solvent and would itself be of interest. The alternatives are shown below.



The synthesis of inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate

This synthesis utilizes the pathway developed by Inch et al. 1,3,2-oxazaphospholidine-2-thione was prepared from thiophosphoryl chloride and ephedrine. The product (8) after separation via liquid chromatography was obtained in good yield. Hydrolysis of the cis chlorocompound (8) in $Li^{18}OH$ has been shown to proceed smoothly and introduce the first isotope as ^{18}O. This step is known to proceed with retention of configuration[80] (see Chapter 2). Therefore the product will be of cis configuration (19).

P-N ring hydrolytic cleavage is effected by treatment of trifluoroacetic acid in $H_2{}^{17}O$. This then introduces the second isotope into the framework. The product (41) formed as shown in illustration 5.7 involves an "in-line" displacement as shown by the studies of Inch et al.[80] This reaction also went as predicted.



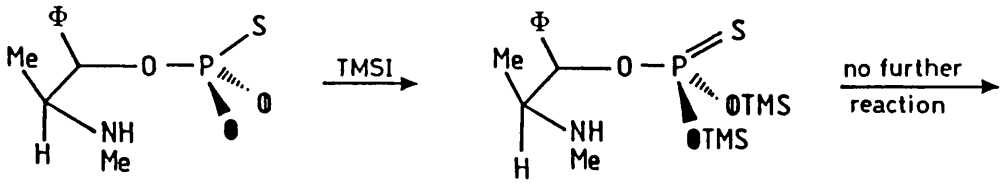
i) Toluene
ii) LiOH
iii) CF_3CO_2H/H_2O^{17}
iv) MeI
v) TMSI

FIGURE 5.7 The synthesis of chiral inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate.

However, the step which involved C-O bond cleavage to release the isotopic inorganic thiophosphate proved difficult. Use of sodium in

liquid ammonia lead to phosphates and possibly phosphoro amidates.

An alternative reagent to effect the C-O cleavage was investigated, trimethylsilyliodide as used by Knowles et al.[30] After the initial reaction no further reaction to effect C-O cleavage could be achieved even with excess reagent.

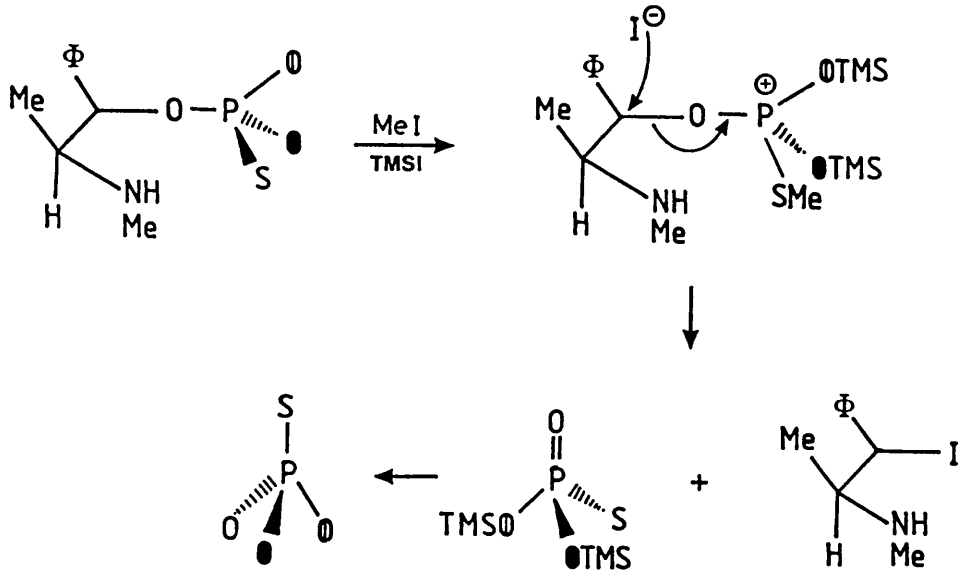


When the reaction was heated loss of sulphur was apparent. ^{31}P nmr of product on heating with TMSI gave peaks at ca. 0 ppm which is consistent with loss of sulphur.

In a silyation reaction a P=S bond is much less reactive than a P=O bond.[93] The reluctance shown by thiophosphates to react with trialkylhalosilanes constitutes a good example of the low nucleophilicity of P=S groups towards silicon, which contrasts the exceptionally high nucleophilicity exhibited by the P=O group.[93]

To overcome this problem the sulphur group was again preferentially methylated using methyl iodide, C-O bond cleavage could now be effected using four equivalents of trimethylsilyliodide. After purification via ion exchange chromatography inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate is obtained in good yield. This reaction involves attack of the iodide on carbon to liberate inorganic thiophosphate (see Chapter 2) and, as such, there is no stereochemical implications at phosphorus. Based on the known stereochemical course of the reaction involved (Scheme 5.7) Sp inorganic [^{16}O,^{17}O,^{18}O] thiophosphate is formed (41). The above synthesis of inorganic thiophosphate was carried out without isotopes.

The analysis of inorganic [^{16}O,^{17}O,^{18}O] thiophosphate requires the following:-



[1] The preparation of a suitable chiral molecule of known configuration.

[2] The efficient attachment of inorganic phosphate to this molecule.

[3] A stereospecific cyclisation.

[4] The assignment of two diastereoisomers of triesters to their respective ^{31}P nmr chemical shifts.

2-O-benzyl-(S)-propanediol was used as the chiral acceptor molecule. The alcohol has a chromophore and so is detectable by ultraviolet monitoring. The chiral molecule has already been used for the configurational analysis of [^{16}O, ^{17}O, ^{18}O] phosphoryl groups[102,103] and therefore seemed the obvious choice in this case.

The procedure for the preparation of 2-O-benzyl-propanediol[100] is shown in Scheme 5.8.

Preparation of 2-O-benzyl-(S)-propane-1,2-diol

The first stage of this reaction involves the preparation of fresh silver oxide (44), which is then used in subsequent reactions to form 2-O-benzyl-(S)-propane-1,2-diol (46). All three stages proceeded smoothly to form the chiral alcohol. Identification of the product was confirmed by ^{1}H nmr. The optical purity was >95% which was sufficient

for use in the analysis.

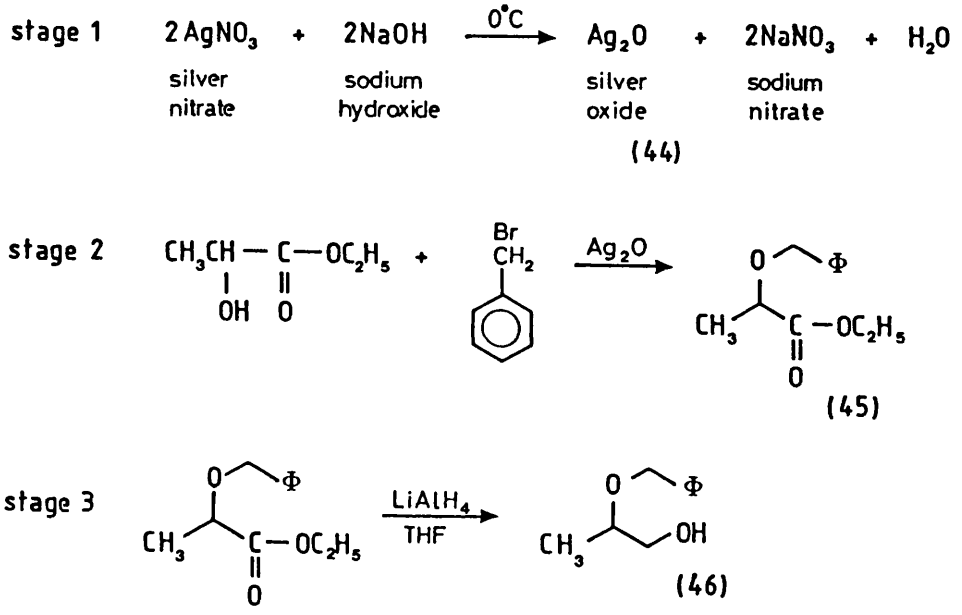


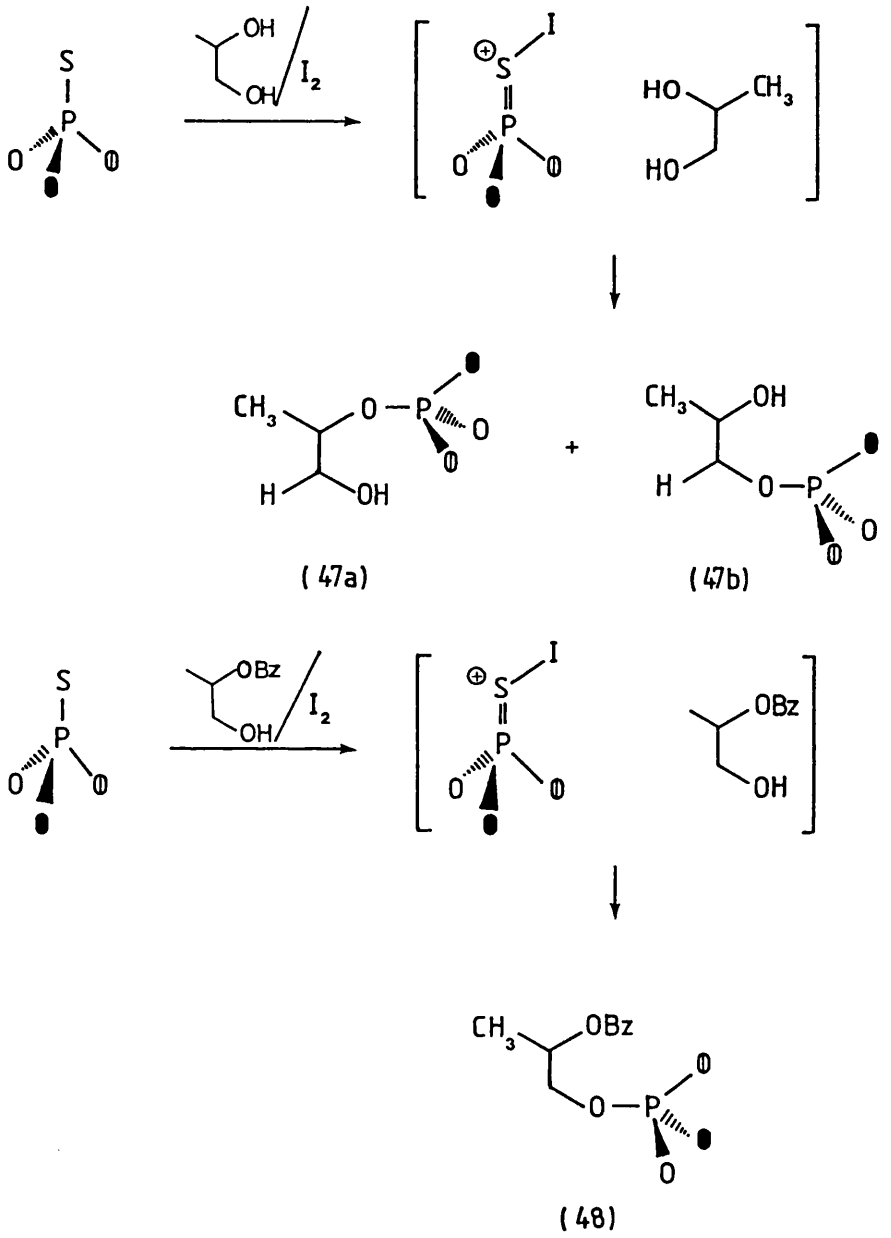
FIGURE 5.8 The preparation of 2-O-benzyl-propanediol.

Phosphoryl transfer

In order to achieve phosphoryl transfer from inorganic thiophosphate to an alcohol molecule, the sulphur group has to be activated by reaction with a halogen.

The sodium salt of inorganic thiophosphate was reacted with several halogens under different reaction conditions in the presence of an alcohol. However, the best conditions appeared to be the reaction of the sodium salt of inorganic thiophosphate with two equivalents of iodine at room temperature in propane-1,2-diol. This lead to the formation of 1- and 2-phospho-(S)-propane-1,2-diol (47).[64] These were identified by ^{31}P nmr and ^{1}H nmr after purification using ion-exchange and the Briggs phosphate test. This test is used to detect phosphates which have no substituents containing chromophores attached to the phosphorus. The reaction is shown in Scheme 5.9.

Having found the ideal conditions for the transfer of the phospho



SCHEME 5.9 Proposed phosphoryl transfer.

group from inorganic thiophosphate, 2-O-benzyl-(S)-propanediol (46) was now used as the chiral acceptor molecule for the phosphoryl group. However, a few problems were encountered in this procedure, namely that the sodium salt of inorganic thiophosphate would not dissolve in the alcohol (46). The sodium cation was then exchanged for the triethylammonium cation using Dowex 50 (NEt_3). The triethylammonium salt of inorganic thiophosphate was now soluble in the 2-O-benzyl-propanediol and treatment

with iodine led to smooth phosphoryl transfer. The products were purified *via* ion exchange using triethylamine buffers and identified using ^{31}P nmr and ^{1}H nmr. This reaction was carried out using unlabelled material. The products that would be formed using ^{17}O and ^{18}O are [1(R/S)^{16}O,^{17}O,^{18}O] phospho-benzyl-(S)-propane-1,2-diol (48). The reaction is shown in Scheme 5.9.

The next step of the analysis involved the removal of the benzyl group. This was achieved *via* hydrogenation using a palladium on charcoal catalyst. However, two hydrogenations were required to totally remove the benzyl group. This is probably because traces of residual sulphur-containing compounds led to poisoning of the palladium catalyst.

The last two stages involve cyclisation of a five-membered ring followed by methylation of the product. The closure of the five-membered ring is stereospecific involving inversion of configuration[101] and is achieved in fairly high yields often in the region of 80%.

The remaining steps of the configurational analysis are literature procedures. Therefore this represents a formally complete route.

CONCLUSION

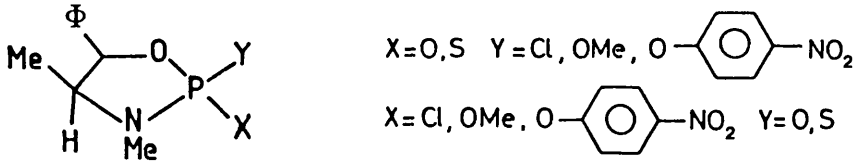
A novel synthesis and configurational analysis of inorganic [^{16}O, ^{17}O, ^{18}O] thiophosphate have been developed. Time did not permit the stereochemical course of the sulphur displacement step to be determined. This approach may still provide an alternative general analytical method to that developed by Arnold and Lowe or, if accompanied by racemisation, may provide evidence for the existence of monomeric metaphosphate in protic solvents.

CHAPTER 6

Studies on the Mechanism of Epimerisation of 1,3,2-Oxazaphospholidin-2-ones and -2-thiones

INTRODUCTION

Compounds of the general type shown below have been widely used in the synthesis of [^{16}O,^{17}O,^{18}O] phosphate monoesters,[45,76] [^{16}O,^{18}O] thiophosphate monoesters,[79] and in the configurational analysis of [^{16}O,^{18}O] thiophosphate monoesters[79,175] as well as in the determination of the enantiomeric excess of chiral amines and alcohols[152-156] [see Figure 6.1].



Each of these uses depend upon the availability of such structures as single enantiomers and upon the stereocontrolled displacement reactions of such systems.

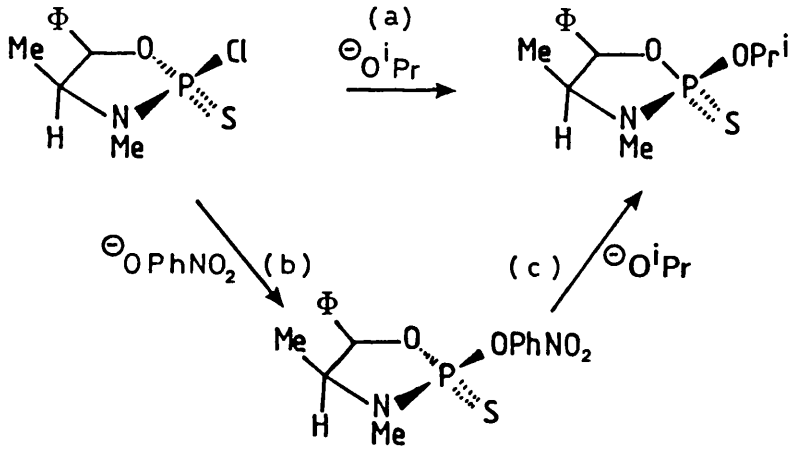
It is generally assumed that the 2-chloro-1,3,2-oxazaphospholidin-2-one and the corresponding -2-thione derived from (-)ephedrine are configurationally stable.[1,80,157] Furthermore, it is a cornerstone of organophosphorus chemistry that exocyclic displacement reactions at phosphorus held in a five-membered ring proceeds with retention of configuration because of the strong preference of the ring to span apical-equatorial positions in the trigonal bipyramid.[23]

During the course of developing the stereochemical analysis described in Chapter 3 it was noted that O-ethyl thiophosphate reacted with the cis chloro compound (24) with some apparent loss of stereochemical control. The origin of this epimerisation reaction and the general configurational stability of these systems were therefore investigated.

5-membered ring phosphorus compounds have been studied by Inch[80] and Westheimer.[1]

Inch et al.[80] have investigated the stereochemistry of 1,3,2-oxazaphos-

pholidin-2-ones and 2-thiones. ^{1}H nmr data and the correlations of specific rotations, provide evidence that nucleophilic exocyclic displacement of good leaving groups generally occurs with retention of configuration.[158] Supporting chemical evidence for this conclusion was obtained when direct displacement of chloride by isopropoxide[159] gave the same product as the displacement of chloride by nitrophenoxide followed by isopropoxide as shown.

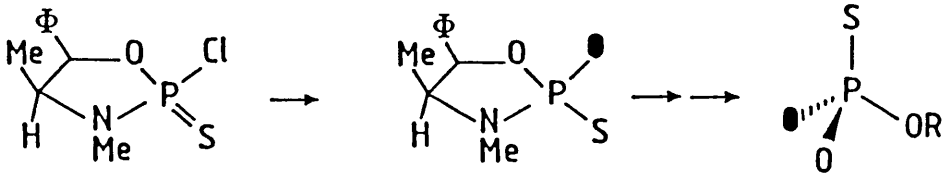


In order for both pathways to produce the isopropoxide product with the same configuration at phosphorus would imply that nucleophilic displacement at the 1,3,2-oxazaphospholidin-2-thione occurs with retention of configuration.

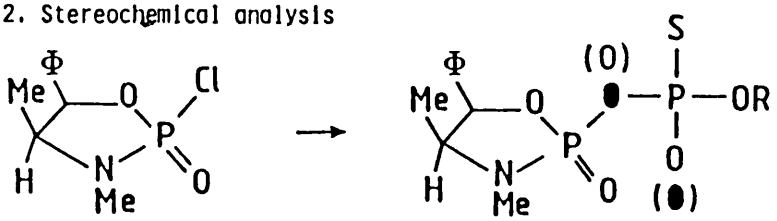
If, however, inversion of configuration is assumed as the outcome of nucleophilic displacement at the 5-membered ring, then the two pathways (a) and (b)(c) would lead to products with different stereochemistries. Pathway (a) would then form the isopropoxide product with inversion of configuration. By the same argument, pathway (b) would form a product with inversion of configuration as would pathway (c). Therefore the overall stereochemical result of pathway (b) and (c) would be retention of configuration at the isopropoxide product.

Therefore, only by concluding that nucleophilic displacement reactions at 1,3,2-oxazaphospholidines occur with retention of configuration can

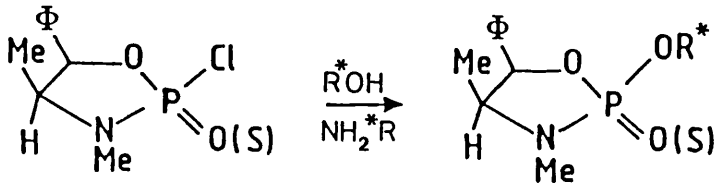
1. Isotopic synthesis



2. Stereochemical analysis



3. Chiral derivatising agent



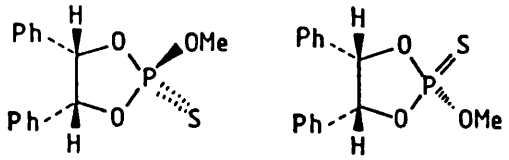
^{31}P NMR determination of enantiomeric excess

Each require exocyclic displacement to occur stereospecifically with retention.

FIGURE 6.1 Stereocontrol in 1,3,2-oxazaphospholidines.

the products of both pathway have the same stereochemistries.

Lowe et al.[88] have shown some interesting work on the studies of the formation of cis-2-methoxy-4,5-diphenyl-1,3,2-dioxaphospholan-2-thione (50a) and its trans isomer (50b).



(50a) cis (50b) trans

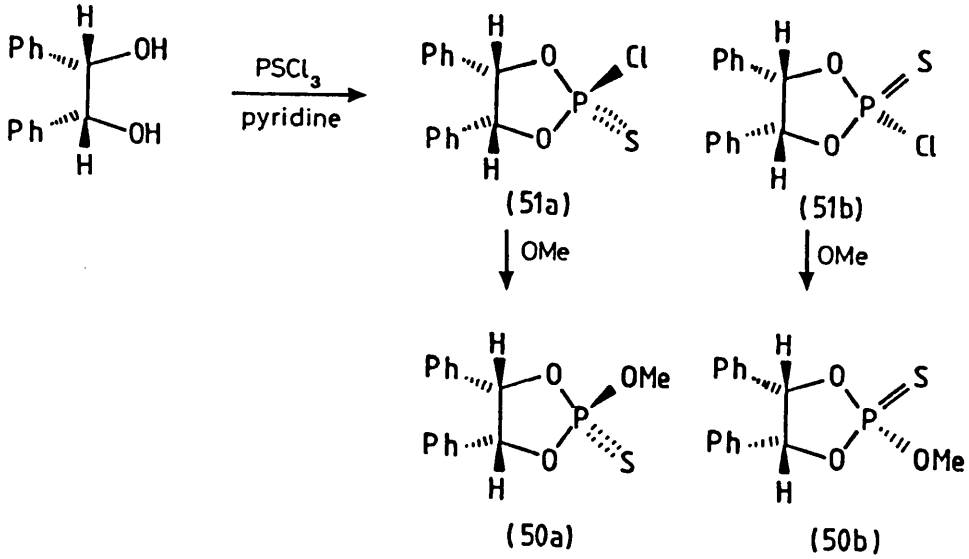
The cis isomer (50a) is formed stereospecifically from the trans isomer. The trans isomer (50b) was obtained by treating meso-hydrobenzoin with thiophosphoryl bromide in the presence of pyridine followed by methanolysis of the resulting product in the presence of triethylamine. Although both isomers (50a/50b) were present in the reaction mixture as a ratio of 1:4, the trans isomer (50b) was the only isolated product. However, when pyridine was used in excess or as solvent with thiophosphoryl bromide or chloride, the predominant product after methanolysis was the cis isomer being favoured (8:1).

These observations apparently cannot be explained by invoking an exocyclic substitution at phosphorus by either chloride or pyridine as both Westheimer and Inch have demonstrated that they occur with retention of configuration.

Lowe explained these reactions by proposing a mechanism involving a reversible ring opening of the phosphorochloridate (or bromidate) in the presence of pyridine allowing the kinetically favoured product to be transformed to the thermodynamically more stable product.

This interpretation was supported by the following evidence. The reaction of meso-hydrobenzoin and thiophosphoryl chloride in the presence of pyridine was monitored by ^{31}P nmr. After 30 minutes cis and trans 2-chloro-4,5-diphenyl-1,3,2-dioxaphospholan (51a/51b) isomers appeared in a ratio of 1:3 but after a further 20 minutes only the cis 2-chloro-4,5-diphenyl-1,3,2-dioxaphospholan remained (51a). When the reaction was repeated and quenched with methanol after 30 minutes the ratio of the cis (50a) and trans (50b) isomers established that the precursor of the trans isomer is the kinetically favoured product and the cis isomer precursor the thermodynamically favoured product.

In contrast, Inch had not observed any analogous epimerisation reactions



which would involve a similar reversible ring opening pathway for the 2-halo-1,3,2-oxazaphospholidin-2-one and 2-thione derived from ephedrine.

Reactions of 1,3,2-oxazaphospholidin-2-one and 2-thione

The complete loss of stereocontrol during the configurational analysis as described in Chapter 3 arose when O-ethyl thiophosphate was reacted with the cis chlorocompound (24) in the presence of pyridine. The diastereoisomers (52a) and (52b) are formed in comparable amounts over several hours. This raises the question of "how is the trans product formed?". The various possibilities in which the trans product may be formed are illustrated in Figure 6.2.

These alternative pathways will now be discussed:-

Pathway A

In this pathway the nucleophile O-ethyl thiophosphate reacts stereospecifically with the cis chlorocompound (24) [as demonstrated by Inch et al.] to form the pyrophosphate product of cis configuration (52a). This product then epimerises to give the trans pyrophosphate product (52b).

Pathway B

The cis chlorocompound (24) reacts with O-ethyl thiophosphate without

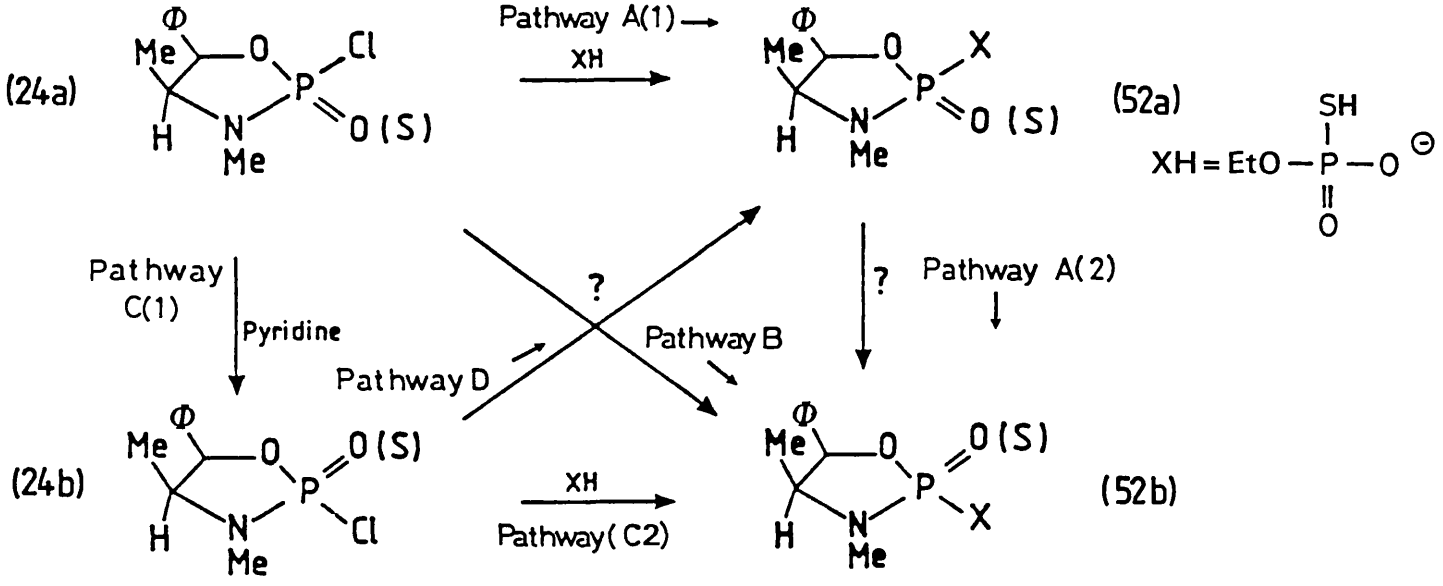


FIGURE 6.2 Proposed pathways for the formation of trans product.

complete stereospecificity to form the trans pyrophosphate (52b) directly (this, however, is not in keeping with previous literature precedent].

Pathway C

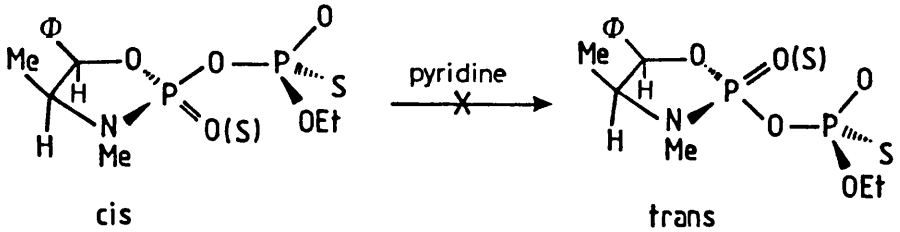
This pathway involves the direct epimerisation of the cis chloro-compound (24a) to the trans chlorocompound (24b) in the solvent pyridine or other nucleophile. The trans chlorocompound (24b) then reacts stereo-specifically with O-ethyl thiophosphate to form the trans pyrophosphate product (52b).

Pathway D

In this case the cis chloro compound (24a) is epimerised by pyridine to form the trans chloro compound (24b) which may then react non stereo-specifically.

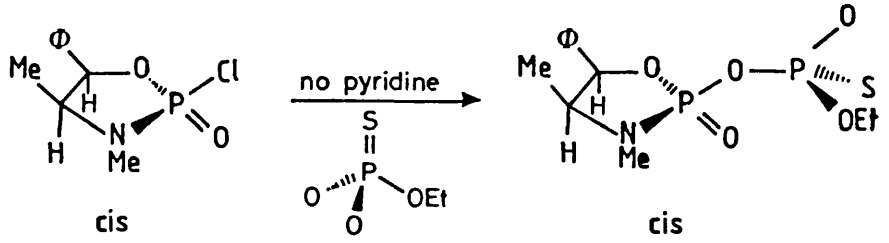
To determine which pathway is followed and whether the reaction path way is affected by the solvent "pyridine" or nucleophile "O-ethyl thio-phosphate" the following studies were undertaken.

In the absence of other nucleophiles cis pyrophosphate product (52a) was dissolved in pyridine and the reaction was monitored via ^{31}P nmr at room temperature over several days. After this time no epimerisation had taken place, i.e. there had been no conversion of cis pyrophosphate product to trans pyrophosphate product. This evidence excludes pathway A.



cis trans

It was found that when the cis chlorocompound (24a) was reacted with O-ethyl thiophosphate in the absence of pyridine the major product formed is the cis pyrophosphate. This strongly suggests that the solvent pyridine



is involved in the epimerisation reaction.

The cis chlorocompound (24a) was dissolved in pyridine and the reaction was monitored by ^{31}P nmr. The cis chlorocompound (+19.54 ppm) was gradually converted to the trans chlorocompound product (+24.0 ppm). This is shown in Figure 6.3. After 24 hours the reaction is complete and the equilibrium mixture favours the trans material by ca. 2:1. This thermodynamic mixture is obtained from either pure cis or pure trans (24a/24b). When (-)ephedrine and phosphoryl chloride were reacted in pyridine the trans product (24b) was obtained as the major isomer directly. The isolated material is identical with the minor isomer reported by Inch [m.p. 110-111°C; δ (^1H) $CDCl_3$; 0.80 (3H, d, J 7Hz); 2.65 (3H, d, J 13Hz); 3.70 (1H, ddq, J 7Hz, 7Hz and 7Hz); 5.54 (1H, dd, J 7Hz and 7Hz); 7.3 (5H, aromatic)].

The corresponding thiochloride (8) was also investigated in this manner. This ring system had been used to synthesise [^{16}O,^{17}O] thiophosphate monoesters [Chapter 2] and any configurational instability of this system would have consequences for the enantiomeric excess in the resulting isotopically chiral phosphate esters. The thermodynamic mixture of epimers at phosphorus again favoured the trans thiochlorocompound on treatment of the cis compound (8a) with anhydrous pyridine, the equilibrium ratio being 3:1 trans to cis. The rate of reaction was considerably slower ($t_{\frac{1}{2}}$ ca. 16 hours at 50°C) than the corresponding reaction of (24a). This difference in reactivity between trisubstituted thiophosphates as compared to phosphates in associative nucleophilic substitution reactions

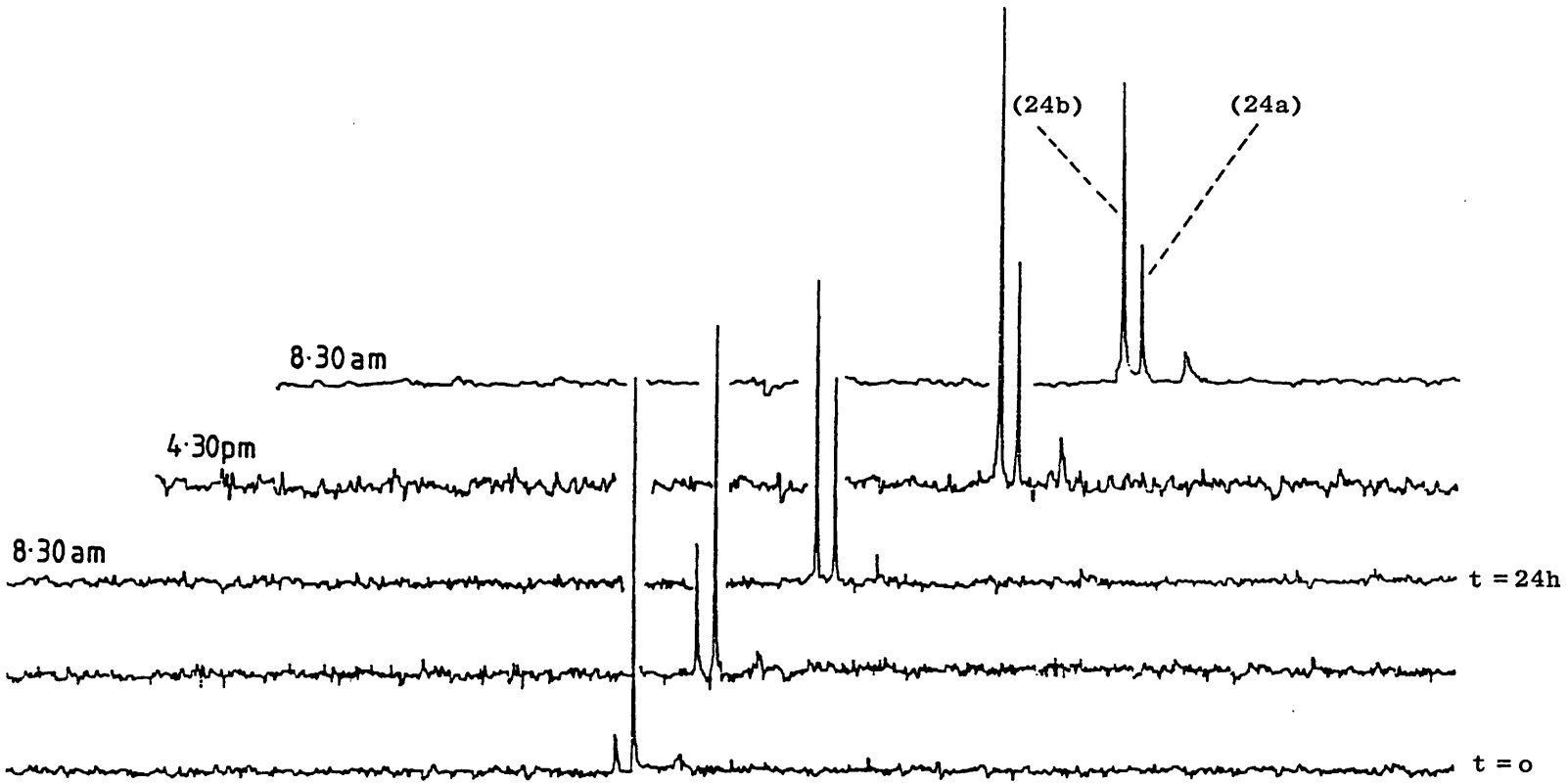
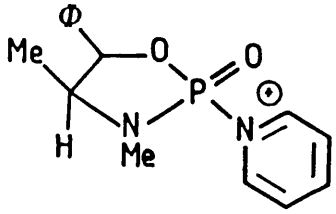


FIGURE 6.3 The epimerisation of cis chlorocampound (24a) to the trans chlorocampound (24b) at room temperature over 24 hours.

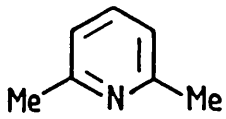
is as expected.[49]

The trans products (8b/24b) are the thermodynamic products and the cis (8a/24a) the kinetically favoured products.

These reactions help support the idea that pyridine must be acting as a nucleophile in the mechanism of these epimerisation reactions, and implies that a pyridinium species of the following structure is formed during the reaction.



Alternatively, we cannot exclude Cl^- as the nucleople, however the chlorocompound (24) does not epimerise in anhydrous acetone in the presence of LiCL, this excludes the formation of traces of base hydrochloride as the cause of epimerisation. Replacing pyridine with a less nucleophilic base such as lutidine (shown below) considerably reduces the rate of epimerisation.



The mechanism of these epimerisation reactions must incorporate the following criterion:-

(1) A mechanism which leads to inversion of configuration.

(2) A pathway which allows the formation of a pyridinium species.

Two principal pathways for this type of reaction can be considered. The mechanism shown in Scheme 6.4 involves a ring-opening pathway, as has been suggested for the epimerisation of the cyclic phosphorochloridate and thiophosphorochloridate derived from meso hydrobenzoin.[80] The mechanism of this reaction is as follows. An adjacent attack of the pyridine on the phosphorus atom of the cis chlorocompound (24a)

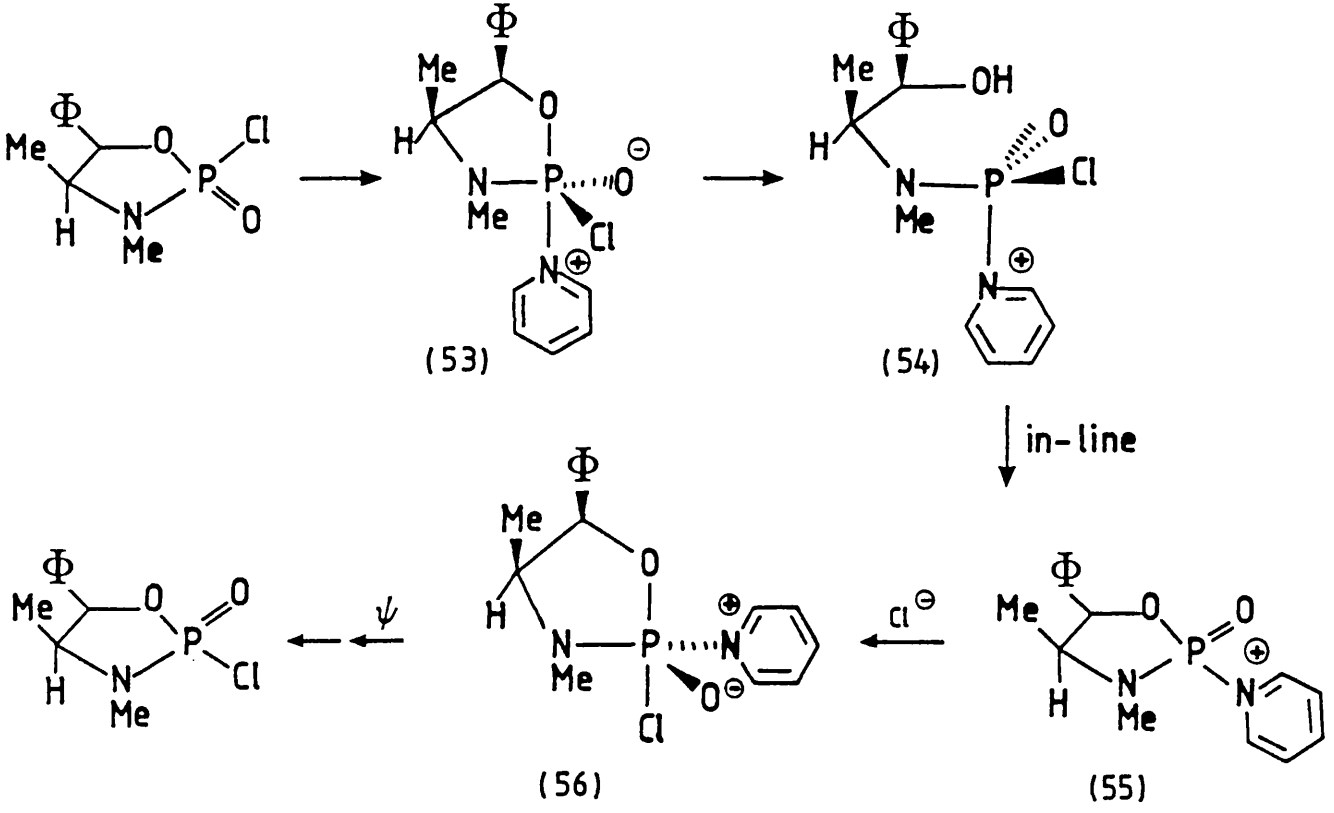


FIGURE 6.4 Epimerisation reaction - ring-opening mechanism.

places the ring axial-equatorial in the trigonal bipyramid (53). The axial P-O bond of the five-membered ring in the trigonal bipyramid is then cleaved to form a ring-opened species (54). Reformation of the five-membered ring by an in-line mechanism leads to loss of the chlorine atom and formation of a trans pyridinium species (55). This is then followed by an adjacent attack of the chloride ion to the pyridine substituent in the trans pyridinium species (56). Pseudorotation then places the pyridinium substituent axially, the pyridinium ion departs to give the trans chlorocompound.

The alternative pathway is shown in Figure 6.5 and involves a direct in-line displacement via a pentacoordinate intermediate. The mechanism of this pathway is as follows. A direct in-line displacement of the chloride ion by pyridine occurs transforming the cis chlorocompound (24) to the trans pyridinium species (58) via a trigonal bipyramid intermediate in which the ring is placed diequatorially (57). Adjacent attack of the chloride ion to the pyridinium substituent leads to the formation of a trigonal bipyramidal species in which the ring is placed axial-equatorial (59). Pseudorotation of such a species places the pyridinium substituent axial, which departs to leave a trans chlorocompound. [The reaction could also occur with initial attack of the pyridine with retention, followed by the chloride ion with inversion of configuration.]

It is difficult to distinguish between these pathways as no intermediates were detected by ^{31}P nmr spectroscopy. Although the chloro substituent makes the phosphorus more susceptible to nucleophilic attack the ring-opening/ring-closure pathway might be expected to occur with substituents other than chloride. Epimerisation would be observed provided that the acyclic pyridinium species analogous to (54) suffers subsequent inversions at phosphorus. The proposed mechanism for the conversion of cis methoxy ester (61a) to trans methoxy ester (61b) is shown in Figure 6.6. Cis 2-methoxy-1,3,2-oxazaphospholidine (61a) suffers

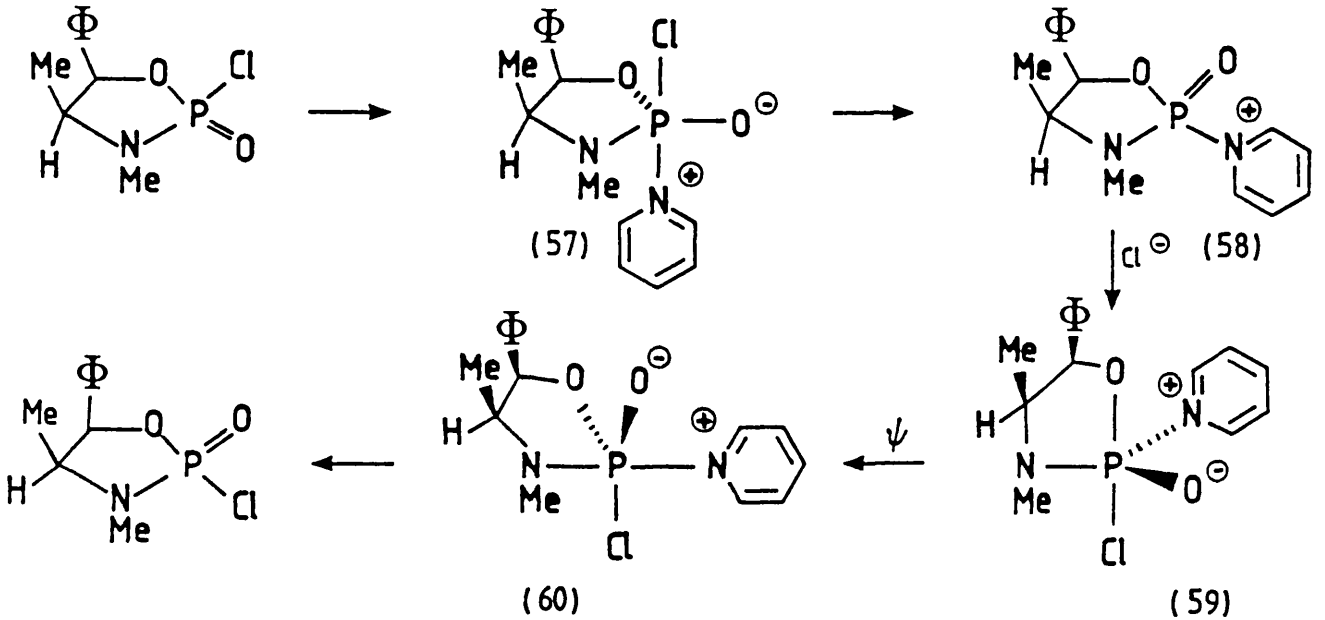
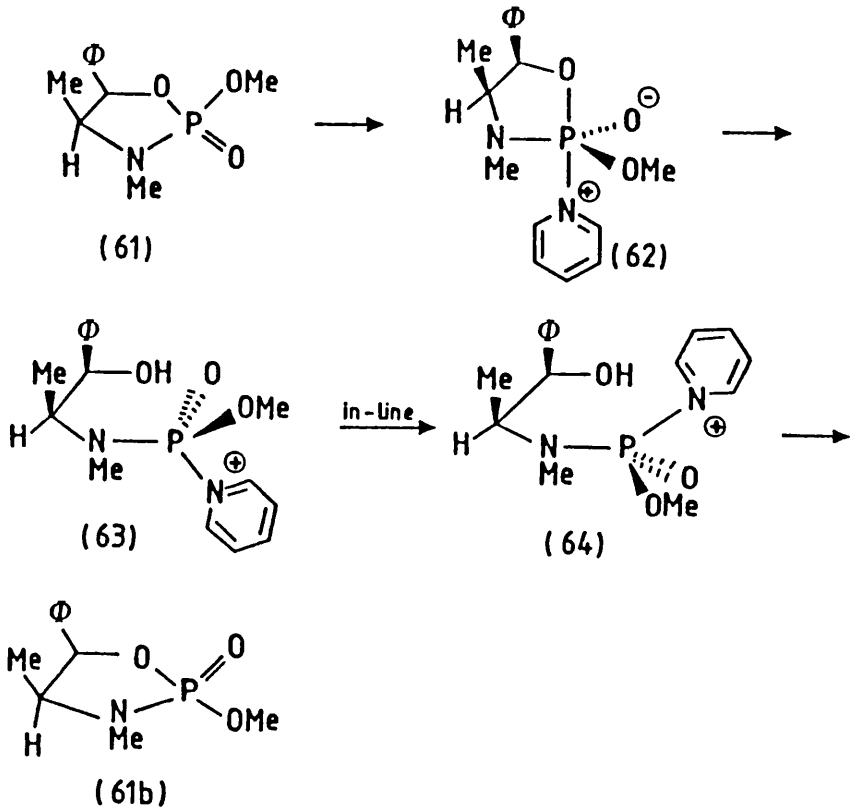


FIGURE 6.5 Epimerisation reaction - via diequatorial ring.

(1) Epimerisation could occur via a ring opening pathway



(2)

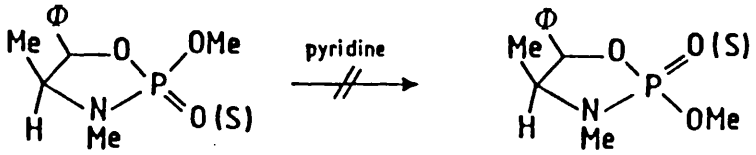


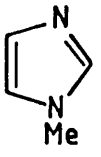
FIGURE 6.6 (1) Proposed mechanism for the epimerisation of cis methoxy ester (61);
(2) Observed reaction of cis methoxy ester (61) in pyridine.

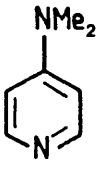
nucleophilic attack at the phosphorus atom adjacent to OMe by pyridine. This forms a trigonal bipyramidal intermediate. In this intermediate the OMe group stays in the preferred equatorial position due to stereo-electronic and apicophilicity requirements [see Chapters 1 and 7]. Therefore ring-opening occurs via P-O bond breaking. Pseudorotation of the substituents around the phosphorus atom after reformation of the ring leads to the loss of the pyridinium ion and the formation of trans 2-

methoxy-1,3,2-oxazaphospholidin-2-one (61b). The formation of (61b) can only be achieved via ring-opening/ring-closure. However, for X = O; Y = OMe, no epimerisation in pyridine was observed under comparable conditions. This was also true for X = S; Y = p-nitrophenyl. This provides circumstantial evidence against a ring-opening mechanism and in favour of an in-line displacement pathway. A pathway involving a diequatorial placement of the five-membered ring would be unusual because of the presumed increased strain in the phosphorane plus the movement of the lone pairs on the heteroatom out of their preferred orientation in the equatorial plane. However, the energy required to move a ring diequatorial from the preferred axial-equatorial conformation in a phosphorane has been estimated to be only ca. 20 kcal mol^{-1}.[23] This barrier may not be too great to be overcome and can, in part, be offset by the relative apicophilicities of the other substituents, i.e. chlorine.

Epimerisation was also expected with other nucleophilic catalysts such as N-methyl imidazole and N,N-dimethylaminopyridines in the presence of both (24) and (8). These catalysts are both more nucleophilic than pyridine and, as such, would be expected to quickly and effectively epimerise the cis compounds of (24) and (8) to their respective trans compounds.

However, in reality, when N,N-dimethylaminopyridine and N-methyl imidazole were reacted with the cis compound (8a) the respective trans compound was not formed but an unassigned resonance in the region of +72 - +75 ppm appeared in both cases.

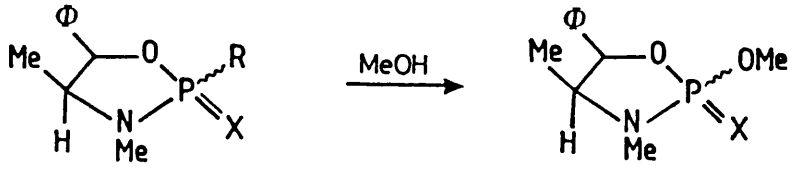




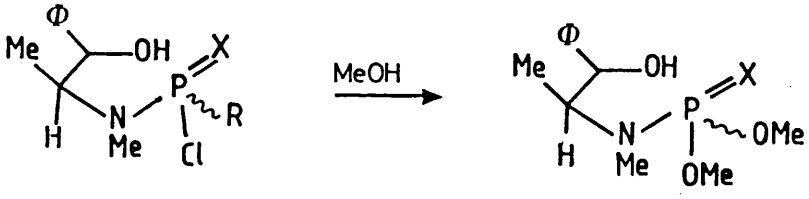
The ^{31}P chemical shift of these resonances suggest a species in which the 1,3,2-oxazaphospholidine ring is still intact.

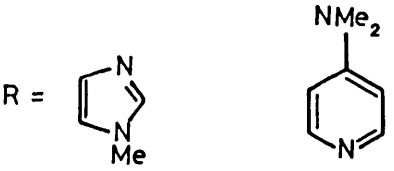
In order to probe the structure of such intermediates, the reactions were quenched with methanol. The most reasonable alternatives are shown below.

(I)



(II)

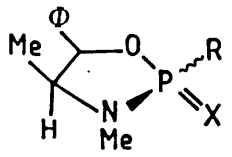




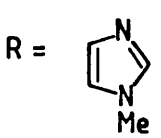
X = O, S

The outcome of this reaction led to the formation of the cyclic triester (61) as a mixture of epimers in the case of (8) with N-methyl imidazole and N,N-dimethylaminopyridine.

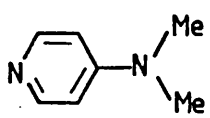
These results are therefore consistent with the intermediates with ^{31}P nmr resonances (+74 to +75 ppm) having a ring-closed structure as shown below.



X=O, S



(65)

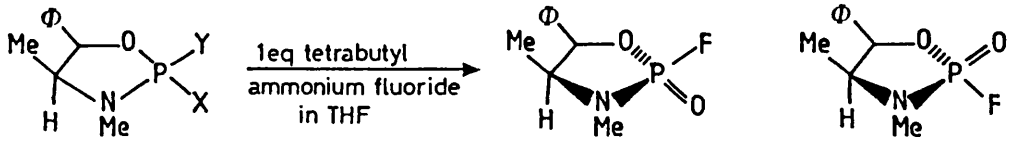


(66)

This implies that both N-methyl imidazole and N,N-dimethylamino pyridine form comparatively stable compounds with the chlorocompounds (8a/8b). This may be due to their greater nucleophilicity, i.e. an increase in the number of nitrogen atoms within the structure.

The possibility that the species of resonance +75 ppm may have been the trans chlorocompound (8b) of a different chemical shift (because of solvent change) was eliminated by the following reaction. A mixture of cis and trans chlorocompounds (8a/8b) reacted with N-methyl imidazole. As the reaction progressed, the trans chloro product (8b) could still be identified while the resonance at +75 ppm appeared. This strongly suggests the existence of a structure of that shown previously.

With exocyclic substituents other than halogen no such epimerisations were observed, however when either (8a) or (24a) is treated with one equivalent of tetrabutylammonium fluoride in THF identical mixtures of the epimeric cyclic fluorides (67a/67b) were formed. The instantaneous reaction meant that it was not possible to determine whether the epimers arise in the first displacement step or as a result of epimerising the initial product. This provides an example of an exocyclic displacement reaction not involving amine nucleophiles that proceed non-stereospecifically.[180]



(67a) (67b)

CONCLUSION

Inversion of configuration has been observed as the outcome of exocyclic displacement reactions at phosphoryl and thiophosphoryl centres held in a five-membered ring. Although the reaction occurs at compara-

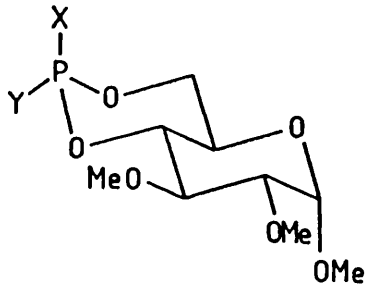
tively slow rates, it is nevertheless an unexpected result. It is important not to assume the stereochemical integrity of intermediates formed when such a system is used to generate isotopic chirality at phosphorus, particularly when the reaction involves a weak nucleophile. The use of pyridine and other nucleophilic catalysts should be avoided when using such systems as chiral auxiliaries to determine the enantiomeric excess of chiral alcohols and amines.

CHAPTER 7

Displacement Reactions at Phosphates held in Six-Membered Rings

INTRODUCTION

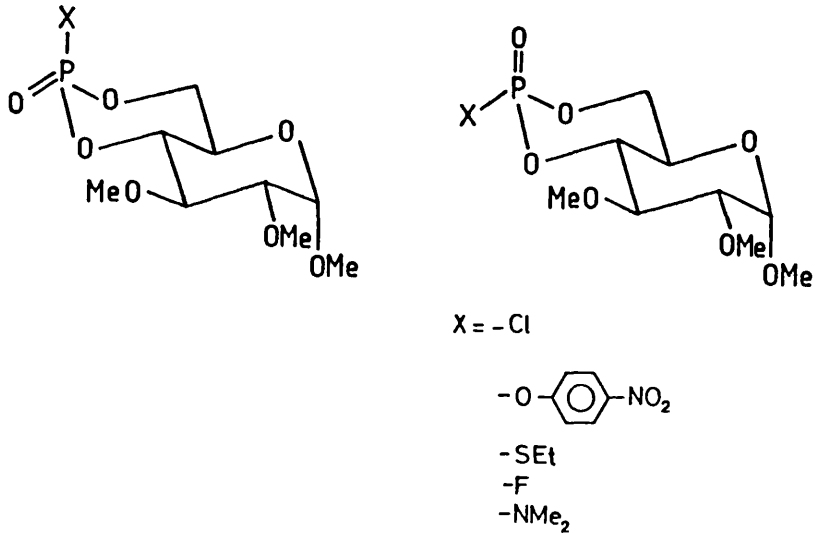
Some years ago Inch and coworkers[160] looked at displacement reactions at phosphates held in a six-membered ring. They studied the 1,3,2-dioxaphosphorinan-2-one system (68) shown below.[161,162]



X=O Y=Cl
X=Cl Y=O

(68)

The reactions undertaken involved the nucleophilic displacement of groups such as Cl, F, p-$O_2N \cdot C_6H_4 \cdot O$ and SR from (68) as shown in Figure 7.1. Such studies were undertaken by Inch in order to probe the possible mechanistic implications of the effect of a six-membered ring on the stereochemistry of nucleophilic displacement reactions at phosphorus.



X = -Cl

-O-(C6H4)-NO2

-SEt

-F

-NMe_2

FIGURE 7.1 Hydrolysis reactions studied.

Depending on the nature of the nucleophile and the leaving group, these reactions followed different (and often mixed) stereochemical courses. These results are shown in Table 7.1.

The 1,3,2-dioxaphosphorinan-2-one (68) is readily available through a

TABLE 7.1

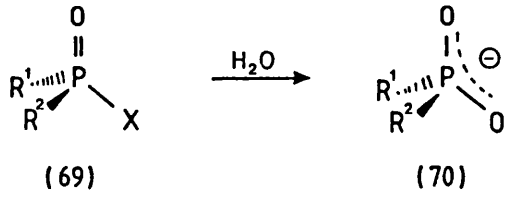
Nucleophilic displacement reactions on 1,3,2-dioxaphosphorin-2-one by Inch

Compound	Leaving group	Nucleophile	Products (%)	
			Inversion	Retention
(72a)	Cl	EtOH	93	4
(72a)	Cl	EtOH-Et_3N	81	trace
(72a)	Cl	EtOH-EtONa	42	54
(73a)	SPr	EtOH-NaOEt	trace	77
(73b)	SPr	EtOH-NaOEt	trace	67
(74a)	p-$O_2N \cdot C_6H_4 \cdot O$	EtOH-NaOEt	trace	88
(74b)	p-$O_2N \cdot C_6H_4 \cdot O$	EtOH-NaOEt	15	75
(75)	F	EtOH-NaOEt	16	46

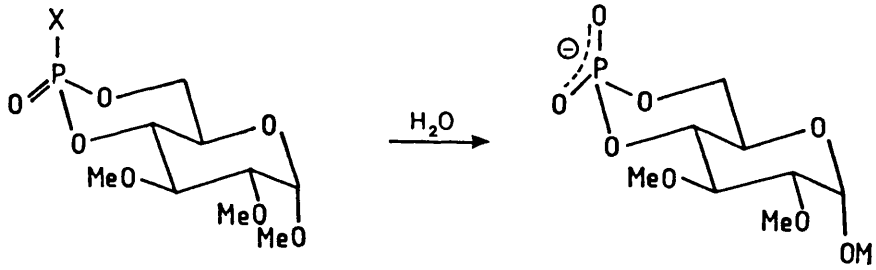
Cl = chloride
SPr = propane thiol
p-$O_2N \cdot C_6H_4 \cdot O$ = p-nitrophenoxide
F = fluoride
EtOH = ethanol
Et_3N = triethylamine
NaOEt = sodium ethoxide

a = axial compounds
b = equatorial compounds

diol[162] which reacts with $POCl_3$ to form the trans fused bicyclic system which provides the chiral framework exploited by Inch. These compounds make excellent systems within which the stereochemistry of displacements at phosphorus may be studied. Many displacement reactions have been studied in this system. Hitherto, it has not been possible to investigate the stereochemical course of the hydrolysis reaction of general phosphorus acids such as (69) as this forms a product (70) that is prochiral at phosphorus.



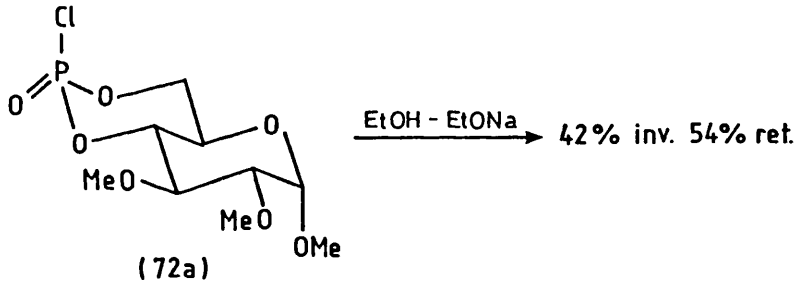
(69) (70)



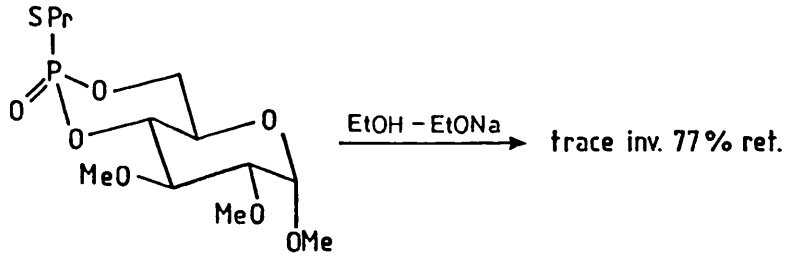
Such reactions are of interest in understanding the factors that determine the mechanistic pathway, e.g. stabilization of 5-coordinate intermediates, dependence on nucleophiles, dependence on leaving groups, stereoelectronic effects, etc. Much of our understanding of organophosphorus reaction mechanisms has come from studies of 5- and 6-ring phosphoryl compounds.[4,5,6,7] Westheimer found that both acid and base catalyzed hydrolysis of cyclic five-membered esters of phosphoric acid proceed 10^6-10^8 times faster than acyclic esters. These results implied the existence of a five-coordinate intermediate with trigonal bipyramidal geometry formed by nucleophilic attack on the central coordinated phosphorus atom to be involved in both endocyclic and exocyclic cleavage.

In contrast the acid and base catalyzed hydrolysis of cyclic six- and seven-membered esters of phosphoric acid in terms of rate are the same as their acyclic counterparts [see Chapter 1].

Studies by Inch et al.[163] have shown that the reaction pathway of displacement reactions of phosphorus acids are affected by the nature of the leaving groups, i.e. when 2-chloro-1,3,2-dioxaphosphorinan-2-one (72a) undergoes nucleophilic displacement with ethanol in sodium ethoxide the % stereochemical outcome is 42% inversion and 54% retention while 2-propylthio-1,3,2-dioxaphosphorinan-2-one (73a) undergoes nucleophilic displacement under the same conditions with only a trace of inversion and 77% retention (see Table 7.1).



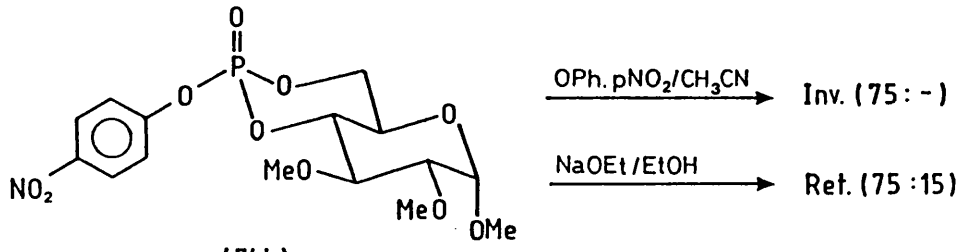
(72a)



(73a)

The nature of the nucleophiles can also affect the reaction pathway. In the example shown below when (74b) was reacted with p-nitrophenoxide ion in acetonitrile the predominant stereochemistry was inversion of configuration (75:-). However, when ethanol in sodium ethoxide was used as the nucleophile, the predominant stereochemistry was in favour of retention of configuration (75:15).

The presence of ring structures can effect the reaction pathway. The

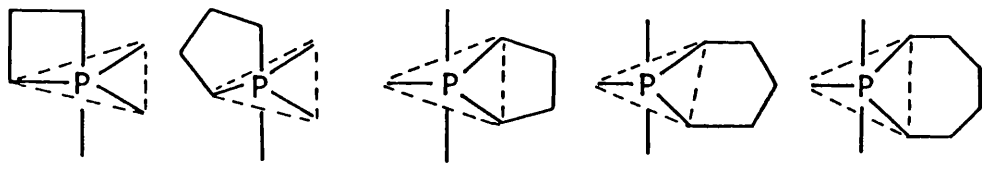


(74b)

size of a planar ring determines very largely the ring angle. This is shown below:-

Ring Size	Preferred Angle
4	90°
5	105°
6	120°
7	129°

The actual angle may differ from these values if the ring is not planar or if it contains different kinds of atoms. Even so, the nature of a ring itself tends to prevent undue distortions of the ring angle from these values. The result for pentacoordinate phosphorus in a ring system is that a four-membered ring prefers an apical-equatorial posture, and six- and seven-membered rings an equatorial-equatorial (not so clearly defined). Five-membered rings suffer a 15°C distortion from the preferred angle of 105°C if they are either apical-equatorial (90°C) or diequatorial (120°C). In this case other factors determine the configuration. The preferred positions are shown below:-



4 5 or 5 6 7

A rigid six-membered ring effectively prevents any pseudorotation as this would result in the ring angle at phosphorus being an unacceptable 90°. The same applies to a seven-membered ring. For a four-membered ring

pseudorotation is not prevented but restricted.

In reality six-membered rings possess a large degree of flexibility and so must the pentacoordinate configuration. Its equatorial angle is not set at a fixed 120° any more than that of a four-membered ring is set at 90°, and a diequatorial positioning of such a ring is feasible. The deforming of both systems necessary to do this will create an energy barrier, the height of which will be proportional to the strains imposed on the ring and trigonal bipyramid intermediate bonding systems.

Some of the observed stereochemical observations can be rationalised in terms of the apicophilicities[23] of the phosphorus ligand [see Chapter 1]. The relative apicophilicity of two groups is the change in energy when these groups exchange apical and equatorial positions in a trigonal bipyramid. Calculations by several groups[163,164] suggest that apicophilicity should be a function of:-

(a) Electronegativity, increasing electronegativity favouring occupation of an apical position.

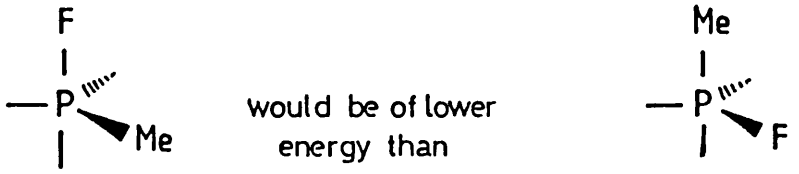
(b) The presence on the atom bonded to phosphorus of a lone-pair of electrons, this favours occupation of an equatorial position.

(c) The presence on the atom or group of a vacant low-lying orbital, thus favouring occupation of an apical position.

With three factors to balance, most experimental data can be rationalized. What is not clear is how far the relative apicophilicity of two groups will vary with the nature of the other groups attached to phosphorus. A wide variation could lead to data which would be extremely difficult to compare. Most of the information of relative apicophilicities has been obtained from nmr studies on stable five-coordinate phosphoranes.[165,166] The order of apicophilicity is as follows:-

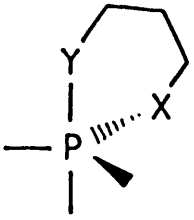
$$F > H > CF_3 > OPh > Cl > SMe > OMe > NMe_2 > Me > Ph$$

Apicophilicity values can be used to predict the relative energies of phosphoranes.

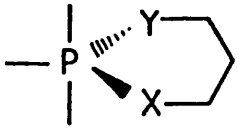


Trippett et al. have also obtained data on the energies required to move various six-membered rings from axial-equatorial to equatorial-equatorial positions from dynamic nmr spectra of a range of stable phosphoranes.

Models using sp^2 hybridised heteroatoms suggest that with strain-free chair conformations occupying either axial-equatorial (76) or equatorial-equatorial (77) positions, the lone-pairs on equatorial heteroatoms are in p-orbitals nearer to an apical plane rather than to the favourable equatorial plane.

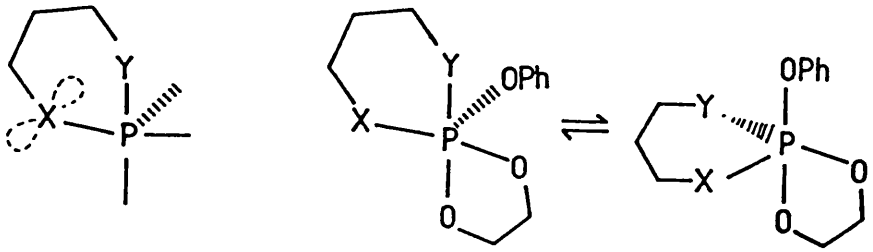


(76)



(77)

However, with an axial-equatorial boat conformation (P and C atoms are at the prow) (78) the lone-pairs on the equatorial substituent, X, can be in the equatorial plane. Certainly there are considerably greater barriers to placing six-membered ring hetero rings diequatorial than would be expected if these were most stable in the chair conformations (76) and, once again, the nature of the heteroatom that remains equatorial significantly affects the height of the barrier. This is shown overleaf.



(78) (79) (80)

(79) ⇌ (80)

The free energies of activation for pseudorotation (79) ⇌ (80)

X	O	N	S	N
Y	O	O	O	N
$\Delta G^{\ddagger}$ (kcal mol^{-1})	6.1	9.5	9.2	<6

Thus for oxygen, oxygen heteroatoms in a ring the $\Delta G^{\ddagger}$ is ≈6.1 (kcal mol^{-1}). This is less than for other heteroatoms.

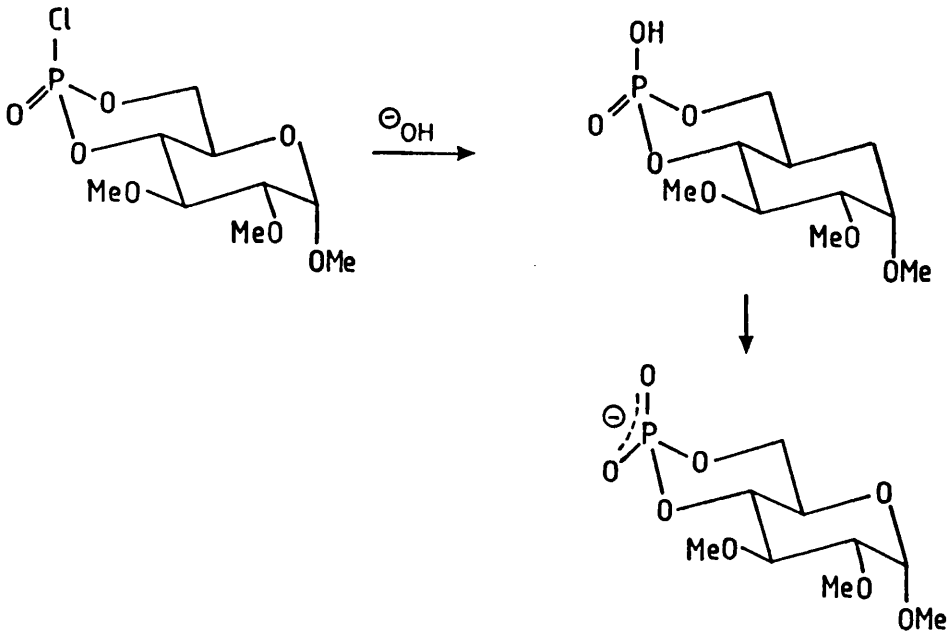
Furthermore, the reaction pathway of phosphorus acids can be affected by solvents and other ionic species present, etc.

Our aim is to study the stereochemical course of a range of hydrolysis reactions of the 1,3,2-dioxaphosphorin system using OH^- and H_2O/base as the nucleophiles with a variety of leaving groups. This is shown in Scheme 7.1.

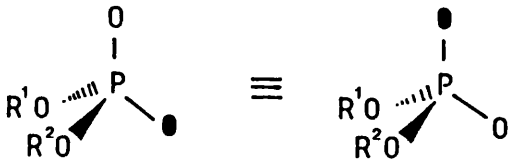
These results will be directly comparable with the results obtained by Inch and used to establish whether there are any mechanistic differences.

In the studies of Inch et al., the mixed stereochemistries could arise from multiple displacements or, possibly, via ring-opened intermediates. The hydrolysis reaction may be easier to study since the product is a diester anion and is not susceptible to further displacements.

The stereochemical course of the hydrolysis reaction can now be determined with the advent of the methodology involving the use of oxygen



isotopes [^{16}O,^{17}O,^{18}O]. Thus the stereochemical course of the reaction can be simply deduced by methylation and subjecting the product to high-field ^{31}P nmr [see Chapter 1].



It is worth noting that the retention and inversion information is contained within a single chemical entity and, therefore, the ratios do not alter after isolation.

Synthesis of 1,3,2-dioxaphosphorinan-2-one

The synthesis of 1,3,2-dioxaphosphorinan-2-one is that followed by Inch et al. exploiting the carbohydrate route and illustrated in Figure 7.2.

Methyl-2,3-dimethyl-4-6-benzylidene-β-D-glucoside (81) was made from methyl benzylidene-β-D-glucoside (71) and dimethyl sulphate. This reaction occurs over a few hours to produce the methylated product in 55% yield as white prisms.

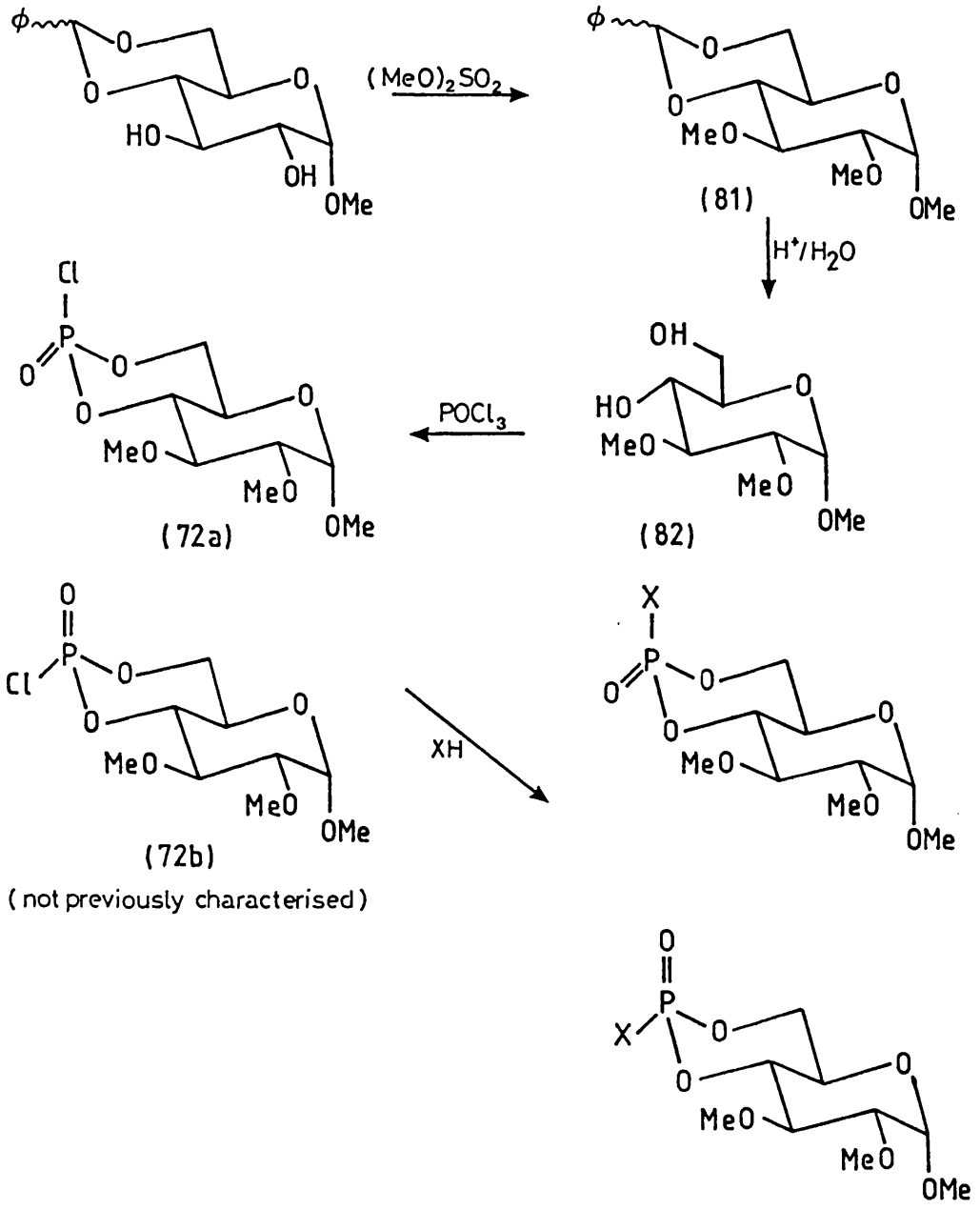


FIGURE 7.2 The synthesis of 2-halo-1,3,2-dioxaphosphorinan-2-one.

The acetal group of this product is removed by acid hydrolysis[167] which proceeded smoothly to form methyl-2,3-dimethyl-β-D-glucoside (82) in a 70% yield.

The phosphorylation of the diol is achieved using phosphous oxychloride in the presence of triethylamine. This reaction was monitored by ^{31}P nmr which showed two resonances at -3.8 ppm (72b) and -5.6 ppm (72a) in a

ratio of approximately 1:2 respectively after 2 hours. The spectrum did not change after this time. The major product was isolated as a crystalline material upon recrystallization from diisopropyl ether and was identified as the axial component (Sp -5.6 ppm) (72a). The supernatant yielded a colourless oil which was mainly the minor product at -3.8 ppm, it also contained some of the axial isomer (72a), 5:1 respectively by nmr integration.

The similarity between spectroscopic and chromatographic properties suggest that the minor product was the equatorial compound. This was confirmed by alkaline hydrolysis of a mixture of (72a) and (72b) to give one peak in the ^{31}P nmr at -3.024 ppm (corresponding to the hydrolysis product). The equatorial compound was not detected by Inch and is therefore a new compound.

The tentative configurational assignments are based on an observation that in 1,3,2-dioxaphosphorin-2-ones the P=O infrared stretching band is at higher frequency and the ^{31}P nmr chemical shift is at higher field when the P=O group is equatorial rather than axial.[168]

A number of problems were encountered during this reaction. The consistency of the crude product varied, sometimes a white solid would be formed and at other times a yellow oil. The crude product was purified by flash chromatography. A reasonable yield of crude compound could be obtained which, upon columning, gave a very low yield of desired product. The product was found to streak on t.l.c. This indicated that the product was decomposing on silica gel. Rapid columning of the product solved this problem.

Furthermore, if the product obtained after flash chromatography was not recrystallised immediately, decomposition would yet again occur. In order to obtain sufficient yield and purity of the chlorocompound (72), the crude

material must be subjected to flash chromatography and recrystallisation on the same day. It is then stable for up to 1 month, if kept refrigerated. The compound is also extremely sensitive to hydrolysis (i.e. Cl is easily displaced) and, as such, must be kept under strictly anhydrous conditions.

All the axial (S) and equatorial (R) derivatives were made from the axial chlorocompound (72a) as described by Inch, except the fluoro compound (75) which was made from the chlorocompound with potassium fluoride rather than the more complex dimethylamidophosphoric difluoride.

Both axial and equatorial p-nitrophenyl compounds (74) were formed using the chlorocompound (72a) and sodium p-nitrophenoxide. An equivalent amount of both reagents formed the (S)-p-nitrophenyl phosphate as a brown solid while an excess of the same reagent produced the (R)-p-nitrophenyl phosphate as a white solid. These compounds were assigned on the basis that axial triesters appear upfield in the nmr spectrum while equatorial triesters downfield.

The formation of both axial and equatorial thiophosphate compounds (73) were derived from reaction of the chlorocompound (72a) with an excess of sodium ethanethiolate.

The reaction of the chlorocompound (72a) with dimethylamine also proceeded smoothly to give the expected (S) and (R) dimethyl phosphoamidates. In the case of the reaction with fluoride ion only the axial diastereoisomer (75) was formed. This is due to the strong apicophilic nature of fluorine favouring an axial position.

In general the crude ratios of the compounds formed were closely related to those obtained by Inch. Obviously, the difficulties which arose on purification, as mentioned earlier, affected the amount of pure material formed.

Once the purity and stereochemistry of the starting materials had been deduced, analysis of the above compounds could now be carried out.

Hydrolysis reactions of 1,3,2-dioxaphosphorinan-2-ones

The objective of this work is to determine the stereochemical course of displacement reactions from the 1,3,2-dioxaphosphoran-2-one system. This is achieved through: (a) hydrolysis, (b) methylation, (c) high-field ^{31}P nmr of the ^{18}O shift in the above system.

The conditions of hydrolysis were established using $H_2{}^{16}O$. The ideal conditions required two equivalents of potassium hydroxide in $H_2{}^{16}O$ for immediate hydrolysis, producing a resonance at -3.02 ppm corresponding to the hydrolysed material. This reaction using ^{18}O isotope was then carried out.

The 1,3,2-dioxaphosphorinan-2-one system was dissolved in dioxan. This solvent is chosen as it maintains a homogeneous solution. Hydrolysis conditions were employed using a 50/50 mixture of $H_2{}^{18}O$ and $H_2{}^{16}O$. This gives rise to isotopomers (83) and (84) in a ratio corresponding to the ratio of retention : inversion for the stereochemical course of the reaction. This product can be purified without risk of perturbing the isotopomer ratios of (83) and (84) as these are chemically identical, as shown in Figure 7.3.

The mixture was then neutralized and 1.5 equivalents of 18-crown-6-ether added. The compound was freeze-dried overnight. 18-Crown-6-ether complexes with the potassium ion and so increases the nucleophilicity of the phosphate anion. Dimethyl sulphoxide is a good dipolar aprotic solvent of moderately high dielectric constant. It solvates cations most strongly and leaves anions relatively unencumbered and highly reactive. The compound is very soluble in dimethyl sulphoxide and this facilitates methylation.[169]

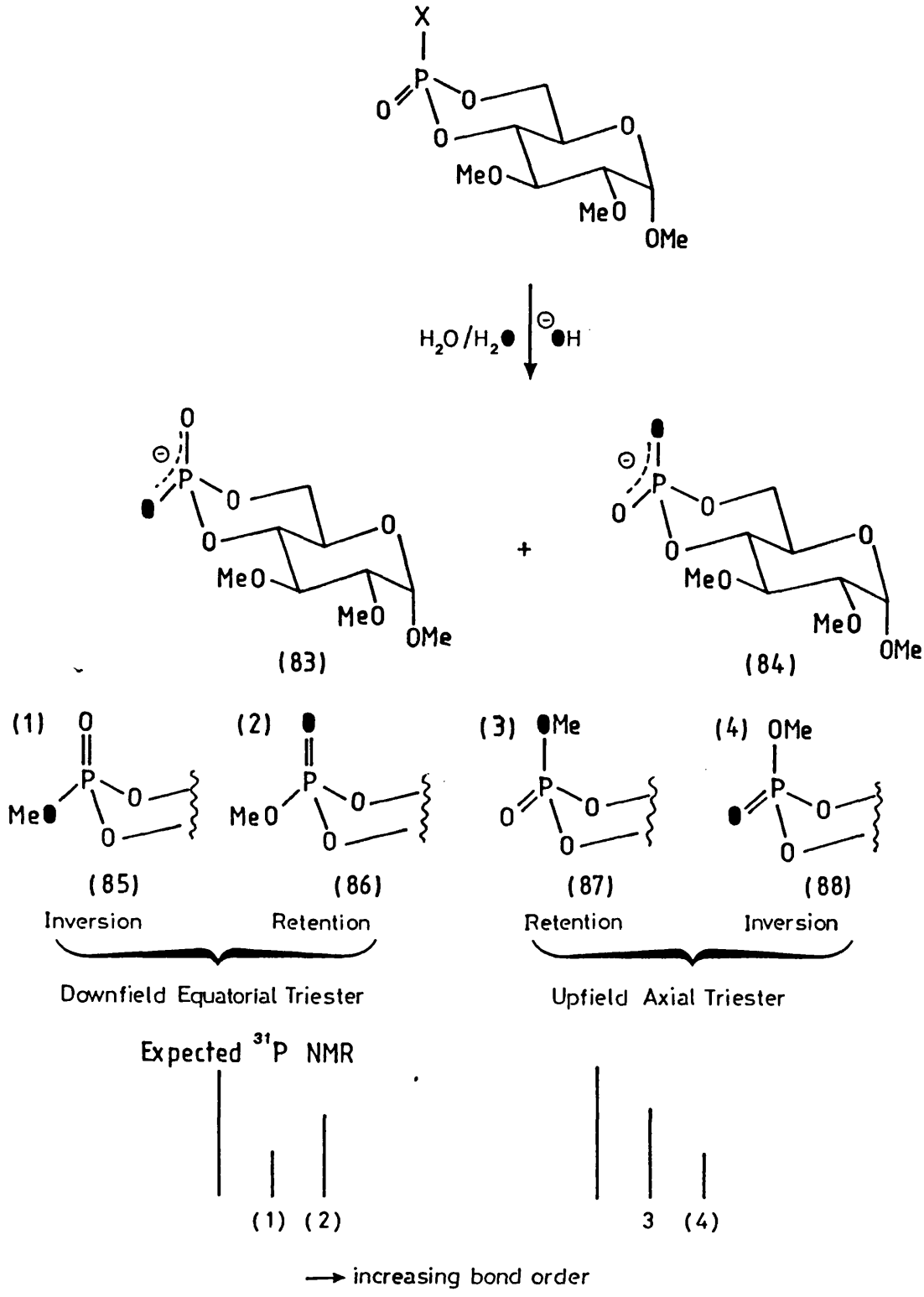


FIGURE 7.3 Proposed analysis of the hydrolysis reactions of 1,3,2-dioxaphosphorinan-2-one system.

Alkylation imposes a different bond order on each of the original phosphoryl oxygens and allows the isotopic identity of the diastereotopic oxygen to be ascertained by measurements of the ^{18}O perturbation on the ^{31}P nmr resonances of each of the esters.

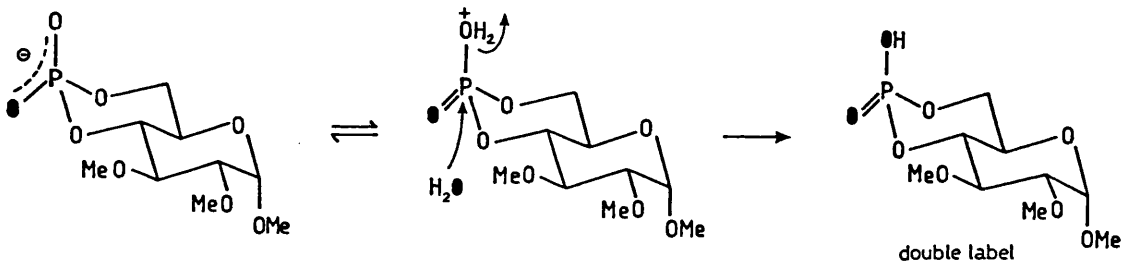
Methylation using methyl iodide^{170} in dimethyl sulphoxide overnight gives rise to four isotopically chiral triester diastereoisomers which can be separated in the ^{31}P nmr (85/86/87/88).

For leaving groups of a basic nature, e.g. NMe_2, hydrolysis under acid conditions have to be employed. In this case, p-toluene sulphonic acid as well as trifluoroacetic acid were used. However, the reactions of 2-dimethylamino-1,3,2-dioxaphosphorinan-2-one proved to be resistant to hydrolysis in the conditions shown in the table below.

Compound	Acid	Time	Outcome
2-dimethylamino-1,3,2-dioxaphosphorinan-2-one	p-toluene sulphonic acid [2 equivalents]	0-3 hours	No reaction
	[4 equivalents]	"	"
	Trifluoroacetic acid [2 equivalents]	"	"

It was feared that the use of stronger acidic conditions would cause acid catalysed $H_2{}^{18}O$ exchange and so lead to double labelled material. This would then prevent identification of the stereochemical pathway. This can be seen below.

Previous work on the stereochemistry of nucleophilic displacement reactions in cyclic phosphate esters has been based upon the determination



of the ratios of geometrical isomers that are formed. This method, however, cannot be used for hydroxide attack or water, since the cyclic diester product has a prochiral centre. Hydrolysis in $H_2{}^{18}O$ water leads to the products (83/84) shown in Figure 7.3. The ^{18}O incorporation into the phosphate diester can be deduced by ^{31}P nmr spectroscopy due to the fact that ^{18}O directly bonded to phosphorus has been demonstrated to exert an upfield shift on the ^{31}P nmr resonance, the double bond shift being approximately twice that of a single bond shift.

Figure 7.3 shows that the four isotopically chiral triesters can be split into two distinct sets, the equatorial triesters appearing at resonance -3.02 ppm while the axial triesters appear at resonance -5.64 ppm. As previously stated, ^{31}P nmr can distinguish ^{18}O perturbations of double and single bond shifts.[32,33,34] Concentrating on the downfield lines at -3.02 ppm in Figure 7.4 it can be seen that the first line corresponds to unlabelled equatorial triester. The second line represents a single bond ^{18}O shift, whereas the third line represents a double bond ^{18}O shift. The ^{18}O in the P=O double bond has a larger phosphorus shift than ^{18}O in the single P-OMe bond.

The same explanation is valid for the axial triester. The first line of the three lines corresponds to unlabelled axial triester. ^{18}O incorporation in a single P-OMe bond, corresponds to the second line. The largest shift from the unlabelled axial triester line has ^{18}O incorporated into a $P={}^{18}O$ bond.

If the starting material has an axial orientation of the leaving group, then ^{18}O incorporation into an axial $P-{}^{18}O$ bond indicates that the stereochemistry of the reaction has occurred with retention of configuration whereas ^{18}O incorporation into an equatorial bond means that inversion of configuration is the outcome of the reaction.

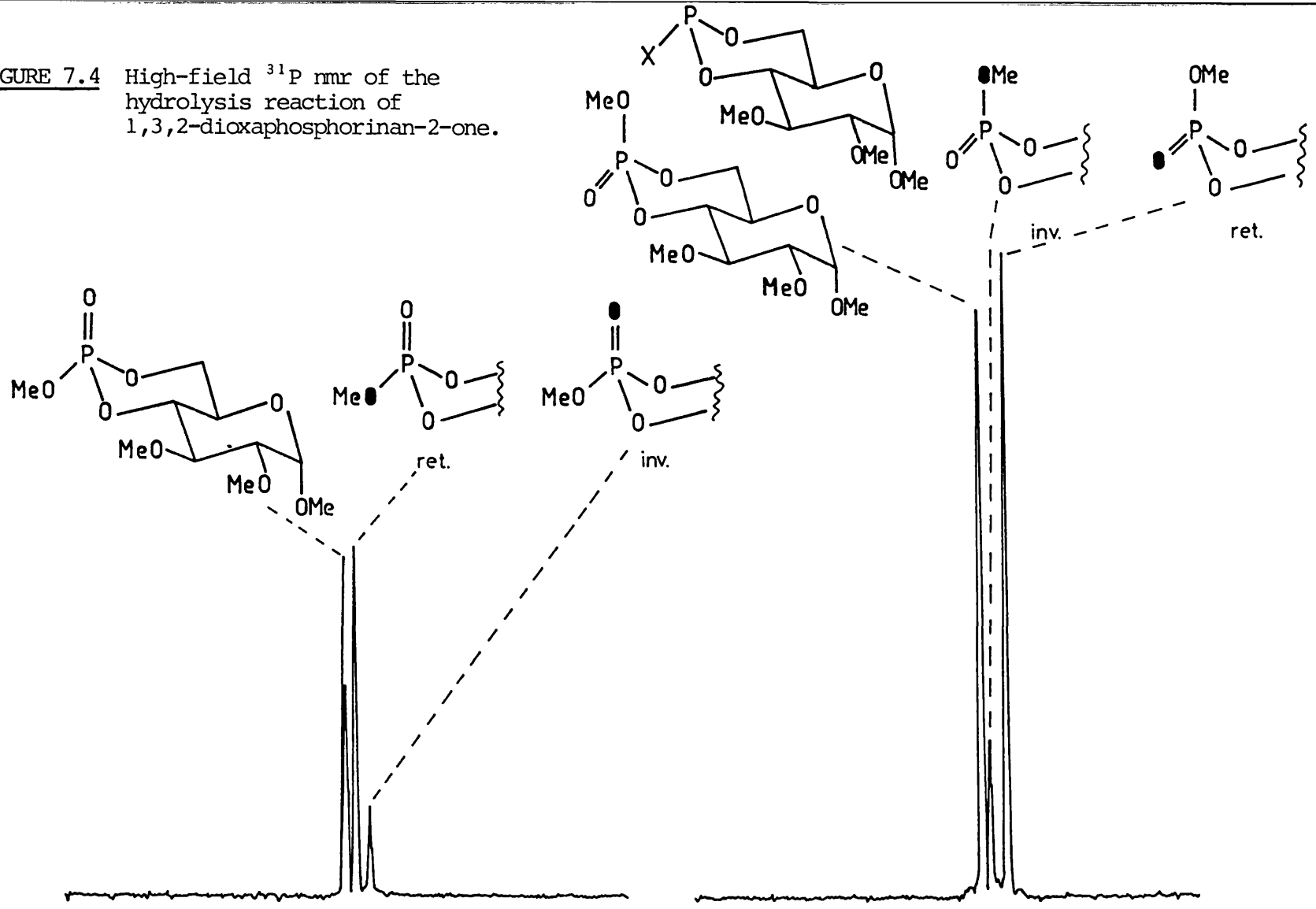


FIGURE 7.4 High-field ^{31}P nmr of the hydrolysis reaction of 1,3,2-dioxaphosphorinan-2-one.

The amount of retention to inversion is based on the peak areas of the resonances in the ^{31}P nmr. For this example it can be seen that there is more retention than inversion of configuration.

It is possible that the reaction could occur via a pyrophosphate mechanism, as shown in Figure 7.5. This would affect the stereochemical outcome of the results obtained. Such a reaction would manifest itself as doubly labelled material in the ^{31}P nmr. However, no such species has been found in our investigations.

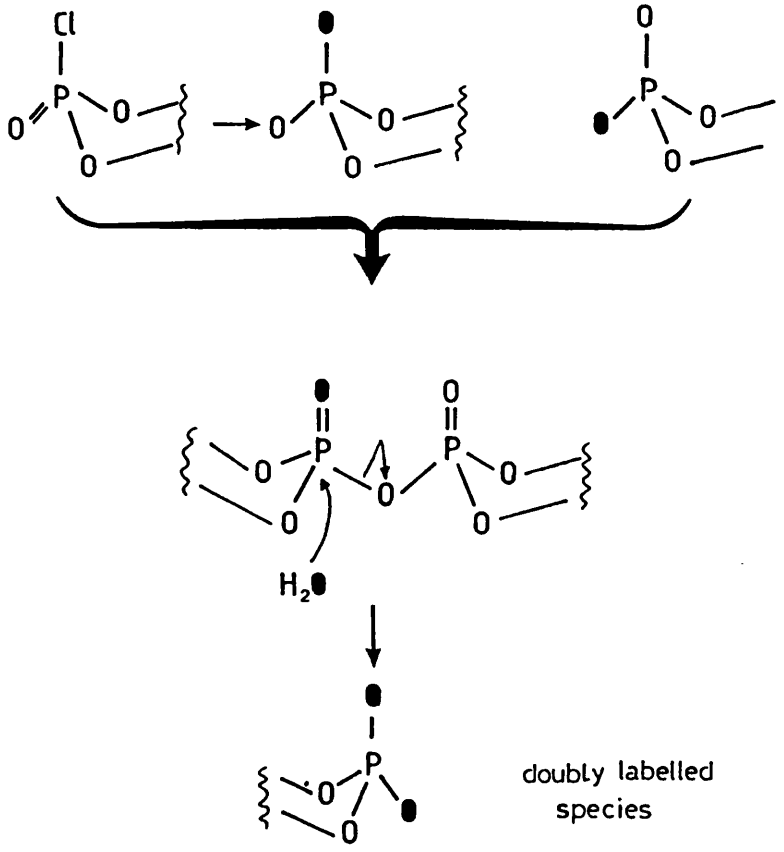


FIGURE 7.5 Formation of possible pyrophosphates during the hydrolysis reaction of 1,3,2-dioxaphosphorinan-2-one.

RESULTS

The stereochemical course of displacement reactions from 1,3,2-dioxaphosphoran-2-one are shown in Table 7.2.

TABLE 7.2

Results of the hydrolysis of 1,3,2-dioxaphosphorinan-2-one

Compound	Nucleophile	Products Ret.	Products Inv.
(72a) -Cl(ax)	OH	25%	75%
	H_2O/Et_3N		⩾95%
	H_2O		⩾95%
(72b) -Cl(eq)	H_2O/Et_3N		⩾95%
(74a) -O-  -NO_2 (ax)	OH	75%	25%
(74b) -O-  -NO_2 (eq)	OH	43%	57%
(73a) -SEt(ax)	OH	85%	15%
(73b) -SEt(eq)	OH	⩾95%	-
(75) F	OH	24%	76%

Firstly, it appears that the stereochemical course of displacement reactions is dependent on the initial axial or equatorial orientation of the leaving group. However, for axial and equatorial chlorocompounds (72a/72b) hydrolysis reactions occur with mainly inversion of configuration and only 25% retention of configuration for the axial compound (72a).

The results obtained using p-nitrophenoxide as a leaving group, shows that the axial compound (74a) gives 75% retention and 25% inversion while the equatorial compound (74b) gives mainly inversion of configuration.

Both axial and equatorial ethane thiols give products with retention of configuration while only 15% inversion is observed for the axial compound (73a). The fluoride compound (75) gives products with mainly inversion of configuration.

These results imply that leaving groups such as chloride and fluoride undergo hydrolysis reactions with overwhelming inversion of configuration and thiols with overwhelming retention of configuration. However, for

leaving groups such as p-nitrophenoxide the distinction is not so clear cut.

This seems to indicate that for better leaving groups (e.g. F, Cl) hydrolysis reactions occur with inversion of configuration and for poor leaving groups (e.g. thiol) hydrolysis reactions with retention of configuration are dominant. While for other leaving groups such as p-nitrophenoxide the mechanism pathway is finely balanced between the two and is, therefore, dependent on other factors, e.g. stereoelectronic effects.

Routes to both inversion and retention pathways are shown in Figure 7.6. The pathway giving rise to inversion of configuration involves an in line attack of the nucleophile opposite the leaving group X. This produces a trigonal-bipyramid with the ring diequatorially placed (89). This may be expected to be a "high energy species", higher in energy than placing the ring axial-equatorial. However, as stated earlier, the energy barrier required for this ($\sim 6.1\,kcal^{-1}$) is not as great as placing a five-membered ring diequatorially ($\sim 20\,kcal^{-1}$). Therefore the preference for X to be axial is greater than that of the ring to span axial-equatorial. X^- then departs to give inversion of configuration.

In order to obtain retention of configuration, the ring must span axial-equatorial in the trigonal bipyramid. The hydroxide nucleophile attacks adjacent to the leaving group, which is equatorially placed in the trigonal bipyramid[17,172] (90). Application of the microscopic reversibility concept means that the leaving group must depart axially. This involves a pseudorotation placing the leaving group axially and allowing it to depart. In this case the preference for the ring to span axial-equatorial takes preference over the initial placement of the leaving group in an apical position.

The actual pathway chosen must be the one which has the lowest energy

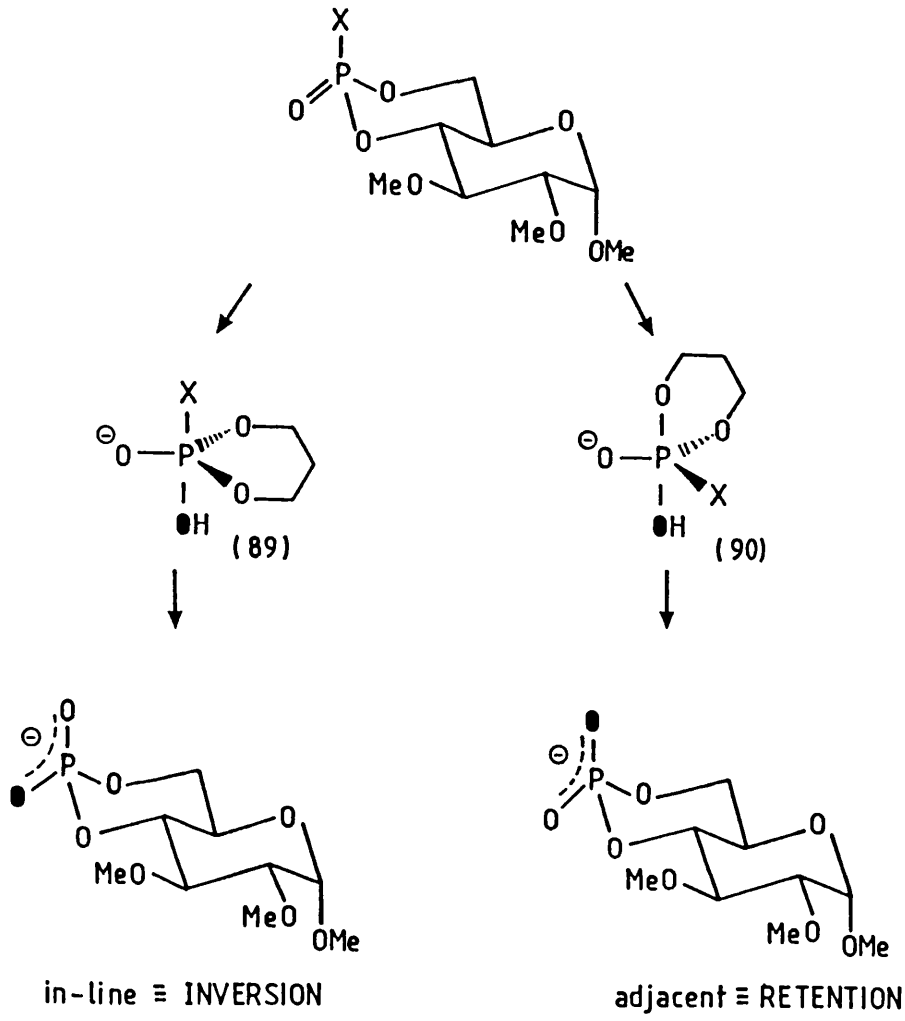


FIGURE 7.6 Alternative pathways for the hydrolysis of 2-halo-1,3,2-dioxaphosphorinan-2-one and 2-thione.

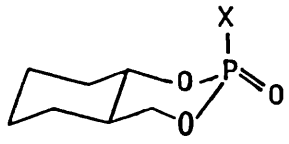
trigonal bipyramidal species. Trigonal bipyramid species in which the ring spans diequatorially is of lowest energy for leaving groups such as F and Cl, while for thiols the lowest energy trigonal bipyramidal species is that which has the ring spanning axial-equatorial. For leaving groups such as p-nitrophenoxide the energies of the two relative trigonal bipyramids must be similar.

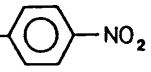
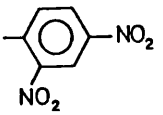
CONCLUSION

The present data with the leaving groups studied suggest that alkoxide

and hydroxide behave comparable in this system. Our results are very close to those obtained by Inch.

Gorenstein et al.[171,173] have carried out similar studies on the closely related six-membered ring system 2-aryloxy-2-oxy-1,3,2-dioxaphosphorinane, as illustrated below.



X = p-MeOPh
= ph
= O- 
= O- 

Reactions involving the methanolysis of this system[171] with the leaving groups shown was initially undertaken. Further studies investigated the hydrolysis of 2-aryloxy-2-oxy-1,3,2-dioxaphosphorinanes.[173]

The results of these studies are shown in Table 7.3.

TABLE 7.3

Compound (leaving group)	Nucleophile	Stereochemical outcome % inversion
p-MeOPh (ax)	NaOMe/MeOH	28%
p-MeOPh (eq)	NaOMe/MeOH	9%
Ph (ax)	NaOMe/MeOH	24%
Ph (eq)	NaOMe/MeOH	4%
PNP (ax)	NaOMe/MeOH	17%
PNP (eq)	NaOMe/MeOH	73%
2,4 DNP (ax)	NaOMe/MeOH	83%
2,4 DNP (eq)	NaOMe/MeOH	100%
p-MeOPh (eq)	$H_2{}^{18}O/OH$	59%
2,4 DNP (ax)	$H_2{}^{18}O/OH$	82%

These results show that there are significant differences between the stereochemistry for hydroxide attack in the p-methoxyphenoxy ester and the stereochemistry of methoxide attack in methanol in the same system. Thus, the methoxide displacement proceeds with only 9% inversion, while

59% inversion is observed in the hydroxide reaction. In contrast, both hydroxide and methoxide displacement of the 2,4-dinitrophenoxy ester yields 82-83% inversion.

EXPERIMENTAL

GENERAL EXPERIMENTAL DETAILS

MATERIALS AND METHODS

Solvents were obtained commercially from a number of sources and purified when necessary before use.

Anhydrous methanol and ethanol were obtained by drying with magnesium and iodine before distillation.

Pyridine was heated under reflux with potassium hydroxide pellets, then distilled and stored over potassium hydroxide pellets (sieves 4A).

Dioxan was purified by passing through an alumina column followed by distillation from sodium metal and benzophenone. The solvent was stored over molecular sieves (4A) and under nitrogen.

Chloroform and dichloromethane were both sodium dried, distilled and stored.

Acetonitrile was distilled and stored.

Petroleum spirit (b.pt. 40-60°) and (60-80°) were fractionally distilled.

Peroxide free ether, benzene and toluene were all dried with sodium wire.

Tributylamine was distilled and stored under nitrogen.

Triethylamine was distilled and stored under nitrogen.

Analytical grade acetone was used throughout.

Deionised water was obtained from a Milli-Q reagent grade water system.

CHEMICALS

Unless otherwise stated, these were at least of laboratory grade and obtained from:- BDH Chemicals Ltd. [Poole, Dorset], Koch-Light Laboratories [Colnbrook, Bucks], Fisons Scientific Apparatus Ltd. [Loughborough], Aldrich Chemical Company [Gillingham, Dorset].

Oxygen-18 enriched water (99.5 atom %) was obtained from Prochem.

Deuterium oxide was obtained from the Ryan Chemical Company [Southampton].

INSTRUMENTATION AND METHOD

Melting points were determined on a Kofler hot-stage apparatus and are quoted uncorrected. The pH of aqueous solutions, including deuterium oxide solutions, were measured with a Radiometer pH meter using a single glass electrode. Optical rotations were determined on a Perkin-Elmer polarimeter.

Infrared spectra were recorded on a Perkin-Elmer 298 instrument. Ultraviolet spectra were recorded on a Shimadzu UV-240 spectrometer. Proton nuclear magnetic resonance spectra were recorded on a Varian EM-390 spectrometer at 90 MHz.

Phosphorus nuclear magnetic resonance spectra were run on a Jeol FX-60 low-field spectrometer at 24.15 MHz, spectra recorded both proton coupled and proton decoupled but, unless otherwise stated, data quoted refers to broad band proton decoupled spectra.

Throughout this thesis, by convention, resonances that appear down-field of the relevant reference signal are assigned a positive chemical shift. Reference compounds used were:- for ^{1}H nmr spectra internal tetramethylsilane (TMS), ^{31}P nmr spectra external reference D_2O.

The Jeol FX-60 requires a deuterium signal for lock, when recording spectra in non-deuterated solvents the lock signal was provided by deuterium oxide.

Solutions for ^{31}P spectra were contained within a 5 mm tube monitored coaxially with a 10 mm diameter tube containing D_2O.

High-field ^{31}P nmr was recorded on a Bruker AM-300 at 121.5 MHz and processed with Gaussian multiplication [Gaussian broadening 0.1 Hz and line broadening -0.3 Hz].

Preparation of Rp-O-phenyl [^{16}O,^{18}O] thiophosphate

Formation of 2-phenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (Route I) (9)

This was prepared by the method of Inch *et al.*[80] To a cooled solution of ephedrine (2g, 0.012 mol) and triethylamine (6 ml) in toluene (50 ml) was slowly added phenyl dichlorophosphite (2.3g, 0.012 mol) in toluene (10 ml). The reaction mixture was kept under nitrogen and allowed to warm to room temperature. The mixture was then stirred for fifteen minutes. After this time the reaction was monitored *via* ^{31}P nmr and was found to have gone to completion. A vast excess of elemental sulphur was added to the reaction mixture (4g, 10-fold excess). The reaction mixture was then stirred for approximately one hour. ^{31}P nmr spectroscopy showed that the reaction had gone to completion. This mixture was then washed with water and concentrated to a yellow solid by evaporation. The yellow solid was purified *via* flash column chromatography using petroleum spirit/diethyl ether (7:3). The cis material was obtained as white square crystals while the trans material was obtained as white needles.

cis[80] (1.8g, 50%), ^{1}H nmr ($CDCl_3$); +0.86 (3H, d, J7Hz, CH_3) +2.90 (3H, d, $^{3}J_P$ 15Hz, N-CH_3) +3.70 (1H, ddq, J7Hz, J6.5Hz, $^{3}J_P$ 29.5Hz, CH-4) +5.80 (1H, dd, J6.5Hz, $^{3}J_P$ 1Hz, CH-5) +7.2 (10H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O = +78.25 ppm.

trans[80] (1.2g, 33%); ^{1}H nmr ($CDCl_3$); +0.79 (3H, d, J7Hz, CH_3) +2.28 (3H, d, $^{3}J_P$ 17Hz, N-CH_3) +3.65 (1H, ddq, J7Hz, J7.5Hz, $^{3}J_P$ 13Hz, CH-5) +5.59 (1H, dd, J7.25Hz, $^{3}J_P$ 6.5Hz, CH-5) +7.2 (10H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +77.65 ppm.

P-N bond cleavage of cis 2-phenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione

This is as reported by Knowles *et al.*[30] The cis diester amidate (9)

(0.200g, 0.66 mmol) was dissolved in dry dioxan (1 ml). To this solution was added trifluoroacetic acid (0.23g, 1.98 mmol) and H_2O (0.5 ml). After one hour ^{31}P nmr showed that no starting material remained. The reaction mixture was then concentrated to dryness with methanol. This gives the diester as its zwitterion (0.150g, 75%).[30]

^1H nmr ($CDCl_3$); +1.20 (3H, d, J7Hz, C$\underline{H}_3$) +3.05 (3H, s, N-C$\underline{H}_3$) +4.30 (1H, dq, J7Hz, J6.3Hz, C$\underline{H}$-4) +5.2 (2H, broad, N-$\underline{H}_2$) +6.1 (1H, dd, J6.3Hz, ^3J_p 2.8Hz, C$\underline{H}$-5) +7.2 (10H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +52.84 ppm.

C-O bond cleavage using strong base

This was carried out as reported by Inch *et al.*[92] To a suspension of the diester as its zwitterion (10) (0.100g, 0.33 mmol) in dichloromethane (0.5 ml) was added sodium hydroxide (0.25 ml, 12M). The reaction was left for twenty minutes. The excess sodium hydroxide was removed. The product appeared as clear liquid. This product was consistent with the reformation of the five-membered ring.

^1H nmr ($CDCl_3$); +0.86 (3H, d, J7Hz, C$\underline{H}_3$) +2.82 (3H, d, ^3J_p 15Hz, N-C$\underline{H}_3$) +3.85 (1H, ddq, J7Hz, J6.5Hz, ^3J_p 29.5Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.5Hz, ^3J_p 1Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +70.79 ppm.

C-O bond cleavage using Na/liq.NH_3

To a suspension of the diester as its zwitterion (10) (0.100g, 0.33 mmol) in tetrahydrofuran (0.5 ml) was added four equivalents of sodium metal (0.030g, 1.3 mmol) in liquid ammonia. The reaction was carried out under nitrogen. The reaction was left for six minutes and quenched with ammonium bicarbonate. The excess ammonia was left to evaporate off and the compound was washed several times with methanol to give a white solid. The product formed was consistent with the loss of sulphur and

this method was no longer used.

^{31}P nmr (CH_2Cl_2) D_2O lock = -19.15 ppm.

C-O bond cleavage using TMSI

This was prepared by the method of Knowles *et al.*[30] To a suspension of the diester (10) as its zwitterion (0.100g, 0.33 mmol) in dichloromethane (0.5 ml) was added trimethylsilyliodide (0.264g, 1.3 mmol). The mixture was left under the following conditions:- (1) room temperature, (2) heating to 35°C, (3) heating to 70°C.

After a few days under the conditions of (1) and (2) monitoring by ^{31}P nmr spectroscopy showed that the reaction did not go to completion.

^{31}P nmr (CH_2Cl_2) D_2O lock = +37.00 ppm.

When the reaction was heated to 70°C the loss of sulphur occurred.

^{31}P nmr (CH_2Cl_2) D_2O lock = -19 ppm.

Preparation of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate

[2R,4S,5R]-3,4-dimethyl-2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thione (14) (alternative route)

The above compound is prepared by the method of Tolkmith *et al.*[181] To a solution of the ephedrine hydrochloride (2g, 0.012 mol) in toluene (5 ml) was added p-nitrophenyl dichlorothiophosphate (3.31g, 0.012 mol) in toluene (5 ml). To this solution is added triethylamine (2.46g, 0.024 mol). The solution was left stirring under nitrogen at room temperature for 12 hours. After this time no starting material remained as monitored *via* ^{31}P nmr. The reaction was stopped, filtered and concentrated to dryness. The mixture was passed through a plug of silica eluted using dichloromethane. The mixture was concentrated to give a pale yellow gum (2.6g, 60% yield) (lit.[80] 80% yield).

^{1}H nmr ($CDCl_3$); +0.78 (3H, d, J7Hz, C$\underline{H}_3$) +2.94 (3H, d, ^3J_p 12Hz, N-C$\underline{H}_3$) +3.80 (1H, ddq, J7Hz, J6.3Hz, ^3J_p 19.4Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.3Hz, ^3J_p 2.8Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph) +6.8, +7.8 (4H, dd, J7.5Hz, J7.5Hz, O$\underline{Ar}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = +78.03 ppm.

P-N bond cleavage of 2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thione (alternative route)

This is carried out as reported by Knowles.[30] The diester amidate (14) (2.0g, 6.55 mmol) was dissolved in dioxan (5 ml) and added to a solution prepared from trifluoroacetic acid (3 equivalents) (2.16g, 19 mmol). After five hours at room temperature the ^{31}P nmr showed the presence of two resonances. Separation by flash chromatography using toluene/acetone (7:4) proved unsuccessful. Therefore, the alternative method was no longer used.

^{1}H nmr ($CDCl_3$);[30] +1.20 (3H, d, J7Hz, C$\underline{H}_3$) +3.05 (3H, s, N-C$\underline{H}_3$) +4.30 (1H, dq, J7Hz, J6.3Hz, C$\underline{H}$-4) +5.1 (2H, broad, N-$\underline{H}_2$) +6.2 (1H, dd, J6.3Hz, ^3J_p 2.8Hz, C$\underline{H}$-5) +7.3 (5H, m, Ph) +6.8, +7.8 (4H, dd, J7.5Hz, J7.5Hz, O$\underline{Ar}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = +54.85 and +52.93 ppm.

[2R,4S,5R] and [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thiones (8)

This was prepared by the method of Inch *et al.*[60] A solution of thiophosphoryl chloride (6.9g, 0.05 mol) in benzene (25 ml) was slowly added to a suspension of (-)ephedrine hydrochloride (8.2g, 0.05 mol) in triethylamine (25g, 0.28 mol) and benzene (150 ml). The mixture was stirred overnight at room temperature and under nitrogen. The mixture was then poured into an excess of water. The aqueous layer was extracted with benzene. The combined extracts were concentrated and the resulting oil was crystallised from di-isopropyl ether to give the product as white crystals. These were

then dried in a vacuum desiccator over P_2O_5. The yield of cis product (8) (7.84g, 60%) (lit.[80] 6.5g, 61%), m.pt. = 125-128°C (lit. 125-128°C).

^1H nmr ($CDCl_3$); +0.87 (3H, d, J7Hz, C$\underline{H}_3$) +2.85 (3H, d, ^3J_p 15Hz, N-C$\underline{H}_3$) +3.85 (1H, ddq, J7Hz, J6.5Hz, ^3J_p 28.5Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.5Hz, ^3J_p 1Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +74.35 (s) ppm.

The mother liquor was concentrated to give the trans compound (0.67g, 6%) (lit.[80] 0.87g, 8%) yield, m.pt. = 57°C (lit. 58°C).

^1H nmr ($CDCl_3$); +0.78 (3H, d, J7Hz, C$\underline{H}_3$) +2.27 (3H, d, ^3J_p 17.5Hz, N-C$\underline{H}_3$) +3.73 (1H, ddq, J7Hz, J6.5Hz, ^3J_p 13.0Hz, C$\underline{H}$-4) +5.59 (1H, dd, J6.5Hz, ^3J_p 7.25Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +79.82 ppm.

[2R,4S,5R]-3,4-dimethyl-2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (14)

Prepared from the method of Inch *et al.*[80] To a solution of the adduct [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (1.0g, 3.83 mmol) in dry acetonitrile (40 ml) was added solid sodium p-nitrophenolate (0.7g, 5 mmol) 10% excess. The mixture was stirred for seven days in a sealed container at room temperature. After this time ^{31}P nmr showed that no more starting material remained. The acetonitrile was then removed under vacuum to yield a viscous yellow oil. This oil was then partitioned between dichloromethane and water. The dichloromethane layer was then concentrated. The product was purified by eluting through a column of silica with chloroform. The product upon concentration to dryness appeared as a light brown crystalline compound (1.67g, 83%) (lit.[80] 54%), b.pt. = 170°C at 0.1 mmHg (lit. = 170°C at 0.1 mmHg), (m.pt. = 110-112°C).

^1H nmr ($CDCl_3$); +0.78 (3H, d, J7Hz, C$\underline{H}_3$) +2.94 (3H, d, ^3J_p 12.5Hz, N-C$\underline{H}_3$) +3.80 (1H, ddq, J7Hz, J6.3Hz, ^3J_p 19.4Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.3Hz, ^3J_p

2.8Hz, CH-5) +7.2 (5H, m, Ph) +6.8, +7.8 (4H, dd, J7.5Hz, J7.5Hz, OAr); ^{31}P nmr = +78.03 ppm.

P-N bond cleavage of 2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thiones

The P-N cleavage is as reported by Knowles *et al.*[30] The diester amidate (14) (0.170g, 0.49 mmol) was dissolved in dry dioxan (1 ml) and added to a solution prepared from trifluoroacetic acid (0.17g, 1.4 mmol) and H_2O (2 ml). After one hour ^{31}P nmr showed that no starting material remained. The reaction mixture was then concentrated to dryness with methanol. This gives the diester as its zwitterion (0.135g, 80% yield).[30]

^{1}H nmr ($CDCl_3$); +1.20 (3H, d, J7Hz, CH_3) +3.05 (3H, s, N-CH_3) +4.30 (1H, dq, J7Hz, J6.3Hz, CH-4) +5.1 (2H, broad, N-H_2) +6.2 (1H, dd, J6.3Hz, $^{3}J_p$ 2.8Hz, CH-5) +7.3 (5H, m, Ph) +6.8, +7.8 (4H, dd, J7.5Hz, J7.5Hz, OAr); D_2O lock = +54.84 ppm.

P-N bond cleavage of 2-p-nitrophenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thiones (using isotopes)

As followed for unlabelled material, i.e. using (0.180g, 0.52 mmol) of starting material. The amount of product formed is (0.140g, 76% yield).

C-O bond cleavage (using TMSI)

This was prepared as reported by Knowles *et al.*[30] To a suspension of the diester as its zwitterion (15) (0.048g, 0.13 mmol) in dichloromethane (0.5 ml) was added trimethylsilyliodide (0.109g, 0.55 mmol). The mixture was left under the following conditions in a sealed tube:- (1) room temperature, (2) heated to 35°C. After a few days no reaction occurred. Therefore an excess of trimethylsilyliodide was used as followed above. No further reaction occurred monitoring *via* ^{31}P nmr. An alternative method was therefore needed.

Methylation of P-N bond cleavage compound

The P-N acyclic compound (15) as its zwitterion (0.135g, 0.35 mmol) was dissolved in methanol (0.5 ml). Two equivalents of methyl iodide (0.099g, 0.70 mmol) was added to the mixture. The reaction was kept in a sealed vessel at room temperature. The reaction was monitored *via* ^{31}P nmr. After 2 hours a small amount of product appeared. The reaction mixture was left at room temperature overnight, after this time no starting material remained. The mixture was concentrated to remove any excess methyl iodide and the solid was then extracted several times with methanol to produce the methylated triester (17) as its iodide salt (0.133g, ~95% yield).

^{1}H nmr ($CDCl_3$); +1.20 (3H, d, J7Hz, C$\underline{H}_3$) +2.35 (3H, d, $^{3}J_P$ 29Hz, S-C$\underline{H}_3$) +3.05 (3H, s, N-C$\underline{H}_3$) +4.30 (1H, dq, J7Hz, J6.3Hz, C$\underline{H}$-4) +5.1 (2H, broad, N-$\underline{H}_2$) +6.2 (1H, dd, J6.3Hz, $^{3}J_P$ 2.8Hz, C$\underline{H}$-5) +7.3 (5H, m, Ph) +6.8, +7.8 (4H, dd, J7.5Hz, J7.5Hz, O$\underline{Ar}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = +27.63 ppm.

Formation of p-nitrophenyl [^{16}O] thiophosphate (after methylation)

Carbon-oxygen bond cleavage as reported by Knowles.[30] To a suspension of the triester as its iodide salt (17) (0.048g, 0.13 mmol) in dichloromethane was added trimethylsilyliodide (0.108g, 0.53 mmol) 4 equivalents. The mixture was left at room temperature overnight and monitored *via* ^{31}P nmr. At this point the ^{31}P nmr showed that no starting material remained. To this reaction mixture was added two drops of mercaptoethanol and two drops of triethylamine in water to hydrolyse the trimethylsilyl esters. The mixture changed from dark red to pale orange, and was then concentrated (to dryness) to give a pale yellow oil. The product (18) was isolated and purified *via* ion-exchange chromatography

Column chromatography (ion-exchange)

A column of DEAE - Sephadex A25 (200 ml, *ca.* 3 x 4 cm diameter) was equilibrated with aqueous triethylammonium bicarbonate buffer (50 mM, pH 7.8). An aqueous solution of the material from above (~50 ml) was applied to the top of the column at a rate of 80 ml per hour. A linear gradient of increasing buffer ionic strength was applied to the column from 50 mM to 250 mM over 24 hours and the effluent from the column collected in fractions (*ca.* 20 ml). The effluent was continuously UV-monitored at 210 nm and the transmittance automatically recorded.

Three peaks were eluted from the column, the third peak spanned fractions 40-60 and, having been identified as the required product, was pooled and evaporated to yield the bis(triethylammonium) salt as an off-white gum. The final traces of triethylammonium bicarbonate were removed by repeated evaporation of dry methanol. The product was obtained (0.052g, ~98% yield).

^{1}H nmr ($CDCl_3$); +6.8, +7.8 (4H, dd, J7.5Hz, J7.5Hz, O<u>Ar</u>); ^{31}P nmr (MeOH) D_2O lock = +40.94 (s) ppm.

Formation of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate

As followed for unlabelled material using (0.148g, 0.41 mmol) (8) of starting material. Product formed (0.152g, ~90% yield).

Preparation of Sp-O-ethyl [^{16}O,^{18}O] thiophosphate

[2R,4S,5R]-2-oxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione

Incorporation of hydroxide as reported by Knowles *et al.*[30] LiOH (0.045g, 1.91 mmol) (2M solution) was dissolved in dioxan (~0.5 ml). This was carried out in a ^{1}H nmr tube. To this was added one equivalent of the starting <u>cis</u> chlorocompound (8) (0.500g, 1.91 mmol). The reaction was

monitored *via* ^{31}P nmr. After several hours the reaction had gone to completion, i.e. no starting material remained. The reaction mixture was then concentrated to dryness and concentrated several times with ethanol. The white monoester amidate lithium salt (19) was used without further purification and was obtained in 0.416g, 90% yield (lit.[30] = 0.46g, 100%). ^{1}H nmr ($CDCl_3$); +0.86 (3H, d, J7Hz, C$\underline{H}_3$) +2.82 (3H, d, $^{3}J_p$ 15Hz, N-C$\underline{H}_3$) +3.85 (1H, ddq, J7Hz, J6.5Hz, $^{3}J_p$ 29.5Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.5Hz, $^{3}J_p$ 1Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +70.79 ppm.

[2R,4S,5R]-2-oxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (using isotopes)

The reaction is as followed for the unlabelled material. Cis chloro-compound is used (0.070g, 0.26 mmol) and $Li^{18}OH/H_2{}^{16}O$ (from Li metal and 33% $H_2{}^{18}O$ (0.006g, 0.26 mmol). Yield obtained (0.066g, ~100%).

P-N bond cleavage of 2-oxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thiones

P-N cleavage is as reported by Knowles *et al.*[30] The monoester amidate lithium salt (19) (0.416g, 1.67 mmol) was dissolved in dry dioxan (1 ml). To this was added a solution prepared from trifluoroacetic acid (0.06g, 5.0 mmol) and ethanol (0.07g, 1.67 mmol). The reaction was carried out in a ^{1}H nmr tube. After one hour the ^{31}P nmr showed that no starting material remained. The reaction was evaporated to dryness and concentrated with ethanol. The diester (20) was formed as its zwitterion which was used without further purification. The solid was obtained (0.479g, 100% yield).[30]

^{1}H nmr ($CDCl_3$); +1.28 (3H, d, J7Hz, C$\underline{H}_3$) +1.29 (3H, t, J6.5Hz, C$\underline{H}_3$) +3.05 (3H, s, N-C$\underline{H}_3$) +3.4 (2H, q, J6.5Hz, OC$\underline{H}_2$) +4.27 (1H, dq, J6.3Hz, J7Hz, C$\underline{H}$-4) +5.2 (2H, broad, N-$\underline{H}_2$) +6.2 (1H, dd, J6.3Hz, $^{3}J_p$ 2.8Hz, C$\underline{H}$-5) +7.3

(5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +52.30 ppm.

Formation of ethyl [^{16}O] thiophosphate (21)

The carbon-oxygen bond cleavage is as followed by Knowles *et al.*[30] To a suspension of the diester (20) as its zwitterion (0.479g, 1.66 mmol) in tetrahydrofuran (0.5 ml) was added four equivalents of sodium metal (0.153g, 6.68 mmol) in liquid ammonia (Birch reduction). The reaction was carried out under nitrogen. The reaction was left for four minutes and quenched with ammonium bicarbonate. The excess ammonia was left to evaporate off and the product was extracted several times with methanol to give the sodium salt of ethyl thiophosphate (21). This was then purified *via* ion-exchange chromatography using 50 mM and 250 mM triethylamine buffers to give the bis(triethylammonium) salt (21) (0.100g, 43%). ^1H nmr ($CDCl_3$); +1.2 (3H, t, J6.5Hz $C\underline{H}_3$) +3.4 (2H, q, J6.5Hz, $OC\underline{H}_2$); ^{31}P nmr = +43.25 ppm (s); ^1H nmr coupled (t, J_{PH} ~10Hz).

Formation of Sp-O-ethyl [^{16}O,^{18}O] thiophosphate

As prepared for unlabelled material. This time using (0.0217g, 0.95 mmol) of sodium and (0.068g, 0.24 mmol) of the diester (20). The product gave (0.032g, 96% yield).

Preparation of racemic ethyl thiophosphate

Formation of ethyl thiopyrophosphate (22) (precursor for racemic ethyl thiophosphate)

To a 25 ml round-bottomed flask was added (0.025g) of ethyl thiophosphate in (0.25 ml) dioxan and (0.30 ml) triethylamine. To this solution was added diphenyl phosphochloridate (0.25 ml). The solution was stirred and monitored at room temperature over several hours by ^{31}P nmr. After this time no starting material remained. The intermediate pyrothiophosphate

(22) was not isolated but was then subjected to hydrolysis.

^{31}P nmr (dioxan) D_2O lock = +47.20 and +24.00 ppm.

Formation of racemic ethyl [^{16}O,^{18}O] thiophosphate (23)

To the pyrothiophosphate intermediate (22) was added triethylamine (0.5 ml) and $H_2{}^{18}O$ (0.02 ml) (98% enrichment). The solution was monitored over 20 hours at room temperature. At this point the reaction had gone to half completion. The solution was then heated at 50°C to speed up the reaction. The reaction was complete after 8 hours. The products were isolated *via* ion-exchange chromatography using 25 and 120 mM buffers.

^{31}P nmr (MeOH) D_2O lock; bis(triethylammonium) salt of diphenyl phosphate = -12.10 ppm; bis(triethylammonium) salt of ethyl thiophosphate = +48.40 ppm.

Ethyl thiophosphate - attempts to dry using dioxan

Ethyl thiophosphate bis(triethylammonium) salt (21) was dried using dioxan. Addition of dioxan to ethyl thiophosphate formed a cloudy product and produced a new resonance in the ^{31}P nmr with the complete disappearance of the starting material. The new product was thought to be a S-S compound (34). This was proved by the addition of one drop of triethylamine (no change in the ^{31}P nmr) and then addition of one drop of mercaptoethanol (this produced a change in resonance to the original starting material resonance, i.e. ethyl thiophosphate). Mercaptoethanol acts as a reducing reagent, i.e. reverses the oxidation to give back the original starting material.

^{31}P of ethyl thiophosphate (EtOH) = +43.58 ppm, ^{31}P nmr of dioxan (EtOH) = +11.49 ppm, ^{31}P nmr of NEt_3 (EtOH) = +11.49 ppm, ^{31}P nmr of NEt_3 + mercaptoethanol (EtOH) = +43.58 ppm.

Starch-iodide test[179]

0.5 ml of dioxan is added to 1 ml of 10% potassium iodide solution acidified with 0.5 ml of dilute (1:5) hydrochloric acid and mixed with a few drops of starch solution just prior to the test. A blue/black coloration appeared in under one minute which therefore showed the presence of peroxides.

Reaction of ethyl [^{16}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

Ethyl [^{16}O] bis(triethylammonium) salt (21) (0.050g, 0.15 mmol) made as previously shown was converted to the bis(t-butylammonium) salt using 0.5 ml t-butylamine and methanol (1 ml). After conversion to the required salt the product was dissolved in dry dioxan (1 ml) and cis chloro-compound (24a) (0.036g, 0.14 mmol) was added to the reaction mixture. The reaction was then left at room temperature overnight. After this time no starting material remained. The dioxan was removed under vacuum. The reaction mixture was dissolved in triethylamine/water (deionized milli-Q water - one drop of triethylamine in 2 ml $H_2{}^{16}O$). This was then washed with ether and subjected to ion-exchange chromatography to give the bis(triethylammonium) pyrophosphate product (0.036g, 54% yield).
^{31}P nmr ($CDCl_3$) D_2O lock, +7.14 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), +46.39 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre).

Reaction of Sp-O-ethyl [^{16}O,^{18}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

As followed for the unlabelled material using (0.100g, 0.30 mmol) of starting material. The product bis(triethylammonium) pyrophosphates (25b/26a) are formed (0.079g, 58% yield).

^{31}P nmr ($CDCl_3$) D_2O lock, +7.14 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one) (^{18}O shift 2.28Hz), +46.39 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre) (^{18}O shift 2.84Hz), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre) (^{18}O shift 4.46Hz).

Preparation of p-nitrophenyl thiophosphate (unlabelled)

[A] Preparation of sodium p-nitrophenolate

The title compound was prepared by placing sodium hydride (0.054g, 2.2 mmol) in a three-necked, round-bottomed flask. The surplus oil from the sodium hydride was removed using petroleum ether. The sodium hydride was then dissolved in acetonitrile (1 ml). The p-nitrophenol (1.585g, 0.0114 mol) was dissolved in acetonitrile (1 ml) and added to the flask. The reaction was monitored *via* ultra-violet spectroscopy. The reaction was complete after 1 hour.

u.v. λ_{max} = 400 nm (ϵ 18,300).

[B] Preparation of dichloride p-nitrophenyl thiophosphorodichloride

The preparation of the title compound was as followed by Breslow et al.[49] Thiophosphoryl chloride (0.5 ml, 5.3 mmol) and triethylamine (0.74 ml, 5.3 mmol) are placed in a round-bottomed flask containing toluene (15 ml). From a dropping funnel sodium p-nitrophenolate (0.737g, 5.3 mmol) dissolved in toluene (8 ml) is added dropwise over several hours to the other reactants. The reaction is carried out under nitrogen. When all the sodium p-nitrophenolate has been added the mixture was left stirring at room temperature overnight. After this time the solution turned brown. The mixture was filtered and concentrated to give a dark brown oil. Distillation at 76° under 12 mmHg gave a clear oil which solidified on cooling to give white crystals (0.793g, 55% yield) (lit.[49] = 0.79g, 55%

yield); m.pt. = 53-54°C (lit. 53-54°C).

^{31}P nmr (toluene) D_2O lock +52.80 (s) ppm.

[C] Preparation of p-nitrophenyl thiophosphate (sodium salt)

The title compound was prepared by the method of Breslow et al.[49] To the dichloride (37) (0.027g, 0.1 mmol) was added sodium hydroxide (16 mg, 0.4 mmol, 4 equivalents) in water (2 ml). After fifteen minutes the reaction had gone to completion with the evolution of hydrogen chloride and the formation of sodium chloride product. The required product was purified using ion-exchange chromatography.

Ion-exchange chromatography

This solution was applied to a column of DEAE sephadex (100 ml, 30 x 4 cm) equilibrated with aqueous ammonium bicarbonate buffer (50 mM, pH 7.8). The column was eluted with a linear gradient of increasing buffer concentrations (50 mM to 250 mM, 2000 ml) and the eluate collected in fractions (80 x 20 ml). Analysis of the product carried out by detection of the eluate on a u.v. spectrometer automatically at 210 nm. Three peaks were collected. The third peak contained the desired product 42-64 [overlap of second peak (p-nitrophenol)]. The fractions 42-64 were concentrated to yield the bis(triethylammonium) salt as a yellow gum (0.036g, 85% yield).

^{31}P nmr (MeOH)(D_2O) lock +40.85 ppm.

Ethanolysis of ethyl Sp [^{16}O,^{18}O] thiophosphate

Ethyl Sp [^{16}O,^{18}O] thiophosphate bis(triethylammonium) salt (0.045g, 0.13 mmol) was dissolved in ethanol (0.5 ml) and 0.3M potassium carbonate (1 ml, pH 9.8 as measured with a glass electrode). The mixture was stirred

for 3 hours at 50°C. The reaction was then concentrated to dryness and subjected to ion-exchange column chromatography.

Ion-exchange chromatography

As followed previously using buffers 50 to 250 mM (0.040g, 89% yield).

^{31}P nmr (EtOH) = +43.58 ppm (s); ^{1}H proton coupled (t, J_{PH} 10Hz).

Analysis of Sp-O-ethyl [^{16}O,^{18}O] thiophosphate

Reaction of ethyl [^{16}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

Ethyl [^{16}O] thiophosphate (isolated under thiophosphoryl transfer conditions) bis(triethylammonium) salt (0.050g, 0.15 mmol) was converted to the bis(t-butylammonium) salt using 0.5 ml t-butylamine and methanol (1 ml). This required several evaporations with methanol before full conversion to the required salt. The ethyl Sp [^{16}O] thiophosphate salt was dissolved in dioxan (1 ml) and cis chlorocompound (24a) was added to the reaction mixture (0.040g, 0.15 mmol). The reaction was left at room temperature overnight. At this point pyrophosphate diastereiosomer derivatives are formed. Upon disappearance of starting material the dioxan was removed under vacuum. The reaction mixture was dissolved in triethylamine/water (1 drop in 2 ml). This was then extracted with ether (several times). The aqueous portion was then purified *via* ion-exchange.

The compound was obtained (0.035g, 50% yield)(25,26).

^{31}P nmr ($CDCl_3$) D_2O lock, +7.14 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), +46.39 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre), diastereoisomer (26), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre).

Ethyl Sp [^{16}O,^{18}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

As followed for unlabelled material using the above quantities.

^{31}P nmr ($CDCl_3$); +7.14 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), (^{18}O shift 2.28Hz), +46.39 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre), (^{18}O shift 2.84Hz), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre) (^{18}O shift 4.46Hz).

Ethanolysis of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate

Ethanolysis of Rp-O-p-nitrophenyl [^{16}O] thiophosphate

A solution of p-nitrophenyl [^{16}O] thiophosphate bis(triethylammonium) salt (18) (0.050g, 0.11 mmol) in ethanol (~0.25 ml) and 0.3M K_2CO_3 (1 ml "pH" 9.7 as measured with the use of a glass electrode) was stirred for 3 hours at 50°C. At this point ^{31}P nmr showed that no substrate remained. The reaction mixture was isolated using ion-exchange chromatography (50 and 120 mM buffers). Product appeared as an off-white gum (0.035g, 90% yield). ^{31}P nmr (EtOH) D_2O lock; +48.58 ppm; ^1H proton coupled (t, J_{PH} 10Hz).

Ethanolysis of p-nitrophenyl R[^{16}O,^{18}O] thiophosphate

As followed above using (0.050g, 0.11 mmol) of starting material gives product (0.035g, 90% yield).

50% Ethanolysis of p-nitrophenyl [^{16}O] thiophosphate

A solution of p-nitrophenyl [^{16}O] thiophosphate bis(triethylammonium) salt (0.050g, 0.11 mmol) in ethanol (~0.25 ml) and 0.3M potassium carbonate (1 ml pH 9.7 as measured with the use of a glass electrode) was stirred for 1½ hours at 50°C. After this time the ^{31}P nmr showed that reaction had gone to half completion. The products were isolated *via* ion-exchange chromatography.

Ion-exchange chromatography

A column of DEAE-Sephadex A25 (100 ml, -30 x 4 cm diameter) was equilibrated with aqueous triethylammonium bicarbonate buffer (50 mM, pH 8.2). The ethanoic solution from above was applied to the top of the gradient at a rate of 80 ml per hour. A linear gradient of increasing buffer ionic strength was applied to the column, from 50 to 250 mM over 24 hours and the effluent from the column collected in fractions (~20 ml). The effluent was monitored continuously using u.v. spectroscopy at 210 nm and the transmittance automatically recorded. Three peaks were eluted from the column. The second peak was identified as p-nitrophenyl thiophosphate and collected as fractions 30-50 and the third peak was identified as ethyl thiophosphate and collected between fractions 55-70. Both compounds were evaporated to yield the bis(triethylammonium) salt as a yellow gum (p-nitrophenyl thiophosphate) and an off-white gum (ethyl thiophosphate). The final traces of the buffer were removed by repeated evaporation of dry methanol.

Products:- p-nitrophenyl thiophosphate bis(triethylammonium) salt (0.022g, ~44% yield), ethyl thiophosphate bis(triethylammonium) salt (0.0182g, 46% yield).

^{31}P nmr (MeOH) D_2O lock:- p-nitrophenyl thiophosphate = +40.92 ppm (s), ethyl thiophosphate = +43.78 ppm (s); ^{1}H proton coupled (t, J_{PH} 10Hz).

Reaction of p-nitrophenyl [^{16}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

p-Nitrophenyl [^{16}O] thiophosphate bis(triethylammonium) salt (18) (0.050g, 0.11 mmol) was converted to the bis(t-butylammonium) salt using 0.5 ml t-butylamine and methanol (1 ml). This required several evaporations before full conversion to the required salt. This was then dissolved in dioxan (1 ml) and the chlorocompound (24a) (0.035g, 0.13 mmol) was added

to the reaction mixture. The reaction was left overnight at room temperature. After this time pyrophosphate diastereoisomer (derivatives) are formed. The dioxan is removed under vacuum to give the required product (0.033g, 52% yield). The mixture was taken up in $CDCl_3$ and analysed in crude form on the AM 300 (high-field nmr).

^{31}P nmr ($CDCl_3$) D_2O lock; +6.5 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), +39.48 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre), +6.0 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +39.38 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre).

Analysis of Rp-O-p-nitrophenyl [^{16}O,^{18}O] thiophosphate

Reaction of p-nitrophenyl R[^{16}O,^{18}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

As followed for unlabelled material.

^{31}P nmr ($CDCl_3$) D_2O lock; +6.5 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), +39.48 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre) (^{18}O shift 4.46Hz), +6.0 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one) (^{18}O shift 2.28Hz), +39.38 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre) (^{18}O shift 2.84Hz).

Reaction of ethyl [^{16}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

Ethyl thiophosphate bis(triethylammonium) salt (21) (0.025g, 0.072 mmol) was converted to the bis(t-butylammonium) salt using 0.5 ml (excess) t-butylamine and methanol (1 ml). The compound required several concentrations using methanol and then dioxan before full conversion to the required salt. Upon conversion to the required salt the compound was dissolved in dioxan (1 ml) and the cis chlorocompound (24a) was added (0.018g, 0.073 mmol). The reaction was left sealed overnight at room temperature. After this time pyrophosphate diastereoisomer derivatives

are formed and no starting material remains. The dioxan from the reaction is removed under vacuum. The product was dissolved in triethylamine/water (milli-Q) mixture (1 drop in 2 ml). This was then extracted with ether (2 ml). The water portion was then purified *via* ion-exchange chromatography.

Ion-exchange chromatography

A column of DEAE sephadex A25 (50 ml, 20 x 4 cm diameter) was equilibrated with aqueous triethylammonium bicarbonate buffer (25 mM, pH 8.0). The aqueous solution from above was applied to the top of the column at a rate of 70 ml per hour. A linear gradient of increasing buffer ionic strength was applied to the top of the column, from 25 to 120 mM over 8 hours and the effluent collected in ~20 ml fractions. The effluent was continuously monitored using u.v. spectroscopy at 210 nm and the transmittance automatically recorded. Two pyrophosphate peaks were eluted. The first peak is a P-N ring-opened compound, the second peak is unopened pyrophosphate and the required product. The product eluted in fractions 12-20 were evaporated to yield the bis(triethylammonium) salt as a colourless gum. The final traces of buffer were removed by repeated evaporation of dry methanol. Product (0.015g, 37% yield).

^{31}P nmr $\delta(CDCl_3)$; +7.00 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +46.19 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre).

Analysis of ethyl [^{16}O,^{18}O] thiophosphate

Reaction of ethyl [^{16}O,^{18}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

The reaction was carried out as for unlabelled material using the above

quantities.

^{31}P nmr $\delta(CDCl_3)$; +7.00 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), (^{18}O shift 2.28Hz), +46.29 (d, J_{pp} 25.9Hz, Rp thiophosphoryl centre), (^{18}O shift 2.84Hz, 4.46Hz), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one) (^{18}O shift 2.28Hz), +46.19 (d, J_{pp} 29.7Hz, Sp thiophosphoryl centre) (^{18}O shift 2.84Hz, 4.46Hz).

Reaction of racemic ethyl [^{16}O,^{18}O] thiophosphate with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one

Racemic ethyl [^{16}O,^{18}O] thiophosphate bis(triethylammonium) salt (0.030g, 0.087 mmol) was converted to the bis(t-butylammonium) salt using 0.5 ml (excess) t-butylamine and methanol (1 ml). This required several concentrations using methanol and then dioxan to give conversion to the required salt. Ethyl thiophosphate is then dissolved in dioxan (1 ml) and cis chlorocompound (23) is added (0.023g, 0.09 mmol). The reaction is left sealed overnight at room temperature, after which time pyrophosphates are formed. The dioxan is then removed and the product is subjected to extraction with ether and purification *via* ion-exchange (as described previously) using 50 and 120 mM buffers. A white solid was formed which was subjected to re-columning using 30 and 180 mM. Again a white solid was formed (10-fold excess). The excess solid could not be removed.

Formation of potassium tertiary butoxide

To freshly distilled tertiary butanol (15 ml) was added (0.600g) of potassium metal in small amounts over one hour. The tertiary butanol was heated to just above its melting point so that the metal could fully dissolve. The apparatus was sealed except for a small outlet for the escaping hydrogen gas. The reaction was complete after several hours and

evaporation of the solvent gives the product as a white crystalline compound (0.650g).

Formation of 2-t-butoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione

Formation of 2-t-butoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (40)

The cis-chlorocompound (8a) (0.260g, 1.0 mmol) dissolved in dichloromethane (5 ml). To this solution was added tertiary potassium butoxide solution (1 ml) (2M) *via* a syringe. This was added a little at a time. The mixture is kept under nitrogen for 20 hours and stirred continuously. After this time no starting material remained (monitored *via* ^{31}P nmr). The solution was then partitioned between water and dichloromethane. The dichloromethane layer is concentrated to dryness. Any impurities in the compound were removed by passing through a short plug of silica with dichloromethane (~400 ml). The solution is then concentrated by evaporation to remove the dichloromethane. The product formed appears as an off-white oil (0.148g, 50% yield).

^{1}H nmr ($CDCl_3$); +0.85 (3H, d, J7Hz, $C\underline{H}_3$) +1.5 (9H, s, $^{t}\underline{Bu}$) +2.85 (3H, d, $^{3}J_p$ 14Hz N-$C\underline{H}_3$) +3.80 (1H, ddq, J7Hz, J6.5Hz, $^{3}J_p$ 22.5Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.5Hz, $^{3}J_p$ 1Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr = +73.61 ppm.

Solvolysis of p-nitrophenyl thiophosphate in aqueous ethanol

To bis(triethylammonium) salt of p-nitrophenyl thiophosphate (18) (0.050g, 0.11 mmol) was added water (0.0057g) and ethanol (0.0146g). The mixture was left stirring for two days under nitrogen at room temperature. The mixture was monitored over this time by ^{31}P nmr to give the amounts of inorganic thiophosphate and ethyl thiophosphate formed. After this time no starting material remained and the reaction was complete.

^{31}P nmr (H_2O/EtOH) D_2O lock; +43.06 ppm (ethyl thiophosphate); ^{1}H nmr coupled (t, J_{PH} ~10Hz), +32.50 ppm (inorganic thiophosphate).

Preparation of [^{16}O] inorganic thiophosphate

Formation of 2-oxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (19)

The cis chlorocompound (8a) (0.050g, 0.19 mmol) was dissolved in dioxan (0.1 ml). To this solution was added lithium hydroxide (10.4 mg in 0.49 ml $H_2{}^{16}O$) in dioxan (0.1 ml). The reaction was monitored *via* ^{31}P nmr. After one hour the reaction had gone to completion. Concentration to dryness and extracting with methanol produced a white lithium salt as product (0.030g, 65% yield).[30]

^{1}H nmr ($CDCl_3$); +0.86 (3H, d, J7Hz, C$\underline{H}_3$) +2.82 (3H, d, $^{3}J_P$ 15Hz, N-C$\underline{H}_3$) +3.85 (1H, ddq, J7Hz, J6.5Hz, $^{3}J_P$ 29.5Hz, C$\underline{H}$-4) +5.81 (1H, dd, J6.5Hz, $^{3}J_P$ 1Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (dioxan) D_2O lock = +70.00 ppm.

Formation of [^{18}O]-2-oxy-1,3,2-oxazaphospholidin-2-thione

As followed for unlabelled material using (0.050g, 0.19 mmol) of cis chlorocompound (8a) in $Li^{18}OH$ (10.4 mg in 0.49 ml $H_2{}^{16}O$). Product formed is (0.030g, 65% yield).

P-N cleavage of 2-oxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione

This is as reported by Knowles *et al.*[30] The lithium salt of the monoester amidate (19) (0.050g, 0.205 mmol) was dissolved in dioxan (0.12 ml). To this solution was added (0.068g, 0.6 mmol) trifluoroacetic acid (0.0002g). The reaction was monitored *via* ^{31}P nmr. After several hours the reaction had gone to completion. The solution was concentrated to dryness and extracted several times with methanol to give the diester as

its zwitterion (0.032g, 60% yield).[30]

^{1}H nmr ($CDCl_3$); +1.27 (3H, d, J7Hz, C$\underline{H}_3$) +3.05 (3H, s, N-C$\underline{H}_3$) +4.24 (1H, dq, J7Hz, J6.3Hz, C$\underline{H}$-4) +5.2 (2H, broad, N-$\underline{H}_2$) +6,2 (1H, dd, J7Hz, ^3J_p 2.8Hz, C$\underline{H}$-5) +7.3 (5H, m, Ph); ^{31}P nmr = +52.00 ppm.

P-N cleavage of [^{18}O]-2-oxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione

As followed for unlabelled material using (0.050g, 0.2 mmol) of [^{18}O]-2-oxy-1,3,2-oxazaphospholidin-2-thione and (0.068g, 0.6 mmol) trifluoro-acetic acid in (0.0002g). Product formed is (0.032g, 60% yield).

To effect C-O bond cleavage using Na/liq.NH_3

To a suspension of the monoester (42) (0.050g, 0.14 mmol) in tetrahydro-furan (0.5 ml) was added four equivalents of sodium metal (0.013g, 0.58 mmol) in liquid ammonia (Birch reduction). The reaction was carried out under nitrogen. The reaction was left for four minutes and quenched with ammonium bicarbonate. The excess ammonia was left to evaporate off and the product was azeotroped several times with methanol to give a white solid. The product gave a ^{31}P nmr = 0 ppm.

This has led to a product with loss of sulphur, therefore this reaction was no longer used.

To effect C-O bond cleavage using TMSI

This was prepared by the method of Knowles *et al.*[30] To a suspension of the monoester (42) (0.050g, 0.14 mmol) in dichloromethane (0.5 ml) is added trimethylsilyliodide (0.109g, 0.55 mmol). The mixture was left in a sealed tube under the following conditions:- (1) room temperature, (2) heated to 35°C, (3) heated to 70°C. After the initial chemical shift from

+52.00 to +40.00 ppm, no further reaction occurred. Upon heating to 70°C the product decomposed.

^{31}P nmr (CH_2Cl_2) D_2O lock = 0 ppm.

Methylation of P-N bond cleavage compound

To a suspension of the cis monoester amidate (42) (0.050g, 0.14 mmol) in methanol (0.5 ml) was added two equivalents of methyl iodide (0.039g, 0.28 mmol). The reaction was kept in a sealed vessel at room temperature. The reaction was monitored *via* ^{31}P nmr. The mixture was left overnight and after this time no starting material remained as monitored by ^{31}P nmr. The mixture was concentrated to remove any excess of methyl iodide. The solid was azeotroped several times with methanol to produce the diester (43) as its iodide salt (0.050g, 99% salt).

^{31}P nmr ($CDCl_3$) D_2O lock = +28.00 ppm.

Formation of [^{16}O] inorganic thiophosphate (after methylation)

(0.050g, 0.14 mmol) of the diester iodide salt (43) was dissolved in dichloromethane (0.5 ml). The compound is sparingly soluble. To this is added (0.109g, 0.55 mmol) of trimethylsilyliodide. This is left in a sealed tube at room temperature for several hours. After this two drops of triethylamine and two drops of isopropanal were added to the reaction mixture along with 1-2 drops of mercaptoethanol. To this was added one spatula of sodium sulphate in water to hydrolyse the trimethylsilyl esters. The reaction was then concentrated to dryness to give the product as an off-white solid. This product was purified *via* ion-exchange chromatography using buffers of ionic strength 50 to 250 mM. The required product was eluted from fractions 10-20 and the solution was evaporated to yield the bis(triethylammonium) salt of the product (0.058g, 68% yield).

^{1}H nmr ($CDCl_3$)(D_2O) proton coupled singlet; ^{31}P nmr = +42.55 ppm (s).

Formation of Sp [^{16}O,^{18}O] inorganic thiophosphate

As followed for unlabelled material using (0.050g, 0.14 mmol) of starting material. Product formed 0.058g, 68% yield.

Preparation of 2-O-benzyl-(S)-propanediol

[A] Sodium hydroxide solution (9.2g, 0.23 mol, 50 ml H_2O) was run into silver nitrate solution (34g, 0.20 mol, 50 ml H_2O) at 0°C with vigorous stirring. The solution was left stirring for two hours at which point the reaction was stopped and a brown precipitate formed. The solid was filtered and washed until the washings became neutral. Acetone (10 ml) and ether (10 ml) were then used to further wash the solid. This is then dried. Product appears as a brown solid (26g, 55% yield).

[B] Preparation of ethyl-2-benzyl-(S)-lactate (45)

This was prepared by Knowles *et al.*[100] Freshly prepared silver oxide (13g, 0.056 mol) was added over thirty minutes to a solution of ethyl-(S)-lactate (6.6g, 0.056 mol) and benzyl bromide (14.3g) in ether (60 ml). When the addition was complete, the mixture was refluxed for approximately thirty minutes. The solid material formed was removed by filtration with ether. The solvent was removed from the combined ether solution and the residue distilled *in vacuo* at a temperature of 150°C. A colourless solution was obtained as ethyl-2-benzyl-(S)-lactate (5.82g, 50% yield).[100]
T.l.c. of product petroleum spirit/ethyl lactate 7:3, R_f = 0.6.
^{1}H nmr ($CDCl_3$); +1.2 (3H, t, J6.5Hz $C\underline{H}_3$) +1.6 (3H, d, J7Hz, $C\underline{H}_3$) +3.5 (2H, q, J6.5Hz, C$\underline{H}$) +3.8 (1H, q, J7Hz, C$\underline{H}$) +4.7 (2H, d, $OC\underline{H}_2$) +7.2 (5H, m, Ph).

[C] Preparation of 2-O-benzyl-(S)-propanediol (46)

This was prepared by Knowles *et al.*[100] In a 3-necked glask fitted with a reflux condenser and graduated dropping funnel is added ethyl-2-benzyl-(S)-lactate (5.2g, 0.025 mmol) in tetrahydrofuran (40 ml) dropwise at 0°C to lithium aluminium lactate (0.822g, 0.030 ml). After addition, the solution is refluxed for approximately ten minutes. After cooling the solution, 10 mls of aqueous sulphuric acid is added. This is then extracted with ether. The ether layer is concentrated and the oil obtained is subjected to column chromatography in petroleum spirit/diethyl ether. The product is then distilled at 150°C (oven temperature) using 0.5 mmHg. 2-Benzyl-(S)-propane-1,2-diol is obtained as a colourless viscous oil (1.64g, 45% yield).

^{1}H nmr ($CDCl_3$); +1.6 (3H, d, J7Hz, $C\underline{H}_3$) +3.4 (1H, tq, J7Hz, J7Hz, C$\underline{H}$) +3.4 (2H, d, J7Hz, $C\underline{H}_2$) +3.5 (1H, s, O$\underline{H}$) +4.4 (2H, d, $OC\underline{H}_2$) +7.2 (5H, m, Ph); [α] 2.84° (neat liquid) (lit.[100] 2.77).

Reactions of Inorganic thiophosphate in alcohol

Reaction of sodium thiophosphate in Br_2/propandiol

This is prepared as reported by Knowles *et al.*[100] Sodium thiophosphate (0.030g, 0.16 mmol) was dissolved in propandiol (1 ml). Liquid bromine (0.128g, 0.8 mmol, 5 equivalents) was added to propandiol (1 ml). The two solutions were mixed and the solution decoloured after a few hours. At this point the ^{31}P nmr showed the presence of a new product and no starting material. Water (10 ml) and ether (10 ml) was then added to the solution. The mixture is separated. The aqueous layer was evaporated to dryness to give an off-white oil as the bromide salt (47) (0.016g, 55% yield).

(47a) T.l.c. acetone/petroleum spirit 7:4, R_f = 0.12.

^{1}H nmr ($CDCl_3$); +1.2 (3H, d, J7Hz, C$\underline{H}_3$) +3.4 (1H, m, C$\underline{H}$) +3.4 (2H, d, J7Hz, C$\underline{H}_2$) +4.4 (1H, s, O$\underline{H}$); ^{31}P nmr (H_2O) D_2O lock = +1.6 ppm.

(47b) T.l.c. acetone/petroleum spirit 7:4, R_f = 0.16.

^{1}H nmr ($CDCl_3$); +1.2 (3H, d, J7Hz, C$\underline{H}_3$) +3.4 (1H, m, C$\underline{H}$) +3.5 (2H, m, C$\underline{H}_2$) +4.4 (1H, s, O$\underline{H}$).

Reaction of sodium thiophosphate in I_2/propandiol

This is prepared as reported by Knowles *et al.*[100] Sodium thiophosphate (0.030g, 0.16 mmol) was dissolved in propandiol (0.5 ml). Iodine (0.061g, 0.24 mmol, 1.5 equivalents) was added to propandiol (0.5 ml). The two solutions were slowly added together. The colourless solution turned blue/black and after one hour the ^{31}P nmr showed that no starting material remained and that the reaction had gone to completion. To the solution, 10 ml water was added and extracted with 10 ml portions of ether. The aqueous layer was evaporated to dryness to give an off-white oil. This was purified using ion-exchange chromatography using 50 and 120 mM buffers. Product obtained as iodide salt (47) (0.018g, 60% yield).

(47a) ^{1}H nmr ($CDCl_3$); +1.2 (3H, d, J7Hz, C$\underline{H}_3$) +3.4 (1H, m, C$\underline{H}$) +3.4 (2H, d, J7Hz, C$\underline{H}_2$) + 4.4 (1H, s, O$\underline{H}$); ^{31}P nmr (H_2O) D_2O lock = +3.0 ppm.

T.l.c. acetone/petroleum spirit 7:4, R_f = 0.12.

^{1}H nmr ($CDCl_3$); +1.2 (3H, d, J7Hz, C$\underline{H}_3$) +3.4 (1H, m, C$\underline{H}$) +3.5 (2H, m, C$\underline{H}_2$) +4.4 (1H, s, O$\underline{H}$).

Briggs-Phosphate Test

From the ion-exchange fractions take ⅓ to ½ ml of solution of every second to third fraction. This solution is evaporated to dryness. Then two drops of concentrated sulphuric acid is added to the test tubes containing the concentrated material. These tubes are heated between

60-100°C for half an hour. To each test tube/fraction is added 0.5 ml of solution (1), 0.25 ml of solution (2) and 0.25 ml of solution (3). The mixtures are shaken well and either left overnight or heated gently. A blue colouring of the solutions in the test tubes is a positive indicator of the presence of phosphate.

Solution (1)

7.5 ml of concentrated sulphuric acid is added slowly, with stirring, to 2.5g of ammonium molybdate in 20 ml of high purity deionised water.

Solution (2)

One drop of concentrated sulphuric acid is added to 0.5g of hydroquinone in 100 ml of water (high purity).

Solution (3)

20 ml of high purity water is added to 4g of sodium sulphate.

Reaction of triethylammonium thiophosphate in iodine/2-O-benzyl-(S)-propanediol

This is prepared as reported by Knowles *et al.*[100] Triethylammonium thiophosphate (0.110g, 0.028 mmol) was dissolved in acetonitrile (0.25 ml). Iodine (0.146g, 2 equivalents 0.056 mmol) was dissolved in benzyl propane-1,2-diol (0.084 mmol, 3 equivalents). The two solutions were added together in a sealed reaction vessel at room temperature. After one hour the reaction had gone to completion. The product was dissolved in water (25 ml) and extracted with ether (three 20 ml portions). The aqueous layer was evaporated to dryness and the residue dissolved in dry methanol (3 ml) and acetone (1 ml). The product was subject to ion-exchange chromatography using a buffer gradient between 50 and 120 mM. The product was obtained as a dark brown gum (48) (0.109g, 75% yield). ^{1}H nmr ($CDCl_3$); +1.2 (3H, d, J6Hz $C\underline{H}_3$) +3.4 (1H, tq, J7Hz, J7Hz, C$\underline{H}$) +3.5

(2H, dd, $^{3}J_{P}$ 9Hz, C$\underline{H}_2$) +4.1 (2H, d, OC$\underline{H}_2$), +7.2 (5H, s, broad); ^{31}P nmr (MeOH) D_2O lock = +0.00 ppm.

Hydrogenolysis of the benzyl group

(0.020g, 0.036 mmol) of bis(triethylammonium) salt of phospho-benzyl-(S)-propane-diol (48) was dissolved in 10 mls of ethanol/water (1:1) mixture and placed in a hydrogenation flask. To this solution is added palladium on charcoal (0.040g) as the catalyst. The flask was sealed and placed on the hydrogenator. The reaction was left overnight. After this time the reactants are filtered through a glass fibre filter paper. The product is washed with 5 ml of concentrated ammonia solution in 75 ml water. The filtrate is concentrated to give the product. However, two hydrogenations are required to fully remove the benzyl group. The product phospho-propanediol is obtained as a gum (0.015g, 88% yield).

Epimerisation reactions of 1,3,2-oxazaphospholidin-2-ones and 2-thiones

[2R,4S,5R] and [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-ones (24)

Prepared by the method of Inch *et al.*[80] Phosphoryl chloride (3.2g, 0.02 mol) was added to a stirred solution of (-)ephedrine hydrochloride (4.1g, 0.02 mol) and triethylamine (10.0g, 0.1 mol) in dry benzene (100 ml) under nitrogen. After a mildly exothermic reaction, the mixture was stirred for a further two hours, stored overnight at room temperature, filtered and concentrated. The residue was chromatographed in diethyl ether/toluene 4.5:1. The product was dried in a vacuum desiccator over phosphorus pentoxide to give products cis (3.2g, 65% yield) (lit. = 3.14g, 63%), m.pt. 88-89°C (lit.[80] 88-89°C).

^{1}H nmr ($CDCl_3$); +0.83 (3H, d, J7Hz, C$\underline{H}_3$) +2.85 (3H, d, $^{3}J_{P}$ 13Hz, N-C$\underline{H}_3$)

+3.85 (1H, ddq, J7Hz, J6.5Hz, ^3J_p 26Hz, C<u>H</u>-4) +5.85 (1H, dd, J6.5Hz, ^3J_p 1Hz, C<u>H</u>-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +18.20 ppm.

<u>trans</u> (0.2g, 4% yield) (lit. 0.3g, 6% yield), m.pt. 111-113°C (lit. 111-113°C).

^{1}H nmr ($CDCl_3$); +0.80 (3H, d, J7Hz, C<u>H</u>$_3$) +2.65 (3H, d, ^3J_p 13Hz, N-C<u>H</u>$_3$) +3.70 (1H, ddq, J7Hz, J7Hz, ^3J_p 14Hz, C<u>H</u>-4) +5.54 (1H, dd, J7Hz, ^3J_p 7Hz, C<u>H</u>-5) +7.3 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +20.3 ppm.

<u>Reaction of 2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one with O-ethyl-thiophosphate in the presence of pyridine</u>

Ethyl thiophosphate bis(triethylammonium) salt (0.025g, 0.072 mmol) was converted to the bis(t-butylammonium) salt using (0.5 ml) excess t-butylamine in methanol (1 ml). The compound (24) required several concentrations using methanol and then dioxan before full conversion to the required salt. This salt was then dissolved in pyridine (1 ml) and the cis chlorocompound (24a) was added (0.018g, 0.073 mmol). The reaction was left sealed overnight at room temperature. After this time pyrophosphate diastereoisomer derivatives are formed (25,26,27,28) in comparable amounts and no starting material remains.

^{31}P nmr δ($CDCl_3$) D_2O lock; +7.14 (d, J_{pp} 25.9Hz, 1,3,2-oxazaphospholidin-2-one), +46.39 (d, J_{pp} 25.9Hz, <u>Rp</u> thiophosphoryl centre), +6.65 (d, J_{pp} 29.7Hz, 1,3,2-oxazaphospholidin-2-one), +46.29 (d, J_{pp} 29.7Hz, <u>Sp</u> thiophosphoryl centre).

<u>Minor isomer</u>

^{31}P nmr ($CDCl_3$) D_2O lock, +6.85 (d, J_{pp} 32.15Hz, 1,3,2-oxazaphospholidin-2-one), +45.98 (d, J_{pp} 32.15Hz, <u>Sp</u> thiophosphoryl centre), 6.75 (d, J_{pp} 31.25Hz, 1,3,2-oxazaphospholidin-2-one), +45.58 (d, J_{pp} 31.25Hz, <u>Rp</u> thiophosphoryl centre).

Reaction of cis pyrophosphate with pyridine

The cis pyrophosphate product (25) (major isomer) (0.1g, 0.18 mmol) was dissolved in pyridine (2 ml). The reaction was monitored by ^{31}P nmr spectroscopy over several days at room temperature. After this time no epimerisation had taken place.

Reaction of [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one with pyridine

2-Chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (24) (0.1g, 0.38 mmol) was dissolved in pyridine (2 ml) and monitored by ^{31}P nmr spectroscopy under the following conditions:-

(a) At room temperature over one day.
(b) At 35°C over a few hours.
(c) At 70°C over one hour.

Epimerisation occurred during these reactions and the equilibrium mixture in all cases estimated from integration of ^{31}P nmr was 1:2 cis:trans.

^{31}P nmr (pyridine) D_2O lock, shift of cis +19.54 ppm to +24.0 ppm.

The reaction was repeated using 2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (8) (0.1g, 0.38 mmol). This time the reaction was carried out at 50°C and ^{31}P nmr spectroscopy showed that the reaction was slower than the oxy compound (24) ($t_{½}$ ca. 16 hours at 50°C). The equilibrium mixture in this case was 1:3 cis:trans.

^{31}P nmr (pyridine) D_2O lock, shift of cis +74.10 ppm to trans +78.50 ppm.

Formation of 2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one in the presence of pyridine

Phosphoryl chloride (3.2g, 0.02 mol) was added to a stirred solution of (-)ephedrine hydrochloride (4.1g, 0.02 mol) in pyridine (80 ml) under nitrogen. After a mildly exothermic reaction the mixture was stirred for 3 hours, stored overnight at room temperature, filtered and concentrated.

The residue was chromatographed in toluene and ether 5:1. The product (24) was dried over phosphorus pentoxide to give a major product of trans configuration; trans (3.4g, 68% yield), m.pt. 111-113°C (lit.[80] 111-113°C). ^{1}H nmr ($CDCl_3$); +0.8 (3H, d, J7Hz, C$\underline{H}_3$) +2.65 (3H, d, ^3J_p 13Hz, N-C$\underline{H}_3$) +3.70 (1H, ddq, J7Hz, J7Hz, ^3J_p 14Hz, C$\underline{H}$-4) +5.54 (1H, dd, J7Hz, ^3J_p 7Hz, C$\underline{H}$-5) +7.3 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +20.3 ppm.
cis (1.2g, 24% yield); ^{1}H nmr ($CDCl_3$); +0.83 (3H, d, J7Hz, C$\underline{H}_3$) +2.85 (3H, d, ^3J_p 13Hz, N-C$\underline{H}_3$) +3.85 (1H, ddq, J7Hz, J6.5Hz, ^3J_p 26Hz, C$\underline{H}$-4) +5.85 (1H, dd, J6.5Hz, ^3J_p 1Hz, C$\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +18.20 ppm.

Epimerisation reaction of [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one using lutidene

The cis chlorocompound (24a) (0.20g, 0.77 mmol) was dissolved in lutidine (0.16g, 1.54 mmol). The reaction was monitored *via* ^{31}P nmr at room temperature over several days during which no epimerisation occurred. The reaction was heated to 35°C but again no epimerisation occurred. The reaction was then heated to 70°C and again no epimerisation occurred.

The above reactions were repeated using 2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (8) (0.20g, 0.76 mmol). The reactions were monitored using ^{31}P nmr - again no epimerisation occurred.

Formation of 2-methoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione (61)

This is followed as reported by Inch.[80] To the cis chlorocompound (24) (0.2g, 0.76 mmol) is added pyridine (1 ml) and methanol (0.024g, 0.76 mmol). The reaction is left stirring for several hours at room temperature under N_2. The reaction is monitored *via* ^{31}P nmr. After this time no starting material remained. The products were formed in a 3:1 mixture of

cis to trans (0.159g, 80% yield).

^{31}P nmr (pyridine) (D_2O) lock; +84.20 ppm (cis OMe), +83.49 ppm (trans OMe). As chemical shifts are extremely close, a known sample of cis methoxide is added to the above solution to accurately distinguish between the isomers.

Formation of cis 2-methoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione

Prepared from the method of Inch *et al.*[80] A solution (*ca.* 2M) of 1 mol equivalent of the methoxide (0.5 ml) in methanol (5 ml) was slowly added to a solution of the cis chlorocompound (8a) (0.26g, 1 mmol). The mixture was stirred for *ca.* 20 hours, poured into water, and extracted with methylene chloride. The extract was concentrated and the product was chromatographed over silica in dichloromethane to give the final product as a clear oil (0.20g, 80% yield) (lit.[80] 0.20g, 80% yield), b.pt. = 140°C at 0.1 mmHg (lit. = 140°C at 0.1 mmHg).

^1H nmr ($CDCl_3$); +0.8 (3H, d, J7Hz, $C\underline{H}_3$) +2.72 (3H, d, ^3J_P 12.5Hz, N-$C\underline{H}_3$) +3.6 (3H, d, ^3J_P 10Hz, O$C\underline{H}_3$) +3.65 (1H, ddq, J7Hz, J6.25Hz, ^3J_P 16.25Hz, $C\underline{H}$-4) +5.60 (1H, dd, J6.25Hz, ^3J_P 4.4Hz, $C\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +84.20 ppm.

Reaction of [2S,4S,5R]-2-methoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-thione with pyridine

[A] Pyridine (1 ml) was added to the methoxy compound (61a) (0.20g, 0.76 mmol). The reaction was stirred at room temperature and kept under nitrogen for several days. Over this time the reaction was monitored *via* ^{31}P nmr. No epimerised product appeared and so the experiment was stopped.

[B] The above reaction was carried out at 35°C. Again no epimerised

product appeared.

[C] The reaction was carried out for the trans isomer (61b). Again no epimerised product appeared.

Epimerisation of [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thiones with imidazole

[1] The chlorocompound (8) (0.036g, 0.14 mmol) was dissolved in dichloro-methane (0.25 ml). To this was added imidazole as follows:-

(a) (0.0242g, 2 equivalents)
(b) (0.048g, 4 equivalents)
(c) (0.096g, 8 equivalents)

(a), (b) and (c) were carried out at room temperature and 35°C. The reaction was monitored over several days by ^{31}P nmr.

[2] The above reaction was carried out in imidazole (0.0242g, 2 equivalents) at room temperature and 35°C.

^{31}P nmr (CH_2Cl_2) D_2O lock; +72.00 ppm (new product).

[3] The reaction [1] to [3] were quenched with methanol (2 equivalents) to form the product cis methoxy ester.[80]

^{31}P nmr (dichloromethane) D_2O lock; +83.00 ppm.

^{1}H nmr ($CDCl_3$); +0.8 (3H, d, J7Hz, $C\underline{H}_3$) +2.72 (3H, d, $^{3}J_p$ 12.5Hz, N-$C\underline{H}_3$) +3.6 (3H, d, $^{3}J_p$ 10Hz, O$C\underline{H}_3$) +3.65 (1H, ddq, J7Hz, J6.25Hz, $^{3}J_p$ 16.25Hz, $C\underline{H}$-4) +5.60 (1H, dd, J6.25Hz, $^{3}J_p$ 4.4Hz, $C\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +83.00 ppm.

Epimerisation of [2S,4S,5R]-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxaza-phospholidin-2-thiones with 4-dimethylamino-pyridine

[1] The chlorocompound (8) (0.028g, 0.12 mmol) was dissolved in (0.25 ml) dichloromethane. To this was added 4-dimethylamino-pyridine in the following quantities.

(a) (2 equivalents, 0.026g)

(b) (4 equivalents, 0.052g)

(c) (8 equivalents, 0.10g)

The reaction was monitored by ^{31}P nmr over room temperature and 35°C.

^{31}P nmr (CH_2Cl_2) D_2O lock; shift +75.63 ppm to +75.22 ppm.

[2] The reaction was then quenched with methanol to give cis-methoxy product.[80]

^{31}P nmr (CH_2Cl_2) D_2O lock; +83.00 ppm.

^{1}H nmr ($CDCl_3$); +0.8 (3H, d, J7Hz, $C\underline{H}_3$) +2.72 (3H, d, $^{3}J_p$ 12.5Hz, N-$C\underline{H}_3$) +3.6 (3H, d, $^{3}J_p$ 10Hz, O$C\underline{H}_3$) +3.65 (1H, ddq, J7Hz, J6.25Hz, $^{3}J_p$ 16.25Hz, $C\underline{H}$-4) +5.60 (1H, dd, J6.25Hz, $^{3}J_p$ 4.4Hz, $C\underline{H}$-5) +7.2 (5H, m, Ph); ^{31}P nmr (CH_2Cl_2) D_2O lock = +83.00 ppm.

Preparation of 1,3,2-dioxaphosphorinan-2-one compounds

Preparation of methyl-2,3-dimethyl-4-6-benzylidene-β-D-glucoside (81)

As prepared by Irvine and Scott.[167] A solution of recrystallised methyl-4-6-benzylidene-β-D-glucoside (10g, 0.037 mmol) in 50 ml of acetone was placed in a three-necked, round-bottomed flask. The flask was equipped with a mechanical stirrer and seal, two burettes and a distillation tube connected to a condenser. The flask was maintained at 50°C. The solution was stirred. To the stirred solution was added dropwise from the burettes dimethyl sulphate (22 ml) and concentrated sodium hydroxide (24.5 ml, 50% excess). The reagents were added and the solution stirred for one hour. The solution was then vigorously stirred and acetone distilled over. The residue was filtered and the white solid was formed. Recrystallisation from benzene/acetone 7:4 gave white prisms (6.36g, 55% yield); m.pt. 122-123°C (lit.[160] 122-123°C). T.l.c. in benzene/acetone (7:4) product R_f = 0.7.

Preparation of methyl-2,3-dimethyl-β-D-glucoside (82)

As prepared by Irvine and Scott.[167] (2.5g, 0.080 mmol) of methyl-2,3-dimethyl-4-6-benzylidene-β-D-glucoside (81) was dissolved in 50 ml of hot acetone. 5 ml of water containing 0.2 ml of concentrated hydrochloric acid was added to the solution. The solution was refluxed for four hours, neutralised with an excess of potassium bicarbonate. The solution was then filtered and 50 ml of toluene was added. A white solid formed on concentration to dryness. Recrystallisation from benzene/hexane 7:4 gave white needle-shaped crystals, (1.32g, 70% yield) (lit. 65%); m.pt. = 80-81°C (lit.[160] 80-81°C). T.l.c. in benzene/acetone 7:3 R_f = 0.3. ^{1}H nmr ($CDCl_3$); +3.75 (3H, s, $OC\underline{H}_3$) +3.80 (3H, s, $OC\underline{H}_3$) +3.83 (3H, s, $OC\underline{H}_3$) +3.80 (4H, broad, C$\underline{H}$) +4.2 (2H, d, J7Hz, $C\underline{H}_2$) +4.6 (2H, s, O$\underline{H}$) +4.8 (1H, d, J7Hz, C$\underline{H}$).

Preparation of methyl-2,3-di-O-methyl-α-D-glucopyranoside (S) and (R) 4,6-phosphorochloridate (72)

As prepared by Inch *et al.*[160] A solution of phosphoryl chloride (0.44 ml) in ether (5 ml) was added dropwise to a hot solution of methyl-2,3-di-O-methyl-α-D-glucopyranoside (1g, 4.3 mmol) (82) in triethylamine (1.28 ml) in ether (15 ml). The reaction was left stirring under nitrogen for two hours. At this point no starting material remained (monitored *via* ^{31}P nmr). The mixture was then filtered, the residue washed with ether and the filtrate and washings were concentrated to give a clear oil. The desired products were isolated *via* flash chromatography and collected as a white solid. Recrystallisation from isopropyl ether afforded the major isomer (ax) 4,6-phosphorochloridate (72a) as white needled crystals. The supernatant yielded a colourless oil which was mainly the minor product (eq) 4,6-phosphorochloridate (72b); (ax 0.53g, 40% yield) (lit. 59%

yield), m.pt. 127-129°C (lit.[160] 127-129°C).

^{1}H nmr ($CDCl_3$); +3.7 (3H, s, $OC\underline{H}_3$) +3.75 (3H, s, $OC\underline{H}_3$) +3.80 (3H, s, $OC\underline{H}_3$) +3.80 (4H, broad, C$\underline{H}$) +4.25 (2H, dd, J7Hz, ^3J_p 16Hz, $C\underline{H}_2$) +4.8 (1H, d, J7Hz, C$\underline{H}$); ^{31}P nmr (ether) D_2O lock = -5.6 ppm.

(eq. 0.070g, 6% yield);

^{1}H nmr ($CDCl_3$); +3.7 (3H, s, $OC\underline{H}_3$) +3.75 (3H, s, $OC\underline{H}_3$) +3.80 (3H, s, $OC\underline{H}_3$) +3.80 (4H, broad, C$\underline{H}$) +4.25 (2H, dd, J7Hz, ^3J_p 16Hz, $C\underline{H}_2$) +4.8 (1H, d, J7Hz, C$\underline{H}$); D_2O lock = -3.8 ppm (minor product).

Methyl-2,3-di-O-methyl-α-D-glucopyranoside (S)-4-6-p-nitrophenylphosphate (74a)

As prepared by Inch *et al.*[160] Sodium hydride (0.038g, 1.58 mmol) was placed in a 100 ml three-necked, round-bottomed flask. To this was added p-nitrophenol (0.11g, 0.79 mmol) dissolved in acetonitrile (2 ml). The chlorocompound (0.2g, 0.79 mmol) (72a) was dissolved in acetonitrile (2 ml) and injected into the flask. This was then left stirring overnight under nitrogen. The solution was then diluted with dichloromethane and washed with water. Concentration of the organic layer gave a brown solid. Purification of the compound was achieved *via* flash chromatography using acetone/hexane (7:4). Recrystallisation from isopropyl ether yielded brown crystals (0.16g, 60% yield) (lit. 60% yield); m.pt. = 110-115°C (lit.[160] 112-115°C).

^{1}H nmr ($CDCl_3$); +3.7 (3H, s, $OC\underline{H}_3$) 3.75 (3H, s, $OC\underline{H}_3$) +3.80 (3H, s, $OC\underline{H}_3$) +3.70 (4H, broad, C$\underline{H}$) +4.2 (2H, dd, J7Hz, ^3J_p 17Hz, CH_2) +4.8 (1H, d, J7Hz, C$\underline{H}$) +6.8, +7.8 (4H, dd, J8Hz, J8Hz, O$\underline{Ar}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = -11.9 ppm. Infrared $\nu_{P=O}$ (KBr) = 1287 cm^{-1}.

Methyl-2,3-di-O-methyl-α-D-glucopyranoside (S)-4-6-p-nitrophenylphosphate (74b)

As prepared by Inch *et al.*[160] Sodium hydride (0.047g, 1.9 mmol) was placed in a 100 ml round-bottomed flask. p-Nitrophenol (0.16g, 1.15 mmol) was dissolved in acetonitrile (4 ml) and injected into the flask together with the chlorocompound (72a) (0.1g, 0.4 mmol) in acetonitrile (2 ml) and left stirring overnight under nitrogen. The solution was then diluted with dichloromethane and washed with water. A white solid formed upon concentration of the dried organic layer. The solid was purified over silica and crystallised from isopropyl ether to give a white crystalline compound (0.06g, 48% yield) (lit.[160] 75% yield); m.pt. = 90-93°C (lit. = 91-93°C). T.l.c. in hexane/acetone (7:4) product R_f = 0.4.
^{1}H nmr ($CDCl_3$); +3.60 (3H, s, $OC\underline{H}_3$) 3.70 (3H, s, $OC\underline{H}_3$) +3.78 (3H, s, $OC\underline{H}_3$) +3.7 (4H, broad, C$\underline{H}$) +4.2 (2H, dd, J7Hz, ^3J_p 17Hz, CH_2) +4.8 (1H, d, J7Hz, C$\underline{H}$) +6.8, +7.8 (4H, dd, J8Hz, J8Hz, O$\underline{Ar}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = -14.7 ppm. Infrared $\nu_{P=O}$ (KBr) = 1295 cm^{-1}.

Methyl-2,3-di-O-methyl-α-D-glucopyranoside (R) and (S)-4-6-S-ethyl-thiophosphate (73)

As prepared by Inch *et al.*[160] Sodium hydride (0.25g, 0.0104 mol) was placed in a 100 ml three-necked, round-bottomed flask. Ethanethiolate (0.37 ml) was dissolved in acetonitrile (10 ml) and injected into the flask. To this was added the chlorocompound (72a) (0.43g, 1.2 mmol) dissolved in acetonitrile (10 ml). The reaction mixture was stored overnight and no starting material remained after this time (monitored *via* ^{31}P nmr). The solution was separated from the sodium salts by decantation and concentrated to a clear oil. Separation of the diastereoisomers was obtained using flash column chromatography using petroleum spirit/acetone 7:4. This afforded the products: equatorial-S-ethyl thiophosphate as a

clear oil and axial ethyl thiophosphate as a white solid, eq (73b) (1.6g, 47% yield) (lit.[160] 47% yield);

^{1}H nmr ($CDCl_3$); +0.9 (3H, t, J7Hz, C$\underline{H}_3$) +1.6 (2H, tq, J7Hz, ^3J_p 8Hz, S-C$\underline{H}_2$) +3.70 (3H, s, OC$\underline{H}_3$) +3.75 (3H, s, OC$\underline{H}_3$) +3.79 (3H, s, OC$\underline{H}_3$) +3.75 (4H, broad, C$\underline{H}$) +4.0 (2H, dd, J7Hz, ^3J_p 18Hz, C$\underline{H}_2$) +4.80 (1H, d, J7Hz, C$\underline{H}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = -29.2 ppm. Infrared $\nu_{P=O}$ (KBr) = 1255 cm^{-1}.

T.l.c. petroleum spirit/acetone (7:4) R_f = 0.34, ax (0.18g, 5% yield), (73a) (lit. 4.4 yield);

^{1}H nmr ($CDCl_3$); +0.9 (3H, t, J7Hz, C$\underline{H}_3$) +1.6 (2H, tq, J7Hz, ^3J_p 8Hz, S-C$\underline{H}_2$) +3.70 (3H, s, OC$\underline{H}_3$) +3.75 (3H, s, OC$\underline{H}_3$) +3.80 (3H, s, OC$\underline{H}_3$) +3.75 (4H, broad, C$\underline{H}$) +4.0 (2H, dd, J7Hz, ^3J_p 18Hz, C$\underline{H}_2$); ^{31}P nmr ($CDCl_3$) D_2O lock = -24 ppm. Infrared $\nu_{P=O}$ = 1285 cm^{-1}. T.l.c. petroleum spirit/acetone (7:4) R_f = 0.4.

Methyl-2,3-di-O-methyl-α-D-glucopyranoside (S) and (R)-4-6-(N,N-dimethyl)-phosphoramidate

The cis-chlorocompound (72a) (0.5g, 1.6 mmol) was treated with an excess of dimethylamine (0.80g, 1.7 mmol) in benzene (5 ml) and stirred under nitrogen. This resulted in the immediate disappearance of the starting material monitored by t.l.c. and ^{31}P nmr. At this point the reaction was stopped and concentrated. The solid formed was recrystallised to afford the (S)-4,6-(N,N-dimethyl)phosphoramidate. Upon concentration of the mother liquors the residual axial compound is separated from the equatorial compound over silica in benzene/ethanol (9:1). After crystallisation from di-isopropyl ether methyl-2,3-di-O-methyl-α-D-gluco-pyranoside (R)-4-6-(N,N-dimethyl)phosphoramidate was obtained as a crystalline compound; however, the ^{1}H nmr was difficult to resolve, eq (0.30g, 60% yield) (lit.

87% yield); m.pt. 145-147°C (lit.[160] 145-147°C); ^{31}P nmr (CH_2Cl_2) D_2O lock = +8.87 ppm, $\nu_{P=O}$ (KBr) = 1250 cm^{-1}.

ax (0.04g, 7%) (lit. 8.7% yield); m.pt. 107-110°C (lit. 105-110°C); ^{31}P nmr (CH_2Cl_2) D_2O lock = +7.46 ppm, $\nu_{P=O}$ (KBr) = 1263 cm^{-1}.

Preparation of 2,3-di-O-methyl-α-D-glucopyranoside (S)-4-6-phosphoro-fluoridate (75)

A solution of 18-crown-6-ether (0.174g) in dioxan (1 ml) and potassium fluoride (0.038g, 0.8 mmol) in dioxan (2 ml) was left stirring under anhydrous conditions for several minutes. To this solution was added (1.00g, 3.8 mmol) of chlorocompound (72a) in dioxan (1 ml). The solution was left stirring for thirty minutes. After this time no starting material remained (monitored by t.l.c). The reaction mixture was then filtered. The white oil obtained was purified over silica and afforded a white crystalline compound (0.56g, 60% yield) (lit. 47% yield);[160] m.pt. 123-125°C (lit. = 126°C); t.l.c. petroleum spirit/acetone (74) R_f = 0.56. ^1H nmr ($CDCl_3$); +3.55 (3H, s, $OC\underline{H}_3$) +3.75 (3H, s, $OC\underline{H}_3$) +3.85 (3H, s, $OC\underline{H}_3$) +3.70 (4H, broad, C$\underline{H}$) +4.3 (2H, dd, J7Hz, ^3J_P 17Hz, $C\underline{H}_2$) +4.8 (1H, d, J7Hz, C$\underline{H}$); ^{31}P nmr (CH_2Cl_2) D_2O lock = +3.43 ppm.

^3J_P - 38.52Hz (P-F splitting).

Stereochemical studies on the hydrolysis of 1,3,2-dioxaphosphorinan-2-one compounds

General conditions for base hydrolysis

The compound to be studied was dissolved in (0.5 ml) dioxan. Then two equivalents of potassium hydroxide in 50/50 $H_2{}^{16}O/H_2{}^{18}O$ is added to the solution. The hydrolysis of the reaction is monitored (using ^{31}P nmr). Upon completion of hydrolysis the now basic solution is neutralised with dry CO_2. Once the solution is neutralised, 1.5 equivalents of 18-crown-

6-ether is added to the solution. This is then frozen in acetone/dry ice and placed on a freeze drier for four hours. Once the solution has cooled 0.5 ml of dimethyl sulphoxide and 0.2 ml of methyl iodide are added to the crystalline compound. The mixture is left stirring overnight. The solution is then subject to ^{31}P nmr (high field).

The attempted hydrolysis of methyl-2,3-di-O-methyl-glucopyranoside (S) 4-6-dimethyl phosphoramidate

The dimethylphosphoramidate compound (0.020g, 0.63 mmol) was dissolved in (0.5 ml) dioxan (0.2 ml H_2O) and to this was added the following reagents under separate experimental conditions:-

(a) 2 equivalents of p-toluene sulphonic acid
(b) 4 equivalents of p-toluene sulphonic acid
(c) 2 equivalents of trifluoroacetic acid.

The above three reactions were carried out at room temperature over three hours and monitored by ^{31}P nmr spectroscopy. The above compound was resistant to hydrolysis and no reaction occurred.

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Stereochemical Studies of Reactions of Phosphate and Thiophosphate Esters

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ABSTRACT

Nucleophilic substitution reactions involving phosphate monoesters have been investigated. Two general syntheses of O-alkyl or O-aryl [^{16}O,^{18}O] thiophosphate monoesters are reported. An independent and general method for the determination of the enantiomeric excess of isotopically chiral thiophosphate monoesters has been developed and the absolute configurations of the diastereoisomers of (2R)-O-(O-ethyl thiophosphoryl)-3,4S-dimethyl-5S-phenyl-1,3,2-oxazaphospholidin-2-one have been assigned.

The solvolysis of p-nitrophenyl [R-^{16}O,^{18}O] thiophosphate in ethanol gives rise to ethyl [^{16}O,^{18}O] thiophosphate with a large degree of racemisation of configuration (~80%). This observation is consistent with the formation of a thiometaphosphate intermediate of finite lifetime which is then trapped by ethanol with accompanying loss of stereochemical integrity. This study provides the first direct evidence for a monomeric thiometaphosphate in protic solvent.

During the course of developing the stereochemical analysis, it was noted that O-ethyl thiophosphate reacts with cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one with ca. 10% inversion and 90% retention of configuration. This system also reacts with fluoride ion with complete loss of stereochemistry. Cis-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one and the corresponding 2-thione are epimerised to the more stable trans isomers by pyridine and other nucleophilic catalysts. These reactions require an in-line exocyclic displacement at a phosphorus centre held in a five-membered ring.

Nucleophilic substitution at di- and tri-esters have also been studied. The stereochemical course of the hydrolysis of the 1,3,2-dioxaphosphorinan-2-one system involving good leaving groups such as chloride and fluoride occur with inversion of configuration via an in-line mechanism, whereas hydrolysis of this system involving poor leaving groups occurs with retention of configuration via a pseudorotation mechanism.