Mechanism and Stereochemistry of Diphosphate Formation from Dioxaphosphorinanes: A Critical Reassessment

Paul M. Cullis,* John Fawcett, Gerald A. Griffith, Martin J. P. Harger,* and Michael Lee

Contribution from the Department of Chemistry, University of Leicester, Leicester, LE1 7RH, U.K.

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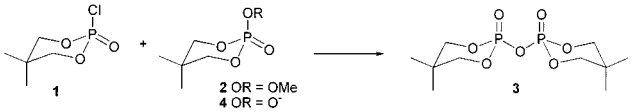
Abstract: The mechanism of diphosphate formation from (*R*)-2-chloro-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane (**5a**) and 2-hydroxy-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane (**6**) has been investigated. The products formed are the ax−ax diphosphate **7a** and the ax−eq diphosphate **7b**, with no evidence in the ^{31}P NMR spectrum for pentacoordinate chlorooxyanionic phosphoranes **9**. The structure of **7b** has been established unambiguously by NMR spectroscopy, mass spectrometry, and elemental analysis, and the structures of **5a** and **7a** have been confirmed by X-ray crystallography. The mechanism of the crucial diphosphate-forming reaction has been probed using ^{18}O-labeling studies. The ^{18}O-labeling patterns are consistent with the unsymmetric ax−eq diphosphate **7b** arising from selective nucleophilic attack of the axial oxygen of **6** on the chloride **5a** with *inversion* of configuration at phosphorus. The symmetric ax−ax diphosphate **7a** can be formed directly, as a result of selective nucleophilic attack of the axial oxygen of **6** on the chloride **5a** with *retention* of configuration, but the majority arises indirectly by isomerization of the ax−eq diphosphate **7b**. The isomerization apparently involves intermolecular exchange, with nucleophilic attack of the phosphate anion **6** on the equatorially substituted phosphorus atom of **7b** with *inversion* of configuration at phosphorus.

Introduction

Polyphosphates (di- and triphosphates) play a crucial role in fundamental biological processes such as energy transduction (ATP), DNA and RNA synthesis (nucleoside triphosphates), cell signaling (GTP), and the assembly of oligosaccharides (NDP-sugars). Both the enzyme-catalyzed formation and reactions of phosphoanhydrides are generally accepted to proceed via simple in-line displacement with a pentacoordinate intermediate and/or transition state (more than one step may be involved if there is a phosphoenzyme intermediate).[1] In contrast, nonenzymic chemical reactions leading to diphosphates have been thought to involve potentially more complex mechanistic pathways.

Simpson and Zwierzak[2] studied the formation of the diphosphate tetraesters **3** from cyclic dialkyl phosphorochloridate **1** and cyclic trialkyl phosphate **2** (Scheme 1). Isotopic labeling studies suggested that both the phosphoryl oxygen *and* the alkoxy oxygen were able to act as nucleophiles in this reaction. Our later reinvestigation using higher isotopic enrichment and high-field ^{31}P NMR spectroscopy revealed extensive isotope scrambling.[3a] The presence of doubly and triply labeled diphosphates indicated extensive *inter*molecular exchange at prolonged reaction times, and there was also some evidence for an *intra*molecular exchange process. In the related but very different reaction of the dialkyl phosphate anion **4** with phosphorochloridate **1** there appeared to be *intra*molecular exchange between the P═O of the phosphorochloridate and the attacking oxygen of the phosphate anion, which suggested the participation of a dioxadiphosphetane intermediate. Subsequent studies on a series of similar reactions leading to unsymmetrical diphosphates, where the ^{31}P NMR spectra are more straightforward to analyze, prompted a reevaluation of the original NMR data and led to the conclusion that dialkyl phosphate anions react with dialkyl phosphorochloridates via a simple direct displacement, without the need to invoke a dioxadiphosphetane intermediate.[3b] The systems studied did not allow us to determine whether displacement occurred with inversion or retention of configuration at phosphorus.

Scheme 1. Diphosphate Synthesis from the Reaction of Phosphorochloridate Diesters with Phosphate Triesters[2,3a] and Phosphate Diesters[3]



Hulst et al.[4] have recently followed up our earlier studies using a system in which the stereochemistry of the displacement reaction can in principle be determined. Using (*R*)-2-chloro-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane (**5a**) and 2-hydroxy-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane (**6**), these authors demonstrated that pyrophosphate formation occurs stereospecifically to give a single diastereoisomer assigned structure **7a** but they appeared to conclude that the displacement reaction is mechanistically complex and *may* involve a dioxadiphosphetane intermediate **8** (Scheme 2) and other more complex pathways. Also, they have detected long-lived intermediates by ^{31}P NMR spectroscopy, at temperatures between 30 and 65 °C and for periods up to several hours, that they believe to be pentacoordinate chloro-

(1) (a) Buchwald, S. L.; Hansen, D. E.; Hassett, A.; Knowles, J. R. *Methods Enzymol.* **1982**, *87*, 279−301. (b) Eckstein, F. *Angew. Chem., Int. Ed. Eng.*, **1983**, *22*, 423. (c) Frey, P. A. *Tetrahedron* **1982**, *38*, 1541. (d) Lowe, G. *Acc. Chem. Res.* **1983**, *16*, 244. (e) Frey, P. A. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1989**, *62*, 119.

(2) Simpson, P.; Zwierzak, A. *J. Chem. Soc., Perkin Trans. 1* **1975**, 201.

(3) (a) Cullis, P. M.; Kay, P. B.; Trippett, S. *J. Chem. Soc., Chem. Commun.* **1985**, 1329. (b) Cullis, P. M.; Kaye, A. D.; Trippett, S. *J. Chem. Soc., Chem. Commun.* **1987**, 1464.

(4) Hulst, R.; Visser, J. M.; de Vries, N. K.; Zijlstra, R. W. J.; Kooijman, H.; Smeets, W.; Spek, A. L.; Feringa, B. L. *J. Am. Chem. Soc.* **2000**, *122*, 3135.

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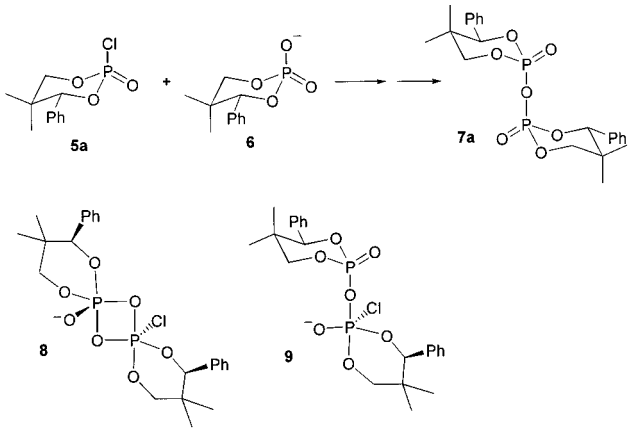
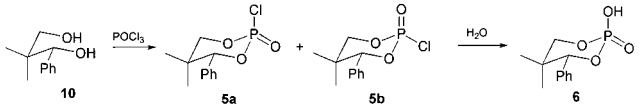
Scheme 2. Reaction Studied by Hulst et al.[4] Showing Proposed Intermediates on the Pathway to Diphosphate **7a**



Scheme 3. Synthesis of (±)-Phosphoric Acid **6**[6]



oxyanionic phosphoranes such as **9**. We would expect such phosphoranes to be high-energy intermediates[5] that will rapidly decompose, with or without pseudorotation depending on the geometry of the initial nucleophilic attack (Cl apical or equatorial). The fact that the principal mechanistic conclusions appear to contradict our most recent study and that the postulated chlorooxyanionic phosphoranes, e.g., **9**, exhibit remarkable longevity prompted a reinvestigation.

Results and Discussion

2-Chloro-2-oxo-5,5-dimethyl-4-phenyl-1,3,2-dioxaphosphorinane (5). The key starting material is the racemic phosphorochloridate **5**, and it was prepared from the (±)-diol **10** with $POCl_3$ in the presence of triethylamine (Scheme 3).[6] The ^{31}P NMR spectrum of the reaction mixture showed production of the two diastereoisomers of **5** (Cl cis or trans to Ph) (δ_P +2.2 and −2.2) in a ratio of ∼3:2. The smaller high-field signal corresponds to the previously characterized trans isomer **5a** which, because the phenyl group acts as a conformational anchor, has the chlorine atom in the axial position. The major low-field signal corresponds to the cis isomer **5b** in which the chlorine is equatorial. This isomer does not seem to have been noted in the reports of earlier studies. Refluxing the 3:2 mixture according to the procedure of ten Hoeve and Wynberg[6] causes the isomer ratio to change to 1:3 in favor of the axial chlorine compound **5a**, in line with the expectation that the thermodynamically more stable isomer is the one in which the electronegative chlorine substituent is axial (anomeric effect). More prolonged heating would most likely have increased still further the proportion of **5a** in the mixture. Clearly **5b** (eq-Cl) is the kinetically preferred product but under suitable conditions it can epimerize (by chloride exchange) to the more stable product **5a** (ax-Cl).[7] Hydrolysis of the racemic phosphorochloridate **5** (mixture of diastereoisomers) gave the corresponding phosphoric acid (±)-**6**, which was easily resolved by the literature method[6] using (1*S*,2*S*)-(+)-2-amino-1-phenyl-1,3-propanediol.

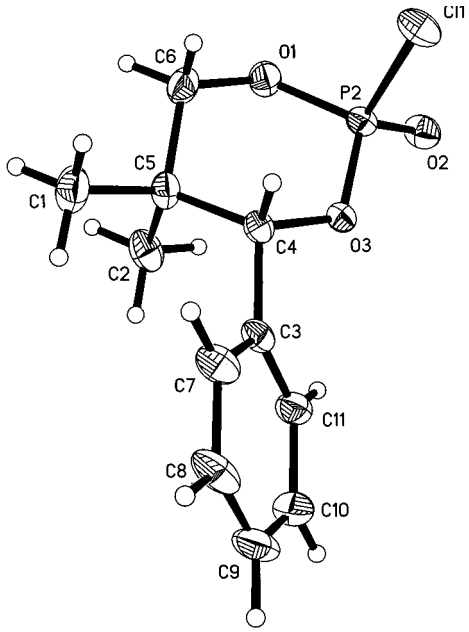


Figure 1. X-ray structure of (±)-**5a** confirming the expected chair conformation with the phenyl group placed equatorial and the chlorine axial. Displacement ellipsoids are shown at the 30% probability level. H atoms are shown as spheres of arbitrary radius.

Enantiomerically pure phosphorochloridate **5a** has been obtained from the resolved acid **6** using PCl_5, but both ten Hoeve and Wynberg[6] and Hulst et al.[4] reported very low yields. A more efficient method was sought, initially using racemic acid **6**. With oxalyl chloride in CH_2Cl_2 containing a catalytic amount of DMF, the acid is converted almost quantitatively into racemic **5a** (very little **5b** is formed) within ∼2 h at room temperature. The structure and geometry of (±)-**5a** was confirmed by X-ray crystallography (Figure 1), showing the phenyl group equatorial in the chair and the chlorine atom axial. Hulst et al.[4] also examined **5a** crystallographically and were able to establish the trans relationship of the phenyl group and the chlorine atom, but they worked with a single enantiomer rather than the racemate and were unable to obtain data of sufficient quality for publication. Our crystallographic study using racemic **5a** gave good-quality data (*R* value of 4%) and established the detailed structure of this key compound. The oxalyl chloride method was then used to convert the resolved acid (−)-(*R*)-**6** into enantiopure **5a**.[8]

Intermediates and Products in the Diphosphate-Forming Reaction. Central to the study of Hulst et al.[4] is the assignment of structures to the species observed by NMR spectroscopy in the reaction between enantiopure samples of the phosphorochloridate **5a** and the phosphoric acid **6**. Repeating the reaction of (−)-**6** and (−)-**5a** (1.25 equiv) in CH_2Cl_2 in the presence of Et_3N (6 equiv) at room temperature we obtained after 20 min

(5) Holmes, R. R. *Pentacoordinated Phosphorus*, ACS Monograph 176; American Chemical Society: Washington, DC, 1980; Vols. I and II.

(6) ten Hoeve, W.; Wynberg, H. *J. Org. Chem.* **1985**, *50*, 4508.

(7) It is estimated that the axial orientation of the Cl atom in **5** is favored by 8.6 kJ mol^{-1}.[4] The conformational energy of a Ph group in cyclohexane is 11.7 kJ mol^{-1} (Eliel, E. L.; Wilen, S. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994: p 697). Isomer **5b** will not be conformationally locked to the same extent as **5a** but is still likely to exist very largely in the eq-Cl (eq-Ph) conformation.

(8) Independent confirmation of the enantiomeric purity of both (−)-**5a** and (−)-**6** is obtained directly from the reaction leading to diphosphates **7a** and **7b** in which only two diastereoisomers are observed. When racemic or partially racemic **5a** and **6** react together, four diastereoisomers are formed and their signals can be resolved in the ^{31}P NMR spectrum of the reaction mixture.[6] In the ^{31}P NMR spectrum reproduced in Figure 2 for the reaction of (−)-**5a** and (−)-**6**, very small doublets (<2%) can be seen upfield of the main pair of doublets, indicating a very small amount of the (*R, S*) diastereoisomer corresponding to **7b**. From this it follows that the reactants (−)-**5a** and (−)-**6** are ≥99% enantiomerically pure.

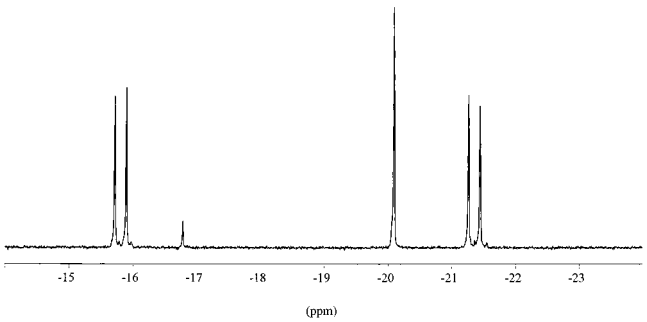


Figure 2. ^{1}H-Decoupled ^{31}P NMR spectrum (122 MHz) of the reaction of (−)-**5a** with (−)-**6** in CH_2Cl_2 at room temperature after 8 h.

a ^{31}P NMR spectrum similar to that previously reported,[4] the principal signals (in addition to unchanged reactants) being a singlet δ_P −20.1 and two doublets δ_P −15.8 and −21.3 ($^2J_{PP}$ 22 Hz)[9] (Figure 2) (a much smaller singlet δ_P −16.8 was also observed and can also be seen in the published spectra[4]). The reaction is rather slow at room temperature but was .∼90% complete after 45 h. Chemical shifts in the region δ_P −15 to −20 ppm are characteristic of diphosphates (pyrophosphates),[10] and the spectrum would be consistent with a mixture of symmetric and unsymmetric diphosphates having respectively equivalent and nonequivalent phosphorus atoms. Hulst et al.[4] did indeed assign the singlet to a symmetrical diphosphate, specifically the axial−axial isomer **7a** based on detailed NMR analysis (2D NOESY NMR and ^{31}P NMR). (To avoid possible confusion we have used axial and equatorial to refer to the orientation of the P−X substitutent throughout, e.g., X = Cl, OR, OP(O)$(OR)_2$.) The pair of doublets, however, they attributed to a deprotonated 5-coordinate chloro hydroxy phosphorane intermediate such as **9**, based largely on its lability as evidenced by the gradual conversion of the pair of doublets into the singlet corresponding to the symmetrical diphosphate **7a**.

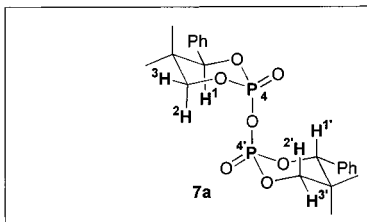
The longevity of the labile species responsible for the pair of doublets (many hours at 30 °C) is, we think, remarkable if it is indeed a chloro hydroxy phosphorane (protonated or deprotonated), and it could have important implications for our understanding of the role of such species as intermediates in nucleophilic substitution at phosphoryl centers. In fact, we found it possible to isolate and purify not only the ultimate diphosphate product but also the labile species, by chromatography on silica gel followed by crystallization. The ultimate product, previously assigned the symmetrical diphosphate structure **7a** by NMR spectroscopy, was eluted first and was fully characterized (mp, $[\alpha]_D$, IR, NMR, MS). The symmetry was evident from the ^{1}H NMR spectrum, with the two phosphorinane rings giving rise to just one set of signals (notably δ_H 1.08, s, 6 H and 0.80, s, 6 H for the four Me groups). The P-coupled CH and CH_2 signals could be satisfactorily simulated using the appropriate values for the chemical shifts and coupling constants for **7a** (Table 1) and taking account of the virtual $^2J_{pp}$ coupling (Figure 3A).

The labile species was chromatographically similar to **7a** but was eluted slightly later. It too was crystalline and was fully characterized. A lack of symmetry in this case is implicit in the ^{31}P NMR spectrum (distinct signals for the two P atoms) and was apparent in the ^{1}H NMR spectrum (notably δ_H 1.10, 1.06, 0.82, and 0.81, all 3 H, s, for the four Me groups), and the P-coupled CH and CH_2 signals could again be satisfactorily simulated using the chemical shifts and coupling constants appropriate to **7b** (Table 1, Figure 3B).

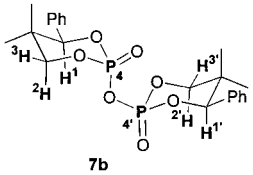
Crucial confirmation that the isolated compounds were indeed the species present in the diphosphate-forming reaction was obtained by adding aliquots of them in turn to the reaction mixture and noting an enhancement of the appropriate ^{31}P NMR signals.

Our data for the symmetrical diphosphate would fit equally well with either the axial−axial structure **7a** or the equatorial−

Table 1. NMR Parameters Used for the Simulated Spectra in Figure 3



Chemical shifts (ppm)	Coupling constants (Hz)
H^1 and $H^{1'}$ = 5.46	$J_{1,4}$ = $J_{1',4'}$ = 1.2
H^2 and $H^{2'}$ = 4.68	$J_{2,3}$ = $J_{2',3'}$ = 11.3
	$J_{2,4}$ = $J_{2',4'}$ = 1.2
H^3 and $H^{3'}$ = 4.07	$J_{3,4}$ = $J_{3',4'}$ = 27.0
P^4 and $P^{4'}$ = – 19.92	$J_{4,4'}$ = 23.3



Chemical shifts (ppm)	Coupling constants (Hz)
H^1 = 5.45; $H^{1'}$ = 5.48	$J_{1,4}$ = 1.2; $J_{1',4'}$ = 1.6
H^2 = 4.48; $H^{2'}$ = 4.45	$J_{2,3}$ = 11.1; $J_{2',3'}$ = 11.2
	$J_{2,4}$ = 1.2; $J_{2',4'}$ = 3.2
H^3 = 4.06; $H^{3'}$ = 4.05	$J_{3,4}$ = 27.1; $J_{3',4'}$ = 24.5
P^4 = – 21.24; $P^{4'}$ = – 15.27	$J_{4,4'}$ = 24.5

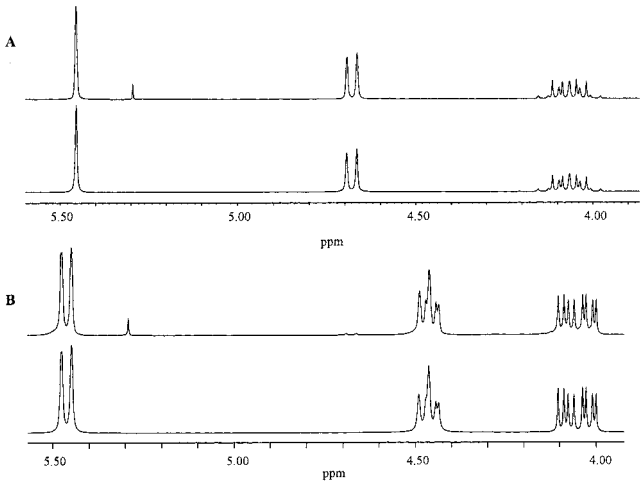


Figure 3. Partial ^{1}H NMR spectra (400 MHz) showing the experimental spectrum (upper trace) and the simulation (lower trace) for (A) the ax−ax diphosphate **7a** and (B) the ax−eq diphosphate **7b**. Simulation parameters are shown in Table 1, simulation program gNMR version 4 (Cherwell Scientific).

(9) The phosphorus−phosphorus coupling constant reported in the text of ref 4 is 33 Hz, but from inspection of the spectrum reproduced in Figure 6 of this reference, which is shown with a scale in hertz, that is clearly in error. The value of $^2J_{PP}$ estimated from the Figure (∼24 Hz) is substantially smaller and similar to the value reported here. It should be noted that the phosphorus−phosphorus coupling constant measured for the purified isolated material in $CDCl_3$ is slightly different ($^2J_{PP}$ 24.5 Hz) from that seen in the reaction medium.

(10) Verkade, J. G.; Quin, L. D. *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis*; VCH: Deerfield Beach, FL, 1987; pp 1−60.

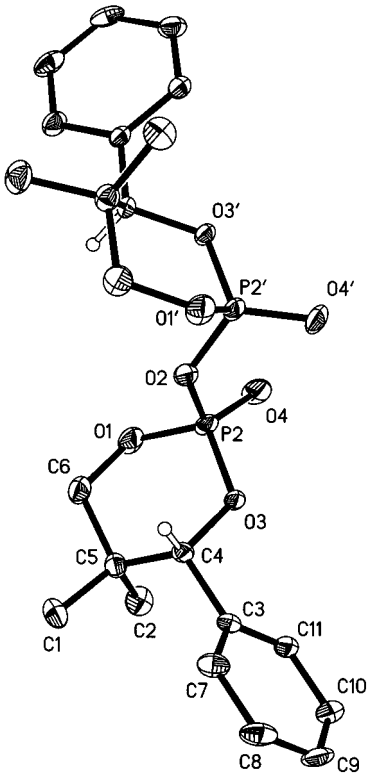
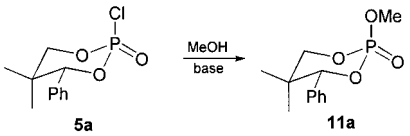


Figure 4. X-ray structure of the ax−ax diphosphate **7a**, showing the atom-labeling scheme and 30% displacement ellipsoids. The chiral center H atoms are shown with open bonds; all other H atoms are omitted for clarity. The atom O2 lies on a 2-fold axis; primed atoms are generated by symmetry ($-x - 1$, y, $-z - 1$). The ring chair conformation can be seen with the phenyl substituents equatorial and the P−O−P linkage axial.

equatorial isomer **7c**. Hulst et al.[4] favored the former from their NMR analysis, and we were now able to establish conclusively the geometry by X-ray crystallography (Figure 4). The two rings are clearly in normal chair conformations and the equatorial phenyl groups that anchor the chairs both have a conformation very similar to that seen in the crystal structure of the phosphorochloridate **5a** (Figure 1). The unsymmetrical species could not be examined crystallographically—it formed only hairlike microcrystals regardless of the crystallization solvent used—and it is not easy to definitively eliminate a phosphorane structure such as **9** on the strength of the NMR and IR data (although no ν_{O-H} was apparent in the IR spectrum) or even the mass spectrum if it is argued that the apparent molecular ion (EI *m*/*z* 466 (8%)) is actually a fragment ion corresponding to loss of chloride. However, we would not expect a chloro hydroxy phosphorane, whether or not it is deprotonated, to survive an aqueous workup and a chromatographic separation, and furthermore, the elemental analysis establishes the absence of chlorine (<1%). Taken as a whole, these data clearly point to a diphosphate isomeric with **7a**. Given the lack of symmetry evident from the NMR spectra, the axial−equatorial diphosphate structure **7b** seems to us the inescapable conclusion. Finally, the minor compound giving rise to a singlet at δ_P −16.8 is almost certainly the other symmetrical diphosphate, the equatorial−equatorial isomer **7c**.

Mechanism and Stereochemistry of the Diphosphate-Forming Reaction. In this diphosphate-forming reaction, where the electrophile is chiral at phosphorus and a new chiral center is generated at the phosphorus atom of the attacking nucleophile, there are a surprising number of mechanistic issues to be addressed. First, there is the question of the stereochemical course of the displacement reaction itself. The stereochemistry of exocyclic displacement reactions at phosphoryl centers held in six-membered rings is known to be strongly dependent on the conditions (leaving group, nucleophile, solvent, etc.).[11] Unlike five-ring phosphoryl compounds, where there is a very marked preference for the ring to span apical−equatorial positions in the trigonal-bipyramidal intermediate, necessitating a pseudorotation step, the six-membered ring can adopt a diequatorial arrangement or span apical−equatorial sites. This means that exocyclic displacement reactions can in this case occur either with in-line geometry, resulting in inversion of configuration, or by an adjacent attack followed by a pseudorotation step, resulting in overall retention of configuration.

Scheme 4. Stereochemistry of the Methanolysis of the Axial Phosphorochloridate **5a**



5a **11a**

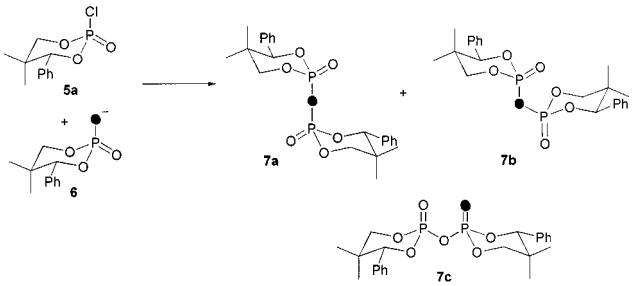
Second, there is the issue of the proportion of nucleophilic attack by the axial and the equatorial O atoms of the nucleophile **6**. From both our study and that of Hulst et al.,[4] it is certain that the final product of the reaction of **5a** with **6**—the symmetrical ax−ax diphosphate **7a**—is formed largely by way of a labile but long-lived intermediate. Our study leaves little doubt that the intermediate is, in fact, the unsymmetrical ax−eq diphosphate **7b** and also that there is a minor pathway leading directly to **7a** without the intervention of **7b**: even in the very early stages of reaction, before there can have been any appreciable isomerization of **7b**, a small amount of **7a** is already apparent (ratio **7a**/**7b** ∼1/5). There is only one way in which the ax−ax product **7a** can be formed directly, this is, attack by the axial O atom of the nucleophile **6** and displacement of the Cl atom from the electrophile **5a** with retention of configuration at phosphorus (but this is a very minor pathway). For the major pathway leading to the ax−eq diphosphate **7b**, however, there are two possibilities: the axial O atom of the nucleophile displacing Cl with inversion of configuration or the equatorial O atom of the nucleophile displacing chlorine with retention. There is not much discrimination between the two O atoms in the reaction of the free acid **6** with diazomethane or in the reaction of its conjugate base with simple alkyl halides (EtI, PrBr), so that the isomeric esters with axial or equatorial OR groups are formed in comparable amounts. However, it does not necessarily follow that the O atoms will still show similar nucleophilic reactivity toward a hard phosphoryl center, as is present in the phosphorochloridate **5a**.

As regards the stereochemical course of the displacement of chloride from **5a**, we find that methanolysis in CH_2Cl_2 in the presence of Et_3N gives very largely one ester, and it is the isomer that appears at higher field (^{31}P NMR) in the mixture obtained from the acid **6** and CH_2N_2. Since 2-oxo-1,3,2-dioxaphorinane derivatives invariably have smaller values of δ_P (higher field) when the P=O group is equatorial,[12] it follows that the methanolysis product is **11a** with the methoxy group axial (Scheme 4). That being so, the methanolysis follows the apparent generalization noted by Hulst et al.,[4] that **5a** reacts

(11) Hall, C. R.; Inch, T. D. *Tetrahedron* **1980**, *36*, 2059.

(12) Mosbo, J. A.; Verkade, J. G. *J. Org. Chem.* **1977**, *42*, 1549. Cooper, D. B.; Inch, T. D.; Lewis, G. J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1043. Weener, J.-M.; Versleijen, J. P. G.; Meetsma, A.; ten Hoeve, W.; van Leusen, A. M. *Eur. J. Org. Chem.* **1998**, 1511.

Scheme 5. Products and Principal Isotope Labeling Patterns Seen during the Reaction of ^{18}O-Labeled (−)-*6* (Label 90% in the Axial Position) with (−)-**5a**[15]



with oxygen nucleophiles with predominant retention of configuration at phosphorus. (Others have noted, however, that with other systems closely related to **5a** inversion becomes increasingly important when less basic oxygen nucleophiles are employed.[13]) To ascertain the stereochemistry of the reaction with hydroxide ion, it was necessary to use ^{18}O-labeling. Thus, enantiopure (−)-**5a** was added to *tert*-butyl alcohol containing a small excess of [^{18}O]hydroxide (from Bu^{t}OK + $H_2{}^{18}O$) and the resulting phosphate anion was protonated (CF_3CO_2H) and treated with CH_2N_2. The ester at low field in the ^{31}P NMR spectrum (eq-OMe) had ∼90% of the ^{18}O in the P═O group ($\Delta\delta$ 6.75 Hz at 162 MHz) and the ester at high field had ∼90% in the P−OMe group ($\Delta\delta$ 2.45 Hz at 162 MHz). Here too, then, the substitution reaction of **5a** proceeds with predominant retention of configuration at phosphorus, presumably by a mechanism involving pseudorotation of a (short-lived) pentacoordinate phosphorane intermediate.

It should be possible to distinguish between the two alternative modes of formation of the unsymmetrical diphosphate **7b** using isotope labeling. Hulst et al.[4] studied the reaction with enantiopure phosphorochloridate **5a** having ^{18}O in the phosphoryl (P═O) group, but labeling of the electrophile cannot shed light on whether it is the axial or equatorial O atom of the nucleophile **6** that attacks. Indeed, as will be seen in the discussion below, it does not even resolve unambiguously the issue of the stereochemical course of displacement of the chlorine atom. We have therefore carried out the complementary labeling experiment, with ^{18}O (∼90 atom %) in the nucleophile (−)-**6** (single enantiomer), predominantly (90%) in the axial position, and unlabeled (−)-**5a** (single enantiomer) (Scheme 5). In the ^{31}P NMR spectrum of the reaction mixture, the two doublets associated with the unsymmetrical diphosphate **7b** (δ_P −15.7 and −21.3) *both* exhibit small upfield shifts and the magnitudes of the shifts ($\Delta\delta$ 2.7−2.9 Hz at 162 MHz) are as expected for P−^{18}O single bonds, Figure 5.[14,15] The implication is clear: the isotope is located in the bridge (P−^{18}O−P) position of the unsymmetrical ax−eq diphosphate **7b**, and the formation of the diphosphate must involve attack by the *axial* O atom of the nucleophile **6** displacing the leaving group from the phosphorochloridate **5a** with *inversion* of configuration. One of the doublets—the one at higher field, corresponding to the axially substituted P atom in **7b**—was accompanied by a much less intense signal having a larger upfield shift ($\Delta\delta$ 6.8 Hz at 162 MHz), indicative of a P═^{18}O double bond.[14] This is to be expected given that one-tenth of the ^{18}O in our labeled **6** is located in the equatorial position, and it is the axial oxygen of **6** that becomes the bridging atom. The other (low-field) doublet, corresponding to the equatorially substituted P atom in the unsymmetrical diphosphate **7b**, was *not* accompanied by an appreciable signal indicative of a P═^{18}O double bond, at least in the early stages (before intermolecular exchange processes become significant). It follows that there is no appreciable attack by the (unlabeled) equatorial O atom of **6** (the label would be left in the axial P═O group of **7b**), and therefore no appreciable formation of **7b** by displacement of the leaving group from **5a** with retention of configuration at phosphorus. This notwithstanding the fact that **5a** reacts with MeOH and hydroxide predominantly with retention of configuration and that it might be supposed it would also do so when the nucleophile is **6**. The spectrum obtained by Hulst et al.[4] using ^{18}O-labeled (−)-**5a** and unlabeled (−)-**6** also accords with our view of how the reaction proceeds. In this spectrum, it is principally the low-field doublet, associated with the equatorially substituted P atom of **7b**, that exhibits an ^{18}O shift. Since in this case the ^{18}O was initially in the equatorial position of the phosphorochloridate **5a**, displacement of the leaving group must have occurred with inversion of configuration at phosphorus. It is difficult to measure accurately the magnitude of the isotopic shift from the published spectrum but it appears to be ∼5 Hz at 121.4 MHz, in accord with a P═^{18}O double bond.

Locating the ^{18}O label in the symmetrical diphosphate **7a** is not straightforward. If the isotope is in the bridge position (P−^{18}O−P), the two phosphorus atoms will retain their equivalence and the singlet in the ^{31}P NMR spectrum will simply experience a small upfield shift (2.7−2.9 Hz at 162 MHz) characteristic of a P−^{18}O single bond. If, on the other hand, the isotope is in one of the phosphoryl groups (P═^{18}O), the equivalence of the phosphorus atoms will be broken. The chemical shift difference ($\Delta\delta$ ∼7 Hz at 162 MHz) will, however, be much smaller than the coupling constant ($^2J_{PP}$ is 22 Hz for **7b** and presumably similar for **7a**), so we will have an extreme AB system.[3b,14c] The outer lines of the (extreme) AB quartet will be vanishingly small, and the appearance of the inner two lines will depend on the natural line widths; in the limit where the line width is greater than 1 Hz, the AB system will appear as a single line halfway between the singlet of the unlabeled (P═^{16}O) compound and the true position of the P═^{18}O-labeled phosphoryl group. It is therefore not easy to distinguish bridge- and nonbridge-labeled **7a** with any real certainty. In the event, the product **7a** formed from unlabeled phosphorochloridate (−)-**5a** and labeled acid (−)-**6** (90% of ^{18}O axial) showed predominantly a single line (δ_P −20.0) with an upfield shift of 2.9 Hz at 162 MHz, but a less intense line was also visible at a slightly higher field ($\Delta\delta$ ∼3.9 Hz at 162 MHz) (Figure 5). These signals are, we think, attributable to bridge-labeled **7a** and the high-field branch of the AB system for P═O-labeled **7a**, respectively. To increase confidence in the analysis, a similar experiment was carried out using less highly enriched acid (−)-**6** (∼50 atom % ^{18}O) to reduce the contribution of doubly labeled product (formed by intermolecular exchange processes), and an extended reaction time (4.7 days) to allow isomerization of **7b** to **7a** to reach effective completion (98%). The product was isolated and purified by crystallization. The ^{18}O content of **7a** from the FAB mass spectrum was 55% no ^{18}O, 42% one ^{18}O, and 3% two ^{18}O atoms (M + H^+ 467, 469, and 471), and the ^{18}O distribution from the ^{31}P NMR spectrum was as shown in

(13) Wadsworth, W. S.; Larsen, S.; Horten, H. L. *J. Org. Chem.* **1973**, *38*, 256.

(14) (a) Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200. (b) Lowe, G.; Potter, B. V. L.; Sproat, B. S.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1979**, 733. (c) Marschner, T. M.; Reynolds, M. A.; Oppenheimer, N. J.; Kenyon, G. L. *J. Chem. Soc., Chem. Commun.* **1983**, 1289.

(15) Some hydrolysis of **5a** to **6** by adventitious moisture seems unavoidable. In experiments using ^{18}O-labeled **6**, this will cause isotopic dilution; hence, the amounts of unlabeled diphosphates **7a** and **7b** apparent in Figure 5 are greater than would otherwise be expected.

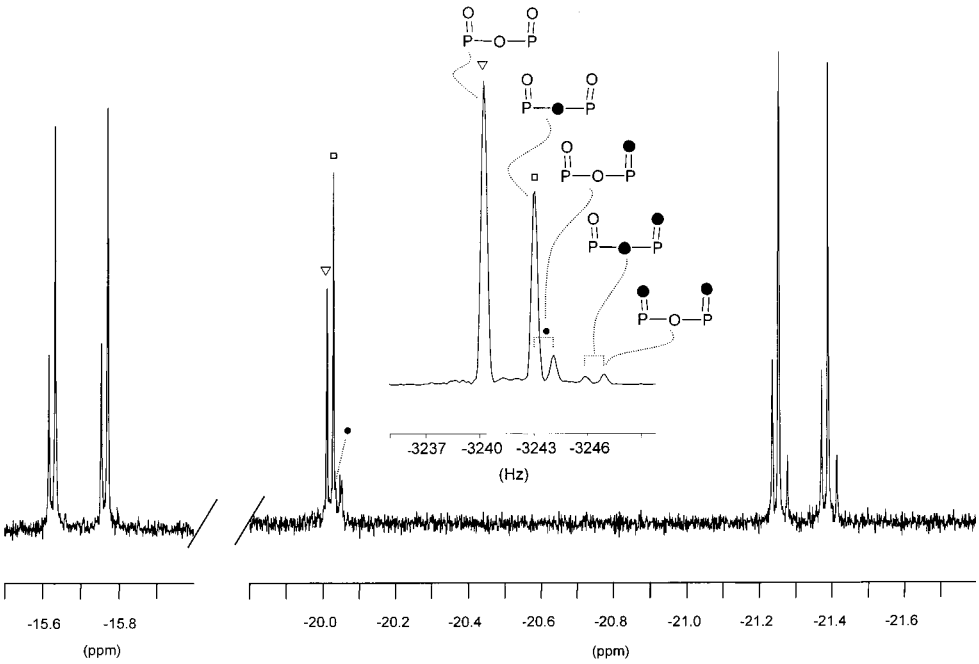
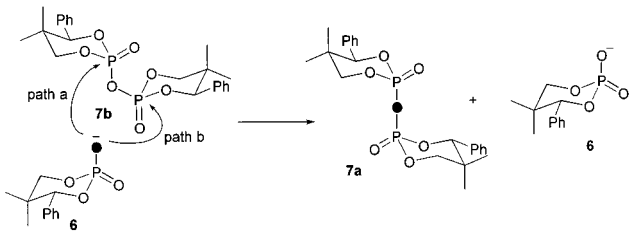


Figure 5. ^{1}H-Decoupled ^{31}P NMR spectrum (162 MHz) of the reaction of (−)-**5a** with ^{18}O-labeled (−)-**6** (90 atom %; distributed 9:1 in favor of the axial position) in CH_2Cl_2 at room temperature after 1.25 h. Inset: Expansion of the signal δ_P −20.0 ppm from an independent reaction of (−)-**5a** with ^{18}O-labeled (−)-**6** (50 atom %; distributed 9:1 in favor of the axial position) allowed to proceed until isomerization of **7b** into **7a** was practically complete (112 h); this material was subjected to methoxide cleavage to confirm the location of the isotope.

Figure 5 (inset) together with our assignment of the peaks. This material was cleaved with NaOMe (very slight excess) in MeOH, and the products were analyzed by ^{31}P NMR spectroscopy. The liberated phosphate anion, which incorporates the former bridging oxygen atom of **7a**, was 36% labeled ($\Delta\delta$ 4.6 Hz at 162 MHz; also 1% double-labeled $\Delta\delta$ 9.7 Hz), while the methyl ester, which incorporates half of the former nonbridge oxygen atoms, was 7% labeled (P═^{18}O; $\Delta\delta$ 6.4 Hz) (this includes a significant contribution from ester derived from doubly labeled **7a**). There can therefore be no doubt that the principal labeled component of the product **7a** has the isotope located in the bridge position or that a substantial minor component has ^{18}O in one of the P═O groups. It is interesting to note that the cleavage of **7a** gave only the axial methyl ester (ax-OMe), implying nucleophilic attack by methoxide with complete retention of configuration at phosphorus.

To the extent that the symmetrical axial−axial product **7a** is formed directly by nucleophilic attack of axially labeled acid **6** on the phosphorochloridate **5a**, the label must be located in the bridge position. Most of the **7a** is not formed directly, however, but by isomerization of the kinetically preferred unsymmetrical ax−eq diphosphate **7b**. In principle, the isomerization could be intra- or intermolecular. The fact that the label ends up predominantly in the bridge position of **7a** is consistent with an intermolecular reaction involving nucleophilic attack by the axial (labeled) oxygen of **6** on the unsymmetrical diphosphate **7b**. In support of intermolecularity, it was seen that the isomerization of pure unlabeled **7b** in the presence of ^{18}O-labeled (−)-**6** (90% of label axial) and Et_3N gave **7a** that was extensively labeled in the bridge position. At the same time, there was very little incorporation of label into the remaining unisomerized **7b**, implying that exchange without isomerization is not an important process. Of the two possible modes of intermolecular isomerization of **7b** to **7a** −displacement of the axial substituent with retention of configuration (path a) or the equatorial substituent with inversion (path b), Scheme 6—it is the latter that intuitively we favor. Greater stability results when

Scheme 6. Possible Mechanisms for the Isomerization of **7b** into **7a** Consistent with the Isotope Exchange Seen in the Reaction Using ^{18}O-Labeled (−)-**6** (90 Atom %; Distributed 9:1 in Favor of the Axial Position)



an electronegative group [Cl, OP(O)(OR)$_2$] is in the *axial* position, so greater *reactivity* is likely to be seen when an electronegative leaving group is in the *equatorial* position. For the phosphorochloridate **5**, we have, in fact, observed greater reactivity (with a phosphate nucleophile) when the Cl leaving group is equatorial, as in **5b**, than when it is axial, as in **5a**. Furthermore, if during the reaction of ^{18}O-labeled (−)-**6** with (−)-**5a** (Scheme 5) the isomerization of **7b** into **7a** had occurred by path a, it would have released (−)-**6** with ^{18}O in the *equatorial* position. This would be expected to lead to extensive scrambling of the isotope out of the bridge in **7a**, which is not seen. After very long reaction times, extensive intermolecular exchange is evident from the appearance of doubly labeled **7a** in the ^{31}P NMR. Comparable intermolecular exchange processes would satisfactorily account for the results of the crossover experiments reported by Hulst et al.[4]

In the reaction of the phosphorochloridate **5a** with the ^{18}O-labeled phosphoric acid **6**, the only way in which any eq−eq diphosphate **7c** could be formed directly would be by attack of the (unlabeled) equatorial oxygen of **6** displacing the chlorine of **5a** with inversion of configuration. Although the designation of the minor compound giving rise to the singlet at δ_P −16.8 as the eq−eq diphosphate **7c** can only be considered tentative, since it has not been isolated and characterized, the isotope

pattern for this signal (not included in Figure 5) in the spectrum of the reaction mixture was in accord with the ^{18}O-labeling pattern that would be expected (Scheme 5).

Finally, it is worth noting that in our experiments conducted at room temperature we have not observed the species with multiple phosphorus couplings that were seen by Hulst et al.[4] in diphosphate-forming reactions conducted at higher temperatures using **5a** generated in situ, and we cannot therefore offer any additional evidence on their possible structures, but it seems unlikely to us that they would be chloro phosphoranes.

Conclusions

A number of important conclusions emerge from this study. First, it confirms that reactions of dialkyl phosphate anions with dialkyl phosphorochloridates occur by a simple displacement mechanism, with no evidence for dioxadiphosphetane intermediates. Second, the ^{31}P NMR signals previously assigned by others to a pentacoordinate chlorooxyanionic phosphorane such as **9** have been unambiguously reassigned to the unsymmetrical ax−eq diphosphate **7b**. Third, and unexpectedly, the stereoselectivity seen in the reaction of (*R*)-2-chloro-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane (**5a**) and 2-hydroxy-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxa-phosphorinane (**6**) has been found to arise more from high selectivity within the nucleophile **6** (axial O atom) than from high stereoselectivity in displacement of the axial leaving group from **5**, with the major in-line displacement pathway (inversion) leading to the ax−eq diphosphate **7b** and the minor adjacent displacement pathway (retention) leading to the ax−ax diphosphate **7a**. Finally, the majority of the symmetric ax−ax diphosphate **7a** is formed from ax−eq diphosphate **7b** by an intermolecular exchange process involving attack of the axial oxygen of **6**, probably on the equatorially substituted phosphorus atom of **7b**. The essentially complete conversion to **7a** is a consequence of the thermodynamic stability of the symmetrical diphosphate in which an electronegative $OP(O)(OR)_2$ group is axial with respect to each of the rings.

Experimental Section

Instrumentation. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured at 589 nm using a 100-mm cell in a Perkin-Elmer 341 polarimeter. ^1H NMR (Me_4Si internal standard) and ^{31}P NMR spectra (negative chemical shifts upfield from external 85% H_3PO_4) were recorded at 250, 300, or 400 MHz using Bruker ARX 250, DPX 300, or DRX 400 spectrometers. ^{31}P NMR spectra are ^1H decoupled unless otherwise indicated and were recorded at 101, 122, or 162 MHz. Mass spectra were obtained in EI (70 eV) or FAB (NBA matrix) mode using a Kratos Concept spectrometer or in ES mode using a Micromass Quattro spectrometer.

Preparation of (±)-2-Hydroxy-2-oxo-5,5-dimethyl-4-phenyl-1,3,2-dioxaphosphorinane (6).[6] A solution of 1-phenyl-2,2-dimethyl-1,3-propanediol[6] (**10**; 36.0 g, 0.20 mol) and Et_3N (42.4 g, 0.42 mol) in CH_2Cl_2 (100 mL) was stirred and cooled in ice, and distilled $POCl_3$ (32.2 g, 0.21 mol) in CH_2Cl_2 (50 mL) was added during 0.5 h (^{31}P NMR: diastereoisomers, δ_P 2.2 and −2.2 ppm, ratio 3:2). Additional CH_2Cl_2 (50 mL) was added to facilitate stirring, and the mixture was heated under reflux for 3 h (^{31}P NMR: δ_P 2.2 and −2.2 ppm, ratio 1:3). When cool, the mixture was filtered and the filtrate was washed with water (2 × 75 mL). The washings were extracted with CH_2Cl_2 (50 mL), and the combined organic portions were dried (Na_2SO_4) and concentrated to give the phosphorochloridate (±)-**5** (mixture of ax and eq diastereoisomers) as a crystalline solid. The crude product **5** was hydrolyzed by portion-wise addition over 0.5 h to a stirred solution of NaOH (24 g, 0.60 mol) in water (240 mL) maintained at ∼95 °C (CAUTION: exothermic reaction), followed by brief heating at the boiling point until a clear solution was obtained. The solution was cooled to ∼60 °C and was acidified with concentrated hydrochloric acid (50 mL). When cool, the colorless precipitate was collected and washed with water (500 mL) and ether (200 mL) and was dried in vacuo at 80 °C over P_2O_5 to give the acid (±)-**6** (41.0 g, 85%): mp 204−205 °C (from EtOH) (lit.[6] mp 224−224.5 °C); ^{31}P NMR ($CDCl_3$ + CD_3OD) −5.4 ppm; ^1H NMR (250 MHz, $CDCl_3$ + CD_3OD) 7.5−7.35 (m, 5 H), 5.33 (s, 1 H, H_4), 4.38 (d, 1 H, J_{HH} = 11 Hz, ax-H_6), 3.98 (dd, 1 H, J_{PH} = 24.5, J_{HH} = 11 Hz, eq-H_6), 1.11 (s, 3 H), 0.86 (s, 3 H); MS (−ES) *m*/*z* 241.

Resolution of 6.[6] The racemic acid (±)-**6** (14.2 g, 59 mmol) and (+)-2-amino-1-phenyl-1,3-propanediol (10.0 g, 60 mmol) were dissolved in hot ethanol (38 mL) containing water (1.5 mL). The solution was stirred and allowed to cool to room temperature. Stirring was continued for a further 2 h before the crystalline salt (8.9 g) was collected and washed with a little ether. The salt was stirred with water (56 mL) containing concentrated hydrochloric acid (17 mL) for 3 h, and the liberated acid was collected, washed with water, and dried in vacuo at 80 °C over P_2O_5 giving (−)-2-hydroxy-2-oxo-5,5-dimethyl-4-*(R)*-phenyl-1,3,2-dioxaphosphorinane (−)-**6** (4.95 g, 35%): mp 210−211 °C (from EtOH); $[\alpha]_{589}$ −60.8° (*c* 0.46, MeOH) (lit.[6] $[\alpha]_{578}$ −60.0°); ^{31}P NMR ($CDCl_3$ + CD_3OD) −5.4 ppm; ^1H NMR as for (±)-**6**.

Preparation of 2-Chloro-2-oxo-5,5-dimethyl-4-phenyl-1,3,2-dioxaphosphorinane (5a) Using Oxalyl Chloride. (a) A suspension of the racemic acid (±)-**6** (1.0 g, 4.1 mmol) in CH_2Cl_2 (20 mL) was stirred, and oxalyl chloride (1.2 g, 9.2 mmol) was added together with DMF (catalyst, 40 μL). A clear solution was obtained in ∼1 h, and after 2 h, the volatile material was evaporated and the residue was crystallized from ether to give the racemic phosphorochloridate (±)-**5a**: mp 121−125 °C (lit.[6] mp 127.5−129.5 °C); ^{31}P NMR ($CDCl_3$) −2.1 ppm; ^1H NMR ($CDCl_3$, 250 MHz) 7.45−7.25 (m, 5 H), 5.30 (d, J_{PH} = 3 Hz, 1 H, H_4), 4.37 (dd, J_{PH} = 3, J_{HH} = 11.5 Hz, 1 H, ax-H_6), 4.09 (dd, J_{PH} = 31, J_{HH} = 11.5 Hz, 1 H, eq-H_6), 1.09 (s, 3 H), 0.86 (s, 3 H); MS (EI) *m*/*z* 260, 262 (1) (M^+), 205, 207 (100) ($M^+ - C_4H_7$); MS (FAB) *m*/*z* 521, 523, 525 (20) ($2M + H^+$), 261, 263 (40) ($M + H^+$), 145 (100). Structure confirmed by X-ray crystallography (Figure 1).

(b) The optically active acid (−)-**6** was similarly treated with oxalyl chloride to give (−)-*(R)*-2-chloro-2-oxo-5,5-dimethyl-4-*(R)*-phenyl-1,3,2-dioxaphosphorinane (−)-**5a**: mp 164−165 °C (softens above 157 °C) (lit.[6] mp 162−164.5 °C); $[\alpha]_{589}$ −94.4° (*c* 0.32, CH_2Cl_2) (lit.[6] $[\alpha]_{578}$ −82.4°); ^{31}P NMR ($CDCl_3$) −2.2 ppm; ^1H NMR and MS (EI) as for (±)-**5a**.

Preparation of 2-(*R*)-[^{18}O]Hydroxy-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane (^{18}O-Labeled Acid (−)-6). A solution of potassium *tert*-butoxide (98 mg, 0.88 mmol) in anhydrous *tert*-butyl alcohol (6 mL) was stirred efficiently (heavy magnet), and ^{18}O-labeled water (22 mg, 1.10 mmol) was added, followed almost immediately (1 min) by **5a** (100 mg, 0.38 mmol). After 1 h, methanol (5 mL) was added to dissolve precipated salts: ^{31}P NMR (101 MHz) −1.76 (without ^1H decoupling, d, J_{PH} = 23.5 Hz); a small peak 3 Hz downfield indicated ∼10% unlabeled product. MS(−ES) *m*/*z* 243 and 241 (ratio 90:10). Volatile matter was evaporated, and the residue was dissolved in water (4 mL). The aqueous solution was cooled in ice and acidified with CF_3CO_2H (114 mg, 1.0 mmol) to precipitate ^{18}O-labeled **6** (84 mg, 91%).

A sample of the labeled acid (−)-**6** was treated with diazomethane, giving a mixture (∼1:1) of the epimeric methyl esters. eq-OMe: ^{31}P NMR (162 MHz; $CDCl_3$) δ_P −1.813, −1.832 (P-^{18}OMe), −1.855 (P=^{18}O) (ratio 10:9:81); ^1H NMR (400 MHz, $CDCl_3$) 7.4−7.25 (m), 5.43 (d, J_{PH} = 2 Hz, H_4), 4.46 (d, J_{HH} = 11 Hz, ax-H_6), 3.94 (dd, J_{PH} = 24, J_{HH} = 11 Hz, eq-H_6), 3.96 (d, J_{PH} = 11 Hz, OMe), 1.025 (s, Me), 0.81 (s, Me); ax-OMe δ_P −5.595, −5.610 (P-^{18}OMe), −5.636 (P=^{18}O) (ratio 10:81:9); δ_H 7.4−7.25 (m), 5.15 (s, H_4), 4.21 (d, J_{HH} = 11 Hz, ax-H_6), 3.94 (dd, J_{PH} = 25, J_{HH} = 11 Hz, eq-H_6), 3.83 (d, J_{PH} = 11, OMe), 1.05 (s, Me), 0.785 (s, Me). [Assignment of ^1H NMR signals to the two epimers was based on comparison with the spectra of mixtures in which the ax-OMe epimer **11a** was largely dominant (≥90%); these were obtained from the methanolysis of the phosphorochloridate **5a** with MeOH/Et_3N in CH_2Cl_2 or KOMe in *tert*-butyl alcohol.]

Condensation of the Phosphoric Acid (−)-6 with the Phosphorochloridate (−)-5a. The phosphoric acid (−)-**6** (250 mg, 1.03 mmol) was treated with an excess of the phosphorochloridate (−)-**5a** (336 mg, 1.29 mmol) in CH_2Cl_2 (12 mL) containing Et_3N (650 mg, 6.4 mmol) at room temperature. After 45 h (^{31}P NMR: δ_P −15.8, d, J_{PP} = 22 Hz; −20.1, s; −21.3, d, J_{PP} = 22 Hz), the volatile material was evaporated and the residue was dissolved in CH_2Cl_2, washed with water, and chromatographed on a column of silica gel 60 (35−70 μm; 200 × 20 mm). Elution with 1:1 hexane/ethyl acetate gave a small amount of the phosphorochloridate followed by the condensation products. Fractions 9−17 contained the pure symmetrical diphosphate **7a** (233 mg, 49%), crystallized from CH_2Cl_2/light petroleum: bp 40−60 °C (1:1); mp 206−208 °C; $[\alpha]_{589}$ −81.2° (*c* 0.29, CH_2Cl_2); MS (EI) *m*/*z* 466 (4) (M^+), 146 (55), 145 (100); IR (Nujol mull, cm^{-1}) 1335, 1325, 1310 (P═O) and 960 (P−O−P); ^{31}P NMR (162 MHz, $CDCl_3$) −19.9 ppm (without ^{1}H decoupling, 6 lines, indicative of virtual coupling); ^{1}H NMR (400 MHz, $CDCl_3$) 7.4−7.2 (m, 10 H), 5.46 (s, 2 H, H_4, $H_{4'}$), 4.68 (d, J_{HH} = 11 Hz, 2 H, ax-H_6, $H_{6'}$), 4.07 [complex (virtual coupling); with ^{31}P decoupling, d, J_{HH} = 11 Hz, 2 H, eq-H_6, $H_{6'}$], 1.08 (s, 6 H), 0.80 (s, 6 H); HRMS *m*/*z* calcd for $C_{22}H_{28}O_7P_2$, 466.131; found 466.131. Structure confirmed by X-ray crystallography, Figure 4.

Fractions 20−50 contained the unsymmetrical diphosphate **7b** (114 mg, 24%) and a trace (≤1%) of **7a**, crystallized from CH_2Cl_2/light petroleum: bp 40−60 °C (1:1); mp 152−154 °C (rapid heating; some softening at 145 °C); $[\alpha]_{589}$ −62.3° (*c* 0.26, CH_2Cl_2); MS (EI) *m*/*z* 466 (8) (M^+), 146 (55), 145 (100); IR (Nujol mull, cm^{-1}) 1310 (P═O) and 975 (P−O−P); ^{31}P NMR (162 MHz, $CDCl_3$) −15.3 (d, J_{PP} = 24.5 Hz) and −21.3 (d, J_{PP} = 24.5 Hz) (without ^{1}H decoupling, both signals dd, $J_{PH} \sim J_{PP} \sim 25$ Hz); ^{1}H NMR (400 MHz, $CDCl_3$) 7.5−7.3 (m, 10 H), 5.48 (d, J_{PH} = 1 Hz, 1 H) and 5.45 (d, $J_{PH} \leq 1$ Hz, 1 H) (H_4,$H_{4'}$), 4.48 (d, J_{HH} = 11 Hz, 1 H) and 4.45 (dd, J_{PH} = 3, J_{HH} = 11 Hz, 1 H) (ax-H_6, $H_{6'}$), 4.06 (dd, J_{PH} = 27, J_{HH} = 11 Hz, 1 H) and 4.045 (dd, J_{PH} = 24, J_{HH} = 11 Hz, 1 H) (eq-H_6, $H_{6'}$), 1.10 (s, 3 H), 1.06 (s, 3 H), 0.82 (s, 3 H), 0.81 (s, 3 H); HRMS *m*/*z* calcd 466.131, found 466.131. Anal. Calcd for $C_{22}H_{28}O_7P_2$: C, 56.65; H, 6.05. Found: C, 56.37; H, 5.74 (Cl, <1%).

Condensation of the ^{18}O-Labeled Phosphoric Acid (−)-6 with the Phosphorochloridate (−)-5a. (a) The ^{18}O-labeled phosphoric acid (−)-**6** (90 atom % ^{18}O distributed 9:1 in favor of the axial position) (15 mg, 61 μmol) was treated with the phosphorochloridate (−)-**5a** (20 mg, 77 μmol) in CH_2Cl_2 (450 μL) containing Et_3N (38 mg, 375 μmol) at room temperature. The location of the ^{18}O label in the diphosphate products **7a** and **7b** during the early stages of reaction was determined by ^{31}P NMR spectroscopy (Figure 5).[15]

(b) A similar experiment using less highly enriched ^{18}O-(−)-**6** (50 atom % ^{18}O distributed 9:1 in favor of the axial position) (22 mg, 91 μmol), and the phosphorochloridate (−)-**5a** (114 μmol) was allowed to proceed until isomerization of the diphosphate product (**7b** → **7a**) was essentially (98%) complete (112 h). Volatile material was evaporated, and the ^{18}O-labeled diphosphate **7a** was isolated by partition between CH_2Cl_2 and H_2O and purified by crystallization from CH_2Cl_2/light petroleum: bp 60−80 °C; MS (FAB) *m*/*z* 467 ($M + H^+$) (55), 469 (42), 471 (3); ^{31}P NMR as shown in Figure 5 (inset).

To confirm the NMR analysis, the product (14 mg, 30 μmol) was cleaved by treatment with NaOMe (34 μmol) in MeOH (240 μL) for 0.25 h: ^{31}P NMR (162 MHz) −1.18 [phosphate **6** anion; isotope shifted peaks $\Delta\delta$ 4.6 Hz (36%) and 9.7 Hz (2 × ^{18}O, 1%)] and −4.16 ppm [methyl ester **11a**; isotope shifted peak $\Delta\delta$ 6.4 Hz (7%)]; MS (−ES) *m*/*z* 241 and 243 (ratio 62:38) (phosphate anion); MS (+ES) *m*/*z* 279 and 281 ($M + Na^+$) (ratio 92:8) (methyl ester). The excess base was quenched with NH_4Cl, the solvent was evaporated, and the methyl ester was isolated by partition between CH_2Cl_2 and H_2O: ^{31}P NMR ($CDCl_3$) −6.2 ppm (ax-OMe isomer) [≤1.5% δ_P −2.4 ppm (eq-OMe isomer)].

Isomerization of the Unsymmetrical Diphosphate 7b in the Presence of ^{18}O-Labeled Phosphoric Acid 6. The ^{18}O-labeled phosphoric acid (−)-**6** (90 atom % ^{18}O distributed 9:1 in favor of the axial position) (2.6 mg, 10.5 μmol) and unlabeled ax−eq diphosphate (−)-**7b** (10.0 mg, 20 μmol) were dissolved in CH_2Cl_2 (250 μL) containing Et_3N (2.2 mg, 21 μmol). The gradual isomerization of (−)-**7b** to the symmetrical diphosphate (−)-**7a** was monitored by ^{31}P NMR spectroscopy (162 MHz). At 20% completion (t = 18 h), the product of (−)-**7a** contained much ^{18}O (δ_P −20.04; isotope shifted peak $\Delta\delta$ 2.8 Hz; ratio 36:64) but the reactant (−)-**7b** (δ_P −15.75 and −21.31; both d, J_{PP} = 22 Hz) contained practically no ^{18}O (≤1%). At 83% completion (t = 6 days), the reactant still contained very little ^{18}O.

Crystal Structure Determination of 5a and 7a. Data for **5a** and **7a** were measured on a Siemens P4 diffractometer with graphite monochromated Mo Kα radiation (λ = 0.7107 Å) using an ω scan technique. Standard reflections monitored every 100 scans showed no significant variation in intensity; the reflections were corrected for Lorentz and polarization effects. The structures were solved by direct methods and refined by full-matrix least squares on F^2 using the program SHELXL-97.[16] All hydrogen atoms were included in calculated positions (C−H = 0.96 Å) using a riding model.

Crystal Data for 5a. $C_{11}H_{14}ClO_3P$, M = 260.64, monoclinic, space group $P2_1/c$, a = 10.920(3) Å, b = 11.836(3) Å, c = 10.554(3) Å, β = 114.65(2)°, V = 1239.7(6) Å^3, T = 200 K, Z = 4, μ(Mo Kα) = 0.426 mm^{-1} colorless needle, and crystal dimensions 0.80 × 0.12 × 0.05 mm. Full-matrix least squares based on F^2 gave $R1$ = 0.045, $wR2$ = 0.134 for all data, and GOF = 1.054 for 145 parameters.

Crystal Data for 7a. $C_{22}H_{28}O_7P$, M = 466.38, monoclinic, space group $C2$, a = 17.579(5) Å, b = 6.749(2) Å, c = 13.064(3) Å, β = 130.83(2)°, V = 1172.6(6) Å^3, T = 200 K, Z = 2, μ(Mo Kα) = 0.225 mm^{-1} colorless block, crystal dimensions 0.55 × 0.41 × 0.28 mm. Full-matrix least squares based on F^2 gave $R1$ = 0.053, $wR2$ = 0.138 for all data, and GOF = 1.032 for 135 parameters. The expected R configuration at C4 was confirmed by the refined Flack parameter, 0.03(15).

Supporting Information Available: Crystal data, a summary of the data collection, and structure refinement parameters for **5a** and **7a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Sheldrick, G. A. SHELXL-97, Bruker AXS Inc., Madison, WI, 1997.