# The Quest for Free Metaphosphate in Solution: Racemization at Phosphorus in the Transfer of the Phospho Group from Aryl Phosphate Monoesters to *tert*-Butyl Alcohol in Acetonitrile or in *tert*-Butyl Alcohol

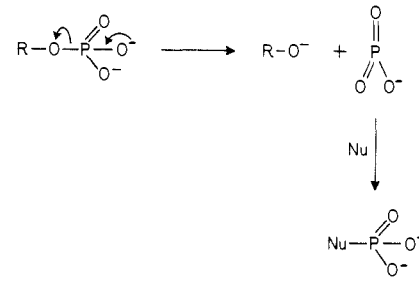
**Jonathan M. Friedman,[†] Sally Freeman,[‡] and Jeremy R. Knowles***

*Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138.* *Received July 17, 1987*

**Abstract:** To test the view that the phosphorylation of *tert*-butyl alcohol is diagnostic of the intermediacy of monomeric metaphosphate, the stereochemical course of the phospho group transfer from two chiral aryl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate monoesters to *tert*-butyl alcohol has been evaluated. First, from the reaction of the dianion of phenyl (*R*)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate with *tert*-butyl alcohol in acetonitrile, the product *tert*-butyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate was 53% $R_P$. Both the substrate and the product were shown in control experiments to be configurationally stable under the conditions of the reaction. Substantial racemization is consistent with the reaction proceeding via monomeric metaphosphate, but the solvent acetonitrile is potentially nucleophilic and the formation of a transient acetonitrile–metaphosphate adduct could also explain this result. [Indeed, the formation of such an adduct could nicely account for the small extent of *retention* observed in this experiment.] To avoid such solvent participation, the stereochemical course of a second reaction, that of the dianion of *p*-nitrophenyl (*R*)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate in neat *tert*-butyl alcohol, has been examined. The product *tert*-butyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate was found in this case to be *completely racemic* under reaction conditions in which both the product and the substrate are configurationally stable. It is clear from these results that phospho group transfer to *tert*-butyl alcohol can proceed through a symmetrically solvated monomeric metaphosphate intermediate.

In 1955, Westheimer[1a] and Bunton[1b] independently proposed that the hydrolysis of phosphate monoesters could proceed by way of a dissociative mechanism. Since that time, the results of a variety of different experiments have been interpreted in support of this formulation, which involves the intermediacy of the monomeric metaphosphate ion, $PO_3^-$ (see Scheme I).[2] Recently, with the advent of synthetic routes to chiral [$^{16}O$,$^{17}O$,$^{18}O$]phosphate monoesters and of methods for their stereochemical analysis,[3] it has been possible to probe the mechanistic importance of the metaphosphate intermediate stereochemically. Thus it is obvious that if in a phospho transfer reaction from a chiral phospho group donor metaphosphate were formed as a symmetrically solvated intermediate, the product of the transfer reaction would be racemic at phosphorus. Initial tests of this expectation were surprising. Stereochemical studies on reactions which by other mechanistic criteria were believed to involve metaphosphate as a reaction intermediate yielded results inconsistent with the intermediacy of a free metaphosphate ion. Thus the methanolysis of the monoanion of phenyl phosphate and of the dianion of 2,4-dinitrophenyl phosphate (for each of which the evidence in favor of a dissociative pathway had seemed compelling) were shown to proceed with clean *inversion* of the stereochemistry at phosphorus.[4] Indeed, even in cases where the donor group reactivity is especially high and the intermediacy of monomeric metaphosphate apparently unambiguous, the Conant–Swan fragmentation of a β-halophosphonate[5] and the methanolysis of an *N*-phosphoguanidine,[4,6] inversion of the phosphorus stereochemistry was also observed. All these stereochemical results are incompatible with the existence of a symmetrically solvated metaphosphate intermediate. The mechanistic dilemma was resolved by the proposal that these reactions may be constrained to preassociative pathways in which the rate-limiting transition state is predominantly dissociative yet the formation of product requires the assembled presence of the acceptor nucleophile.[4,7] Such preassociative pathways can in principle be stepwise, when product only forms when the metaphosphate intermediate is generated in the presence of the acceptor nucleophile, or concerted, which involves a loose $S_N2$-like transition state where there is but little bonding to phosphorus from either the donor or acceptor group.

**Scheme I**




All of these stereochemical experiments employed hydroxylic solvents or high concentrations of alcohol acceptors, and it appears that under such conditions, liberated metaphosphate is not formed. But what about other processes where metaphosphate has been proposed as an intermediate? For example, it seems likely that the lifetime of a putative metaphosphate intermediate could be increased by lowering the concentration and/or the reactivity of the acceptor nucleophile. Examples of two such studies that are commonly cited as providing evidence for monomeric metaphosphate are the three-phase test of Rebek and his group[8] and the phosphorylation of sterically hindered nucleophiles such as *tert*-butyl alcohol studied by Ramirez and his collaborators.[9] The

[†] Present address: Department of Molecular Biology and Biochemistry, Yale University, New Haven, CT 06511.

[‡] S.E.R.C. (U.K.) Postdoctoral Fellow. Present address: Pharmaceutical Science Institute, Aston University, Birmingham, U.K.

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study of most relevance to the present work is the latter, in which the reaction of aryl phosphate monoesters with a variety of aliphatic alcohols was investigated. It was found that in acetonitrile solution, the rates of reaction of aryl phosphate monoester dianions with *tert*-butyl alcohol and with water are similar,[9d] despite the large difference in steric factors. Moreover, when such reactions were done in the presence of competing alcohol nucleophiles, reaction with methanol was only slightly more favored than reaction with *tert*-butyl alcohol.[9f] This similarity contrasts sharply with the results from the alcoholysis of aryl phosphate monoester dianions in *aqueous* solution.[10] Thus, while methanol and ethanol compete effectively with water to form methyl and ethyl phosphate, respectively, isopropyl phosphate can barely be detected if isopropyl alcohol is present.[10] These considerations led Ramirez to suggest that the formation of *tert*-butyl phosphate is evidence that the dianions of aryl phosphate monoesters react by a dissociative pathway in aprotic media. Indeed, it was proposed that the phosphorylation of *tert*-butyl alcohol could be used as a *criterion* for the intermediacy of monomeric metaphosphate.[9] In this paper, we report the results of experiments designed to test the validity of this mechanistic criterion by evaluating the stereochemical course of *tert*-butyl phosphate formation in nonaqueous solution. Preliminary reports of some of this work have been published.[11]

## Experimental Section

**Materials.** All chemicals and enzymes were from Aldrich, Sigma, or Alfa-Ventron and were used as received unless otherwise noted. Isotopically enriched water was obtained either from Monsanto Research Laboratories ($H_2{}^{17}O$ or $H_2{}^{18}O$) or from Merck, Sharp and Dohme ($H_2{}^{17}O$). Molecular sieves (3 or 4 Å) were washed thoroughly with methanol, dried at 100 °C, and activated by heating at 250 °C under vacuum for 24 h. Tri-*n*-butylamine, tri-*n*-hexylamine, and tri-*n*-octylamine were passed through Wöhler neutral alumina prior to use. Trifluoroacetic anhydride was distilled under dry $N_2$. Potassium *tert*-butoxide was sublimed in vacuo at 120 °C just prior to use. Diphenyl phosphorochloridate was distilled under reduced pressure and was stored in a desiccator containing $CaCl_2$. Acetonitrile was distilled through a Vigreux column from $CaH_2$ and was stored over 3-Å molecular sieves; *N,N*-dimethylformamide was stirred over KOH for 30 min, and after decantation the liquid was distilled through a Vigreux column from BaO under reduced pressure and then stored under Ar over 3-Å molecular sieves. Dioxane was distilled from sodium; triethylamine, benzene, and *tert*-butyl alcohol were distilled from $CaH_2$ under $N_2$; and diethyl ether was distilled under $N_2$ either from $CaH_2$ or from Na/benzophenone. A diethyl ether solution of diazomethane was prepared from *N*-methyl-*N*-nitrosourea by the method of Arndt[12] and was used after extraction and drying over KOH. All enzymes were dialyzed at 4 °C against the appropriate buffer.

**Methods.** $^1H$ NMR spectra were recorded on Bruker WM-300, AM-300, and AM-250 and JEOL 270 spectrometers. Chemical shifts are reported in parts per million ($\delta$), employing as a standard tetramethylsilane ($\delta = 0$, for samples in nonaqueous solvents) and (trimethylsilyl)propanesulfonic acid or HOD ($\delta = 0$ or 4.75, respectively, for samples in $D_2O$). $^{31}P$ NMR spectra were recorded on Bruker WM-300 and AM-300 (121.5 MHz) and Varian XL-100 (40.5 MHz) spectrometers. Chemical shifts are reported in parts per million relative to external 85% $H_3PO_4$. Downfield shifts are positive.

Integrable broad-band proton-decoupled $^{31}P$ NMR spectra ($^{31}P\{^1H\}$ spectra) were obtained by using a pulse sequence involving inverse-gated heteronuclear decoupling (decoupler on only during data acquisition) to minimize nuclear Overhauser effects. For the high-resolution $^{31}P$ NMR spectra the line widths at half-height were identical for the resonances of the various isotopically labeled species, and peak heights could be used reliably for integration. For determinations of the configuration at phosphorus, the collected free induction decay data were transformed and rephased by using at least six different apodization functions. $^{13}C$ NMR spectra were recorded on Bruker AM-300 and WM-300 (75.5 MHz) and JEOL 270 (67.8 MHz) spectrometers, and chemical shifts are reported in parts per million downfield from tetramethylsilane.

Electron impact, chemical ionization (CI) with isobutane as a reagent gas, and fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS-50 instrument. For EI or CI, compounds containing phosphorus oxyanions were converted to their pertrimethylsilylated or permethylated derivatives. Pertrimethylsilylated compounds were obtained from the triethylammonium salts of the phosphorus compounds with bis(trimethylsilyl)trifluoroacetamide in triethylamine containing 4-(dimethylamino)pyridine.[13] Permethylated phosphorus compounds were obtained by conversion of the substrate to the free acid form with Dowex 50W-X8 cation-exchange resin (80–200 mesh, $H^+$ form), followed by treatment of the free acid in methanol with ethereal diazomethane. FAB mass spectra were obtained on the underivatized sodium or potassium salts. All mass spectral data reported are the average of 10–40 scans. The extent of isotopic labeling for all compounds was determined from averaged mass spectra by a least-squares fitting procedure using an algorithm analogous to that of Sukharev and Nekrasov.[14]

High-pressure liquid chromatography (HPLC) was carried out with a Waters Associates ALC/201/R-401/6000 system.

Spectrophotometric measurements were performed on a Perkin-Elmer 554 spectrophotometer.

Analytical thin-layer chromatography of phosphoric acid esters was accomplished with Merck silica gel G. The solvent systems used were 1-butanol–glacial acetic acid–water (5:3:2 (by volume) (system A)) and propan-2-ol–aqueous ammonium hydroxide–water (7:1:2 (by volume) (system B)). System A was used for separating *tert*-butyl phosphate ($R_f \sim 0.7$) from the mixture of 1-phospho- and 3-phospho-1,3-butanediol ($R_f \sim 0.50$) and from inorganic phosphate ($R_f \sim 0.35$). System B was used to separate inorganic phosphate ($R_f \sim 0.0$) from 1-phospho- and 3-phospho-1,3-butanediol ($R_f \sim 0.25$–$0.3$), *tert*-butyl phosphate ($R_f \sim 0.25$–$0.3$), aryl phosphates ($R_f \sim 0.4$–$0.55$), 2-hydroxy-4-methyl-1,3,2-dioxaphosphorinane 2-oxide ($R_f \sim 0.7$–$0.8$), and phosphotriesters ($R_f \sim 0.9$–$1.0$). Phosphate esters and ester amidates were visualized by the two-step procedure of Vaskovsky and Latyshev[15] in which silica plates were first sprayed with concentrated perchloric acid and heated on a hot plate just until all of the perchloric acid smoking had subsided. The resulting materials were then visualized by spraying the cooled silica plates with a mixture of sodium molybdate and malachite green in dilute HCl.

Flash column chromatography[16] was carried out with Merck silica gel 60 (230–400 mesh). Ion-exchange chromatography was carried out with either AG1-X8 (Biorad, 200–400 mesh) or Dowex 50W-X8 (80–200 mesh). Triethylammonium bicarbonate buffer was prepared by adding the appropriate amount of triethylamine to the appropriate volume of water and bubbling gaseous carbon dioxide through the mixture at 0 °C until the pH of the solution approached pH 7.0.

Phosphate esters were quantitated by spectrophotometry of the reduced phosphomolybdate complex, calibrating the assay against standard solutions of potassium phosphate.[17]

**Enantiomeric Excess of 1,3-Butanediol.** (3*S*)-1,3-Butanediol was obtained from Aldrich. The 1-triphenylmethyl ether was made by allowing the diol (89 μL, 0.99 mmol) to react with recrystallized triphenylmethyl chloride (300 mg, 1.1 mmol) in freshly distilled pyridine (1.5 mL) in the presence of recrystallized 4-(dimethylamino)pyridine (14 mg). After the mixture was stirred overnight, the reaction was quenched by pouring the solution onto a slurry of ice (3.5 g) and water (2.5 mL). The product was extracted from this solution into methylene chloride (5 times). The combined organic extracts were washed twice with aqueous $NaHSO_4$ (5% w/v)) and three times with aqueous $NaHCO_3$ (5% (w/v)). The methylene chloride solution was then dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The resulting yellow oil was dissolved in a small amount of methylene chloride and applied to a silica gel column. The column was eluted with chloroform, and (3*S*)-1-(triphenylmethoxy)-3-butanol was isolated (263 mg, 0.79 mmol, 80% yield) by combining appropriate fractions and removing the solvent by evaporation. $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 7.44–7.23 (m, 15 H), 3.97 (br m, 1 H), 3.35 (m, 1 H), 3.21 (m, 1 H), 2.85 (d, $J = 2.0$ Hz, 1 H), 1.85–1.655 (br m, 2 H), 1.15 (d, $J = 6.3$ Hz, 3 H).

The enantiomeric excess of the resulting (3*S*)-1-(triphenylmethoxy)-3-butanol was determined by $^{31}P$ NMR after reaction of this alcohol

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(260 mg, 0.78 mmol) with (4*R*,5*R*)-2-chloro-4,5-dimethyl-1,3,2-dioxaphospholane 2-oxide (the Anderson–Shapiro reagent) (140 mg, 0.82 mmol). The identity of the two $^{31}P$ signals corresponding to the two diastereomers had been confirmed by derivatizing *rac*-1,3-butanediol and observing a 1:1 ratio for the integrals of the two $^{31}P$ signals. This control experiment confirmed the lack of diastereoselectivity of the reaction with the Anderson–Shapiro reagent. (4*R*,5*R*)-2-[(1′*R*)-1′-methyl-3′-(trityloxy)propoxy]-4,5-dimethyl-1,3,2-dioxaphospholane 2-oxide: $^{31}P(^1H)$ NMR (121.5 MHz, benzene-$d_6$) $\delta$ 13.032. (4*R*,5*R*)-2-[(1′*S*)-1′-methyl-3′-(trityloxy)propoxy]-4,5-dimethyl-1,3,2-dioxaphospholane 2-oxide: $^{31}P(^1H)$ NMR (121.5 MHz, benzene-$d_6$) $\delta$ 13.006. Observed enantiomeric excess: 82.0 ± 2.0%.

**Phenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate** was prepared by the method of Abbott et al.,[18] using phenol in place of 2-*O*-benzyl-(*S*)-propane-1,2-diol. The yield was 34% (based on $H_2{}^{17}O$). The product was characterized as the bis(tetra-*n*-butylammonium) salt. $^{31}P$ NMR ($CD_3CN$) $\delta$ 0.28 (s); $^1H$ NMR (270 MHz, $CD_3CN$) $\delta$ 7.30 (d, $J$ = 7.9 Hz, 2 H), 7.14 (t, $J \sim 8$ Hz, or unresolved d of d, $J_1$ = 7.3 Hz, $J_2$ = 8.6 Hz, 2 H), 6.75 (t, $J$ = 7.3 Hz, 1 H), 3.24 (br t, $J$ = 8.6 Hz, 16 H), 1.68 (br quin, 16 H), 1.41 (sextet, $J$ = 7.6 Hz, 16 H), 1.02 (t, $J$ = 7.3 Hz, 24 H). The isotopic content was determined by mass spectrometry (dimethyl derivative, EI ionization): $m/s$ ($M^+$) 201 (2.7%), 202 (7.8%), 203 (36.1%), 204 (100.0%), 205 (76.0%), 206 (15.1%), 207 (2.2%); these data correspond to [$^{16}$O,$^{16}$O,$^{16}$O] 1.3%, [$^{16}$O,$^{16}$O,$^{17}$O] 3.6%, [$^{16}$O,$^{16}$O,$^{18}$O] 16.9%, [$^{16}$O,$^{17}$O,$^{18}$O] 46.1%, and [$^{16}$O,$^{18}$O,$^{18}$O] 32.2%.

***p*-Nitrophenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate** was prepared by a similar method to that described for 2,4-dinitrophenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate.[4] A solution of *p*-nitrophenol (recrystallized from chloroform) (4.7 g, 0.034 mol, 1.2 equiv) in dry acetonitrile (20 mL) was added dropwise over 10 min to a stirred solution of a mixture of (2*R*,4*S*,5*R*)- and (2*S*,4*S*,5*R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-[$^{17}$O]one (from reaction of [$^{17}$O]phosphoryl chloride with (−)-ephedrine) (6.94 g, 0.028 mol) in dry acetonitrile (15 mL) containing triethylamine (5.7 g, 0.056 mol, 2 equiv) at 0 °C. After 1 h at room temperature, $^{31}P$ NMR showed that the reaction was clean and complete: $\delta$ (MeCN) 15.7 (anti isomer, 20%) and 15.1 (syn isomer, 80%). The solvent was removed and the residue was dissolved in diethyl ether (100 mL). After this solution was washed with aqueous $NaHCO_3$ (5% (w/v), 3 × 20 mL), the organic layer was dried over $MgSO_4$ and the solvent removed. The solid residue was dissolved in a minimum of chloroform and subjected to flash chromatography on a column (950 mL) of silica gel, eluting with ethyl acetate–hexane (2:1 (v/v)). The minor isomer (2*S*,4*S*,5*R*)-2-(*p*-nitrophenoxy)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-[$^{17}$O]one eluted first and was isolated as a yellow solid (1.16 g, 3.34 mmol, 12%): $R_f$ (ethyl acetate) 0.62; $^{31}P$ NMR ($CDCl_3$) $\delta$ 12.67 ($^{16}$O, 35%), 12.63 ($^{18}$O, 65%); $^1H$ NMR ($CDCl_3$) $\delta$ 8.24 (2 H, d, $J_{HH}$ = 9.2 Hz), 7.45–7.30 (7 H, m), 5.57 (1 H, m), 3.76 (1 H, m), 2.81 (3 H, d, $J_{PH}$ = 10.7 Hz), 0.86 (3 H, d, $J_{HH}$ = 6.6 Hz). The major isomer (2*R*,4*S*,5*R*)-2-(*p*-nitrophenoxy)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-[$^{17}$O]one was isolated as a colorless solid (3.90 g, 0.0112 mol, 40%): $R_f$ (ethyl acetate) 0.36; $^{31}P$ NMR ($CDCl_3$) $\delta$ 12.28 ($^{16}$O, 35%), 12.24 ($^{18}$O, 65%); $^1H$ NMR ($CDCl_3$) $\delta$ 8.27 (2 H, d, $J_{HH}$ = 8.5 Hz), 7.50–7.20 (7 H, m), 5.81 (1 H, d, $J_{HH}$ = 6.2 Hz), 3.80 (1 H, m), 2.87 (3 H, d, $J_{PH}$ = 10.5 Hz), 0.82 (3 H, d, $J_{HH}$ = 6.8 Hz).

Freshly distilled trifluoroacetic anhydride (1.18 g, 5.60 mmol, 0.5 equiv) was added to $H_2{}^{18}O$ (5 mL, 95.0% $^{18}$O) under $N_2$. This mixture was then added by syringe to a stirred solution of the major diester amidate (3.90 g, 11.2 mmol) in dry dioxane (15 mL) at 0 °C. After 50 min, TLC analysis (on silica, eluting with ethyl acetate) showed that no starting material remained. The reaction mixture was filtered, and the precipitate was washed with dry ether and then dried under vacuum to give the zwitterionic diester as a white solid (3.95 g, 10.7 mmol, 96%).

Trimethylsilyl bromide (11.6 g, 75.8 mmol, 7 equiv) was added to a suspension of the zwitterionic diester (3.95 g, 10.7 mmol) in chloroform (50 mL), and the mixture was stirred at room temperature for 20 h. Analysis by $^{31}P$ NMR showed that the reaction was complete, and the solvent and excess trimethylsilyl bromide were removed with a brisk stream of $N_2$. Ether (50 mL) was added and the insoluble ephedrine bromide hydrobromide was removed by filtration. The ethereal solution was concentrated, and methanol (30 mL) was added. After 5 min the methanol was removed under reduced pressure and the residue was dissolved in 2 M NaOH solution (15 mL). This solution was diluted to 1600 mL with $H_2O$ and subjected to ion-exchange chromatography on AG1-X8 (100 mL), eluted with a linear gradient of triethylammonium bicarbonate buffer, pH 7 (1100 + 1100 mL, 50–600 mM). The appropriate fractions were combined and concentrated, taking care to keep the pH above 7 by adding 2 M NaOH. This gave pure *p*-nitrophenyl phosphate (0.0108 mol, 96%): $^{31}P(^1H)$ NMR (121.5 MHz, $D_2O$, disodium salt) $\delta$ 0.27 (s, corresponds to [$^{16}$O,$^{16}$O,$^{18}$O] species, 35%), 0.25 (s, corresponds to [$^{16}$O,$^{18}$O,$^{18}$O] species, 65%); $^1H$ NMR (250 MHz, $D_2O$, disodium salt) $\delta$ 8.15 (2 H, d, $J_{HH}$ = 9.1 Hz), 7.25 (2 H, d, $J_{HH}$ = 9.1 Hz).

***tert*-Butyl ($S_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate** was prepared by adapting our general synthetic route.[18] A solution of potassium *tert*-butoxide (0.73 g, 2 equiv) and 18-crown-6 (0.53 g, 2 equiv) in benzene (4 mL) was added dropwise to the [$^{17}$O]phosphochloramidate adducts of (−)-ephedrine, (2*RS*,4*S*,5*R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-[$^{17}$O]one (0.79 g, 3.24 mmol, 1 equiv) in benzene (12 mL). The mixture was stirred at 4 °C for 90 min. More benzene was then added and the resulting organic phase ($\sim$100 mL) was washed five times with aqueous sodium bicarbonate (5% w/v)) and then five times with water. The benzene solution was dried and concentrated, and the resulting oil was subjected to flash chromatography on silica gel, eluting with petroleum ether–ethyl acetate (2:1 (v/v)), followed by ethyl acetate. (2*S*,4*S*,5*R*)-2-*tert*-Butoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-[$^{17}$O]one (major adduct): $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 7.2–7.4 (br m, 5 H), 5.43 (d of d, $J_1$ = 3.95 Hz, $J_2$ = 6.26 Hz, 1 H), 3.58 (d of quin, $J_1$ = 10.55 Hz, $J_2$ = 6.26 Hz, 1 H), 2.62 (d, $J$ = 10.55 Hz, 3 H), 1.54 (s, 9 H), 0.76 (d, $J$ = 6.59 Hz); $^{13}C(^1H)$ NMR (67.8 MHz, $CDCl_3$) $\delta$ 136.4 (d, $J$ = 7.3 Hz), 128.2, 128.0, 126.3, 81.6 (d, $J$ = 7.3 Hz), 80.0, 58.8 (d, $J$ = 12.8 Hz), 29.9 (d, $J$ = 3.7 Hz), 28.8 (d, $J$ = 3.7 Hz), 14.3; $^{31}P$ NMR (40.5 MHz, $CDCl_3$) $\delta$ 16.07 (d of quint, $J_1$ = 3.9 Hz, $J_2$ = 10.3 Hz); mass spectrum $m/s$ ($M^+$ − 15) 268 (32.9%), 269 (100.0%), 270 (88.0%), 271 (12.6%), 272 (1.2%); these data correspond to [$^{16}$O] 16.7%, [$^{17}$O] 47.8%, and [$^{18}$O] 35.5%. (2*R*,4*S*,5*R*)-2-*tert*-Butoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-[$^{17}$O]one (minor adduct); $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 7.2–7.4 (br m, 5 H), 5.58 (d of d, $J_1$ = 2.3 Hz, $J_2$ = 5.9 Hz, 1 H), 3.62 (d of quint, $J_1$ = 12.5 Hz, $J_2$ = 6.6 Hz, 1 H), 2.67 (d, $J$ = 10.5 Hz, 3 H), 1.57 (s, 9 H), 0.72 (d, $J$ = 6.6 Hz, 3 H); $^{13}C$ NMR (67.8 MHz, $CDCl_3$) $\delta$ 136.2 (d, $J$ = 7.3 Hz), 127.8, 127.5, 125.3, 81.0 (d, $J$ = 7.3 Hz), 80.0, 58.8 (d, $J$ = 12.8 Hz), 29.5 (d, $J$ = 3.7 Hz), 28.5 (d, $J$ = 3.7 Hz), 12.2; $^{31}P$ NMR (40.5 MHz, $CDCl_3$) $\delta$ 16.11 (d of quint, $J_1 \sim 2.7$ Hz, $J_2 \sim 7.8$ Hz). The major *tert*-butyl phosphoramidate adduct was then hydrolyzed and hydrogenolyzed in a manner similar to that described previously to give *tert*-butyl ($S_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate (82%), which was characterized as its bis(tetra-*n*-butylammonium) salt: $^1H$ NMR (270 MHz, $CD_3CN$) $\delta$ 3.19 (br t, $J$ = 9 Hz, 16 H), 1.66 (br quin, 16 H), 1.40 (m) and 1.39 (s) (25 H together), 1.02 (t, $J$ = 7 Hz, 24 H); $^{31}P$ NMR (40.5 MHz, $CD_3CN$) $\delta$ −3.7.

**Bridge-labeled [$^{18}$O]*tert*-butyl phosphate** was prepared by a method similar to that described for *tert*-butyl ($S_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate except that [$^{18}$O]*tert*-butoxide, prepared from [$^{18}$O]acetone, was used. [$^{18}$O]Acetone was prepared by the method of Risley and Van Etten[19] except that the toluenesulfonic acid catalyst was replaced with camphorsulfonic acid. [$^{18}$O]*tert*-Butyl alcohol was prepared by the method of Grignard.[20] The second distillation of [$^{18}$O]*tert*-butyl alcohol was carried out over 3-Å molecular sieves. The yield of [$^{18}$O]*tert*-butyl alcohol was 52% based on [$^{18}$O]water. Potassium [$^{18}$O]*tert*-butoxide was prepared by slowly adding [$^{18}$O]*tert*-butyl alcohol (1.9 g) to a mixture of metallic potassium (1 equiv) and 18-crown-6 (1 equiv) in benzene (45 mL). The mixture was heated under reflux for 2 h and was then allowed to cool to room temperature before addition of the unlabeled phosphochloramidates, (2*R*,4*S*,5*R*)- and (2*S*,4*S*,5*R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholindin-2-one (approximately 1 equiv). The bridge-labeled [$^{18}$O]*tert*-butyl phosphate was characterized as its potassium salt: $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.28; $^{31}P$ NMR ($D_2O$, 121.5 MHz) $\delta$ −3.0 (broad s); $^{13}C(^1H)$ NMR, inverse-gated decoupling (75.5 MHz, $D_2O$) $\delta$ 78.123 (d, $J$ = 7 Hz, $^{16}$O isomer 35%), 78.083 (d, $J$ = 7 Hz, $^{18}$O isomer 65%), 29.86 (d, $J$ = 3.9 Hz); $^{13}C$ NMR, undecoupled (75.5 MHz, $D_2O$) $\delta$ 78.0 (m), 29.75 (q of sx, $J_1$ = 126.2 Hz, $J_2$ = 3.9 Hz); mass spectrum (FAB), $m/s$ ($M^+$ + 39) 269 (47.6%), 270 (4.5%), 271 (100.0%), 272 (5.0%), 273 (20.9%), 274 (0.9%), 275 (3.1%), 276 (0.2%); ($M^+$ + 1, relative to $m/s$ 233) 231 (49.7%), 232 (4.7%), 233 (100.0%), 234 (4.7%), 235 (14.4%), 236 (0.4%), 237 (0.5%); mass spectrum (sodium salt, FAB) $m/s$ ($M^+$ + 23) 221 (53.8%), 222 (4.7%), 223 (100.0%), 224 (4.0%), 225 (5.0%); ($M^+$ + 1, relative to $m/s$ 201) 199 (53.9%), 200 (4.7%), 201 (100.0%), 202 (3.9%), 203 (0.8%). These data correspond to [$^{16}$O] 34.5%, [$^{17}$O] 1.5%, and [$^{18}$O] 64.0%.

**Reaction of Phenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]Phosphate with *tert*-Butyl Alcohol in Acetonitrile.** Phenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate (5 mmol) was converted to the free acid form by passing an aqueous solution of the triethylammonium salt through a column (200 mL) of Dowex 50W-X8 ($H^+$ form) at 4 °C and washing the column with glass-distilled water (10

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column volumes). The combined eluates were concentrated by evaporation under reduced pressure, and the pH was raised to 11 at 0 °C with tetra-*n*-butylammonium hydroxide (1 M) in methanol. The solvent was then removed by evaporation under high vacuum. Final traces of water were removed with five additions and sublimations of *tert*-butyl alcohol at room temperature. Excess *tert*-butyl alcohol was then removed by five additions and evaporations of dry acetonitrile under reduced pressure. The bis(tetra-*n*-butylammonium) salt of phenyl($R_P$)-[$^{16}O,^{17}O,^{18}O$]phosphate was then transferred to a $^{31}P$ NMR tube with acetonitrile-$d_3$ (2 g). The mixture was diluted to a total volume of 10 mL with acetonitrile-$d_3$, and dry *tert*-butyl alcohol (1.0 mL, 10.6 mmol) was then added. The tube was immersed in a thermostated oil bath at 70 °C for 6 h, after which time the tube was cooled to 0 °C.

Analysis by $^{31}P(^1H)$ NMR with inverse-gated decoupling revealed that 55 ± 2% of the bis(tetra-*n*-butylammonium) salt of phenyl ($R_p$)-[$^{16}O,^{17}O,^{18}O$]phosphate had decomposed. The components of the mixture were separated by chromatography on a column (150 mL) of AG1-X8 ($HCO_3^-$ form) eluted with a linear gradient (2 L + 2 L) of triethylammonium bicarbonate buffer, pH 7 (25–400 mM). The isolated products were inorganic phosphate (1.45 mmol), *tert*-butyl phosphate [1.31 mmol, $^{31}P$ NMR ($D_2O$) $\delta$ −2.0 (s)], and unreacted phenyl phosphate (2.27 mmol).

**Reaction of *p*-Nitrophenyl ($R_P$)-[$^{16}O,^{17}O,^{18}O$]Phosphate with *tert*-Butyl Alcohol.** The triethylammonium salt of *p*-nitrophenyl ($R_P$)-[$^{16}O,^{17}O,^{18}O$]phosphate (167 mL of an aqueous solution, containing 3.60 mmol) was concentrated by evaporation under reduced pressure. Isopropyl alcohol (15 mL) was added and removed by evaporation (twice) to eliminate excess triethylammonium bicarbonate. The phosphate ester was then converted to the free acid form by passing the aqueous solution (100 mL) through a column (100 mL) of Dowex 50W-X8 ($H^+$ form) at 4 °C and washing the column with water (1000 mL). The eluate was concentrated under high vacuum, and final traces of water were removed by two additions and sublimations of *tert*-butyl alcohol (6 mL).

Meanwhile, a methanolic solution (1 M) of tetra-*n*-butylammonium hydroxide (10 mL) was concentrated and the last traces of methanol were removed by three additions and sublimations of *tert*-butyl alcohol (6 mL). The concentration was brought to 1 M by the addition of *tert*-butyl alcohol, and this solution (7.2 mL, 7.2 mmol, 2 equiv of tetra-*n*-butylammonium cation) was added to the sample of *p*-nitrophenyl phosphate (free acid form). *tert*-Butyl alcohol was quickly added to give a total volume of 14.4 mL, such that the concentration of phosphate ester was 0.25 M. Analysis by $^{31}P$ NMR showed that the substrate had not detectably decomposed. The solution was then heated in a thermostated water bath at 30 °C, and at regular intervals, portions (2 μL) were removed and quenched by adding to an optical cuvette containing 600 mM potassium bicarbonate buffer, pH 9.9 (4 mL), $MgCl_2$ (30 pmol) and $Zn(OAc)_2$ (0.3 pmol). The absorbance was measured at 405 nm, and alkaline phosphatase (from *E. coli*, 3 μL, 1 unit) was then added to hydrolyze the remaining *p*-nitrophenyl phosphate. Comparison of the final absorbance at 405 nm with that measured prior to the addition of enzyme gave a measure of the *p*-nitrophenyl phosphate remaining. By this assay it was shown that after 220 min, approximately half of the substrate had decomposed. Analysis by TLC and by $^{31}P$ NMR ($D_2O$) showed that more than 80% of the product was *tert*-butyl phosphate. The components of the reaction mixture were separated by ion-exchange chromatography on a column (60 mL) of AG1-X8 ($HCO_3^-$ form) eluting with a linear gradient (2 L + 2 L) of triethylammonium bicarbonate buffer, pH 7, (10–600 mM). The isolated products (in order of elution from the column) were *tert*-butyl phosphate [1.13 mmol, 31%, $^{31}P$ NMR ($D_2O$, potassium salt) $\delta$ 1.28 (s)], methyl phosphate [0.15 mmol, 4%, $^{31}P(^1H)$ NMR ($D_2O$, triethylammonium salt) $\delta$ 4.92 (s) (q, $J_{PH}$ = 9.8 Hz undecoupled), presumably deriving from residual methanol], inorganic phosphate [0.105 mmol, 3%, $^{31}P$ NMR ($D_2O$, triethylammonium salt) $\delta$ 2.96 (s)], recovered *p*-nitrophenyl phosphate [$^{31}P$ NMR ($D_2O$, triethylammonium salt) $\delta$ −0.2 ppm (s)], and uncharacterized material [6%, $^{31}P$ NMR −9.6 and −16.2]. Further purification of the *p*-nitrophenyl phosphate by ion-exchange chromatography on AG1-X8 gave pure *p*-nitrophenyl phosphate [1.31 mmol, 36%, $^{31}P$ NMR ($D_2O$, triethylammonium salt) $\delta$ 0.17].

**Stability of *tert*-Butyl ($S_P$)-[$^{16}O,^{17}O,^{18}O$]Phosphate under the Conditions of Reaction in Acetonitrile.** *tert*-Butyl ($S_p$)-[$^{16}O,^{17}O,^{18}O$]phosphate (750 μmol) was converted to the bis(tetra-*n*-butylammonium) salt via the free acid as described above for phenyl phosphate with due concern for the lability of *tert*-butyl phosphate at low pH. After the sample was dried by repeated additions and evaporations of *tert*-butyl alcohol and of acetonitrile, the bis(tetra-*n*-butylammonium) salt of *tert*-butyl ($S_p$)-[$^{16}O,^{17}O,^{18}O$]phosphate was dissolved in acetonitrile-$d_3$ (6 g) and *tert*-butyl alcohol (0.76 mL). The resulting solution was incubated at 70 °C for 6 h and the unchanged *tert*-butyl [$^{16}O,^{17}O,^{18}O$]phosphate (92%) was isolated by ion-exchange chromatography.

**Stability of *tert*-Butyl ($S_P$)-[$^{16}O,^{17}O,^{18}O$]Phosphate under the Conditions of Reaction in Decalin.** *tert*-Butyl ($S_P$)-[$^{16}O,^{17}O,^{18}O$]phosphate (750 μmol) was converted to the bis(tetra-*n*-hexylammonium) salt by treatment of the free acid with tetra-*n*-hexylammonium hydroxide (prepared in isopropyl alcohol by elution of tetra-*n*-hexylammonium iodide from a column of AG1-X8 ($OH^-$ form) with isopropyl alcohol) in a manner analogous to that described for phenyl phosphate. After drying as described above, the salt was dissolved in decalin (18.1 mL) containing *tert*-butyl alcohol (2.41 mL), and the solution was incubated at 80 °C for 20 h. The unchanged *tert*-butyl [$^{16}O,^{17}O,^{18}O$]phosphate was recovered by ion-exchange chromatography: $^1H$ NMR (300 MHz, $D_2O$) $\delta$ 1.27 (s); $^{31}P$ NMR (121.5 MHz, $D_2O$) $\delta$ −2.65.

**Alkaline Phosphatase Catalyzed Transfer of the Phospho Group of Phenyl [$^{16}O,^{17}O,^{18}O$]Phosphate to (3*S*)-1,3-Butanediol.** The phospho group of the phenyl [$^{16}O,^{17}O,^{18}O$]phosphate recovered from the reaction with *tert*-butyl alcohol in acetonitrile and the phospho group of the *p*-nitrophenyl [$^{16}O,^{17}O,^{18}O$]phosphate recovered from the reaction in neat *tert*-butyl alcohol were in each case transferred to (3*S*)-1,3-butanediol using alkaline phosphatase from *E. coli*[21] except that (3*S*)-1,3-butanediol was substituted for 1,2-propanediol. The [$^{16}O,^{17}O,^{18}O$]phospho-(3*S*)-1,3-butanediol obtained from the alkaline phosphatase transfer reaction was actually a mixture of the 1-phospho and the 3-phospho isomers. The 1-phospho compound was always the major isomer.

[$^{16}O,^{17}O,^{18}O$]Phospho-(3*S*)-1,3-butanediol deriving from recovered phenyl [$^{16}O,^{17}O,^{18}O$]phosphate after partial reaction in acetonitrile: $^{31}P(^1H)$ NMR (121.5 MHz, $D_2O$) $\delta$ 4.65 ($^{16}O,^{18}O$ isomer, 1-phospho), 4.63 ($^{18}O,^{18}O$ isomer, 1-phospho), 4.29 ($^{16}O,^{18}O$ isomer, 3-phospho), 4.27 ($^{18}O,^{18}O$ isomer, 3-phospho) (1-phospho/3-phospho ratio, 19.5/1); $^1H$ NMR (270 MHz, $D_2O$, 1-phospho) $\delta$ 3.92 (m, 1 H), 3.76 (m, 2 H), 1.65 (m, 2 H), 1.12 (d, 3 H); mass spectrum (dimethyl derivative, CI), *m/s* ($M^+$ + 1) 199 (6.7%), 200 (6.1%), 201 (39.5%), 202 (100.0%), 203 (72.8%), 204 (5.8%); these data correspond to [$^{16}O,^{16}O,^{16}O$] 3.1%, [$^{16}O,^{16}O,^{17}O$] 2.6%, [$^{16}O,^{16}O,^{18}O$] 18.2%, [$^{16}O,^{17}O,^{18}O$] 45.4%, and [$^{16}O,^{18}O,^{18}O$] 30.7%.

[$^{16}O,^{17}O,^{18}O$]Phospho-(3*S*)-1,3-butanediol deriving from recovered *p*-nitrophenyl [$^{16}O,^{17}O,^{18}O$]phosphate after partial reaction in *tert*-butyl alcohol: $^{31}P(^1H)$ NMR (121.5 MHz, $D_2O$, potassium salt) $\delta$ 4.76 (1-phospho), 4.35 (3-phospho) (1-phospho/3-phospho ratio, 15/1); $^1H$ NMR (250 MHz, $D_2O$, potassium salt) $\delta$ 3.94 (1 H, tq, $J_{HH}$ = 6.4 Hz), 3.77 (2 H, m), 1.67 (2 H, m), 1.14 (3 H, d, $J_{HH}$ = 6.3 Hz); mass spectrum (potassium salt, glycerol, FAB), *m/s* ($M^+$ + 39) 285 (3.6%), 286 (9.9%), 287 (40.5%), 288 (100%), 289 (81.5%), 290 (26.4%), 291 (18.1%), 292 (2.9%), 293 (1.7%); these data correspond to [$^{16}O,^{16}O,^{16}O$] 1.7%, [$^{16}O,^{16}O,^{17}O$] 4.5%, [$^{16}O,^{16}O,^{18}O$] 18.1%, [$^{16}O,^{17}O,^{18}O$] 44.3%, and [$^{16}O,^{18}O,^{18}O$] 31.4%.

**Acid Phosphatase Catalyzed Transfer of the Phospho Group of *tert*-Butyl [$^{16}O,^{17}O,^{18}O$]Phosphate to Butanediol.** The configuration at phosphorus in the isolated *tert*-butyl [$^{16}O,^{17}O,^{18}O$]phosphate samples was determined by transferring the [$^{16}O,^{17}O,^{18}O$]phospho group to (3*S*)-1,3-butanediol using acid phosphatase (from wheat germ). Triethylammonium bicarbonate buffer (150 mM) containing 0.1 or 0.5 M NaCl was saturated with $CO_2$ (g) at 0 °C to pH 6.6. *tert*-Butyl [$^{16}O,^{17}O,^{18}O$]phosphate was transferred into an NMR tube with several portions (2.0-mL total) of the above buffer followed by two portions (1.0-mL total) of $D_2O$. (3*S*)-1,3-Butanediol (4.0 mL) was added and the reaction was starting by adding a solution of acid phosphatase (250 mg in 1 mL, freshly dialyzed against the above triethylammonium bicarbonate buffer). The reaction was monitored by $^{31}P$ NMR, and after 2 days more phosphatase (1 mL of a dialyzed solution prepared from 250–300 mg of phosphatase) was added. After 5 days, when approximately half of the *tert*-butyl phosphate had reacted, the enzyme was removed by ultrafiltration.

The products 3-[$^{16}O,^{17}O,^{18}O$]phospho- and 1-[$^{16}O,^{17}O,^{18}O$]phospho-(3*S*)-1,3-butanediol were separated from unreacted *tert*-butyl [$^{16}O,^{17}O,^{18}O$]phosphate and from inorganic phosphate by chromatography on a column (30 mL) of AG1-X8 ($HCO_3^-$ form) using a linear gradient (1 L + 1 L) of triethylammonium bicarbonate buffer (25–350 mM). The 3-phospho compound was the major isomer. The ratio of regioisomers was 6:1 from the *tert*-butyl phosphate derived from the phenyl phosphate solvolysis and 4:1 from the *tert*-butyl phosphate derived from the *p*-nitrophenyl phosphate solvolysis, as assessed by $^{31}P(^1H)$ NMR with inverse-gated decoupling. [$^{16}O,^{17}O,^{18}O$]Phospho-(3*S*)-1,3-butanediol (mainly the 3-isomer): $^1H$ NMR (270 MHz, $D_2O$) $\delta$ 4.15 (br sp, $J$ = 5.9 Hz, 1 H), 3.61 (m, 5 lines, 1 H), 3.53 (m, 5 lines, 1 H), 1.59 (br sp t, 2 H), 1.12 (d, $J$ = 6.3 Hz, 3 H); $^1H$ NMR (270 MHz, $D_2O$) irradiated at $\delta$ 1.6, $\delta$ 4.15 (d of q, $J_1$ = 8.6 Hz, $J_2$ = 5.9 Hz, 1 H), 3.62 (d, $J$ = 11.5 Hz, 1 H), 3.50 (d, $J$ = 11.5 Hz, 1 H), 1.1 (d, $J \sim$ 6 Hz,

(21) Jones, S. R.; Kindman, L. A.; Knowles, J. R. *Nature (London)* **1978**, *275*, 564.

3 H); $^1$H NMR (270 MHz, $D_2O$) irradiated at $\delta$ 4.15, $\delta$ 3.6 (m, 5 lines, 1 H), 3.5 (m, 5 lines, 1 H), 1.56 (m, 8 lines, 2 H), 1.10 (s, 3 H); $^{31}$P($^1$H) inverse-gated decoupling (121.5 MHz, $D_2O$, EDTA added) $\delta$ 1.35 ($^{16}$O,$^{18}$O isomer, 1-phospho), 1.32 ($^{18}$O,$^{18}$O isomer, 1-phospho), 0.59 ($^{16}$O,$^{16}$O isomer, 3-phospho), 0.56 ($^{16}$O,$^{18}$O isomer, 3-phospho), 0.54 ($^{18}$O,$^{18}$O isomer, 3-phospho); $^{31}$P undecoupled (121.5 MHz, $D_2O$, EDTA added) $\delta$ 1.28 (t, $J$ = 6.0 Hz, $^{16}$O,$^{18}$O isomer, 1-phospho), 1.25 (t, $J$ = 6.0 Hz, $^{18}$O,$^{18}$O isomer, 1-phospho), 0.49 (d of d, $J_1$ = 2.9 Hz, $J_2$ = 8.3 Hz, $^{16}$O,$^{18}$O isomer, 3-phospho), 0.48 (d of d, $J_1$ = 2.9 Hz, $J_2$ = 8.1 Hz, $^{18}$O,$^{18}$O isomer, 3-phospho).

Two methods were employed for separating 1-[$^{16}$O,$^{17}$O,$^{18}$O]phospho- from 3-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol. The first method involved isocratic elution of the mixture of labeled phosphobutanediols from a Whatman Partisil SAX-M9 semipreparative anion-exchange column with 250 mM sodium acetate buffer, pH 4.0, at a flow rate of 3.5 mL/min. Components were visualized by using a Waters R401 differential refractive index detector. The second method involved isocratic elution of the mixture of labeled phosphobutanediols from an analytical scale Pharmacia FPLC Mono-Q anion-exchange column with 25 mM sodium acetate buffer, pH 5.5, at a flow rate of 0.5–2.0 mL/min. Components were visualized by using a Waters 490 programmable multiwavelength detector at 205 nm. Preparative-scale separations required several injections (approximately 10–20 $\mu$mol of phosphate ester per injection) and two to three recycles per injection. Acetate buffer was removed from the pooled fractions by anion-exchange chromatography on AG1-X8 ($HCO_3^-$ form) in triethylammonium bicarbonate buffer.

Purified 3-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol deriving from recovered *tert*-butyl [$^{16}$O,$^{17}$O,$^{18}$O]phosphate after reaction with *tert*-butyl alcohol in acetonitrile: mass spectrum (dimethyl derivative, CI), *m/s* ($M^+$ + 1) 199 (4.3%), 200 (5.1%), 201 (36.6%), 202 (100.0%), 203 (78.6%), 204 (5.8%), 205 (0.6%); these data correspond to [$^{16}$O,$^{16}$O,$^{16}$O] 2.0%, [$^{16}$O,$^{16}$O,$^{17}$O] 2.3%, [$^{16}$O,$^{16}$O,$^{18}$O], 16.9%, [$^{16}$O,$^{17}$O,$^{18}$O] 45.5%, and [$^{16}$O,$^{18}$O,$^{18}$O] 33.4%.

Purified 3-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol deriving from *tert*-butyl [$^{16}$O,$^{17}$O,$^{18}$O]phosphate formed from *p*-nitrophenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate in *tert*-butyl alcohol: $^1$H NMR (250 MHz, $D_2O$, potassium salt) $\delta$ 4.25 (1 H, m), 3.63 (2 H, m), 1.68 (2 H, dt, $J_{HH}$ = 6.4 Hz), 1.20 (3 H, d, $J_{HH}$ = 6.3 Hz); mass spectrum (potassium salt, glycerol, FAB), *m/s* ($M^+$ + 39) 285 (2.2%), 286 (8.1%), 287 (38.9%), 288 (100%), 289 (80.5%), 290 (25.0%), 291 (17.2%), 292 (2.6%), 293 (1.3%); these data correspond to [$^{16}$O,$^{16}$O,$^{16}$O] 1.0%, [$^{16}$O,$^{16}$O,$^{17}$O] 3.8%, [$^{16}$O,$^{16}$O,$^{18}$O] 18.0%, [$^{16}$O,$^{17}$O,$^{18}$O] 45.5%, and [$^{16}$O,$^{18}$O,$^{18}$O] 31.7%.

**Ring Closure of [$^{16}$O,$^{17}$O,$^{18}$O]Phospho-(3*S*)-1,3-butanediols.** The 1-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol derived from recovered phenyl [$^{16}$O,$^{17}$O,$^{18}$O]phosphate or from *p*-nitrophenyl [$^{16}$O,$^{17}$O,$^{18}$O]phosphate with alkaline phosphatase, and the 3-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol derived from *tert*-butyl [$^{16}$O,$^{17}$O,$^{18}$O]phosphate with acid phosphatase, were each subjected to ring closure. Typically, 1-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol (100 $\mu$mol) was converted to the free acid form by elution through a column of Dowex 50W-X8 ($H^+$ form). Tri-*n*-octylamine (43.7 $\mu$L, 1 equiv) and tri-*n*-butylamine (23.8 $\mu$L, 1 equiv) were added to the concentrated solution, and the resulting dianion was dried by repeatedly adding dioxane (5 mL) and evaporating this solvent under reduced pressure five times. *N,N*-Dimethylformamide (2.5 mL) and dioxane (2.5 mL) were then added, and the mixture was stirred for 4.5 h over molecular sieves (4 Å). Diphenyl phosphorochloridate (18.6 $\mu$L, 0.9 equiv) was added, and the reaction mixture was stirred for 20 min. After this time a solution of potassium *tert*-butoxide (5 equiv) in *N,N*-dimethylformamide (5.7 mL) was added, and the reaction mixture was stirred for a further 30 min. The reaction was quenched by adding dried Dowex 50W-X8 resin (15 mL, pyridinium form, prepared by washing pyridine through a column in the $H^+$ form, followed by drying at 105 °C under vacuum). After the mixture was stirred for 30 min, the resin was removed by filtration and washed successively with dioxane (2 × 25 mL) and water (300 mL). The combined filtrates were concentrated and diluted to 400 mL with water, and the components were isolated by chromatography on a column (30 mL) of AG1-X8 resin preequilibrated with 10 mM triethylammonium bicarbonate and eluted with a linear gradient (1 L + 1 L) of triethylammonium bicarbonate (10–125 mM). The ring-closure reaction of 3-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(3*S*)-1,3-butanediol derived from *tert*-butyl phosphate formed in the solvolysis of *p*-nitrophenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]-phosphate in neat *tert*-butyl alcohol at 30 °C gave [$^{16}$O,$^{17}$O,$^{18}$O]-(4*S*)-2-hydroxy-4-methyl-1,3,2-dioxaphosphorinane 2-oxide (potassium salt) (60 $\mu$mol, 60% yield): $^{31}$P($^1$H) NMR (121.5 MHz, $D_2O$) $\delta$ −2.04 ($^{16}$O,$^{16}$O isomer), −2.07 ($^{16}$O,$^{18}$O isomer), −2.09 ($^{18}$O,$^{18}$O isomer); $^{31}$P NMR (undecoupled) (121.5 MHz, $D_2O$) $\delta$ −2.14 (br d, $J$ = 20 Hz); mass spectrum (potassium salt, glycerol, FAB), *m/s* ($M^+$ + 39) 229 (18.4%), 230 (39.8%), 231 (100%), 232 (41.3%), 233 (38.2%), 234 (6.7%), 235 (4.4%); these data correspond to [$^{16}$O,$^{16}$O] 9%, [$^{16}$O,$^{17}$O] 19.0%, [$^{16}$O,$^{18}$O] 46.4%, [$^{17}$O,$^{18}$O] 15.0%, and [$^{18}$O,$^{18}$O] 10.6%.

**Methylation of [$^{16}$O,$^{17}$O,$^{18}$O]-(4*S*)-2-Hydroxy-4-methyl-1,3,2-dioxaphosphorinane 2-Oxide.** The potassium salt of the cyclic phosphodiester (4*S*)-2-hydroxy-4-methyl-1,3,2-dioxaphosphorinane 2-oxide (approximately 50 $\mu$mol) was dried by five repeated additions and evaporations of freshly distilled dioxane. 18-Crown-6 (29.3 mg, 111 $\mu$mol), dimethyl-$d_6$ sulfoxide (2.5 mL) and iodomethane (250 $\mu$L, 570 mg, 5 mmol) were added. After 18–20 h the reaction mixture was transferred to an NMR tube and the $^{31}$P($^1$H) spectrum of the diastereomeric triesters [(2*R*,4*S*)- and (2*S*,4*S*)-2-methoxy-4-methyl-1,3,2-dioxaphosphorinane 2-oxide] was obtained. The configuration at phosphorus could be assessed by analysis of these $^{31}$P NMR spectra by the modified method of Buchwald and Knowles.[22]

The unpurified mixture of triesters was not stable and had a half-life of about a week at room temperature. The potassium iodide product could be removed by flash chromatography of the triester solution, eluting with ethyl acetate. This purification alleviated the problem of instability and allowed reproducible $^{31}$P NMR spectra to be obtained even after several months of storage at −70 °C.

**Stability of Bridge-Labeled [$^{18}$O]*tert*-Butyl Phosphate under the Conditions of Reaction in *tert*-Butyl Alcohol.** The bis(tetra-*n*-butylammonium) salt of bridge-labeled [$^{18}$O]*tert*-butyl phosphate (380 $\mu$mol) was prepared in a manner similar to that described for the reaction of phenyl phosphate with *tert*-butyl alcohol in acetonitrile. *tert*-Butyl alcohol (3.3 mL) was added, and the solution was heated at 70 °C. At various intervals the reaction mixture was cooled to room temperature for the removal of samples. The *tert*-butyl phosphate was isolated by ion-exchange chromatography and then converted to the sodium salt for analysis by FAB mass spectrometry. By monitoring the loss of the [$^{18}$O] label with time, the rate constant for the phospho group transfer reaction of *tert*-butyl phosphate with *tert*-butyl alcohol was (2.8 ± 0.2) × $10^{-5}$ $s^{-1}$ at 70 °C.

**Stability of Bridge-Labeled [$^{18}$O]*tert*-Butyl Phosphate under the Conditions of Reaction in Decalin.** Decalin (5.56 mL) and *tert*-butyl alcohol (0.74 mL) were added to the bis(tetra-*n*-hexylammonium) salt of bridge-labeled [$^{18}$O]*tert*-butyl phosphate (232 $\mu$mol). The solution was heated at 80 °C, and at various times the reaction mixture was cooled to 0 °C for the removal of samples. The water-soluble products were extracted into 25 mM triethylammonium bicarbonate buffer, pH 7.0, and isolated by ion-exchange chromatography. Each product was then converted to its sodium salt for FAB mass spectrometric analysis. The rate of loss of the [$^{18}$O] label was (3.3 ± 0.1) × $10^{-5}$ $s^{-1}$ at 80 °C.

**Stability of Bridge-Labeled [$^{18}$O]*tert*-Butyl Phosphate with *p*-Nitrophenol in *tert*-Butyl Alcohol.** The bis(tetra-*n*-butylammonium) salt of bridge-labeled [$^{18}$O]*tert*-butyl phosphate (763 $\mu$mol) was prepared from the triethylammonium salt by a method similar to that described for phenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate. After drying by four repeated additions and sublimations of *tert*-butyl alcohol (4 × 6 mL), the phosphate was dissolved in *tert*-butyl alcohol (6.6 mL) containing *p*-nitrophenol (freshly recrystallized from chloroform, 1 equiv, 763 $\mu$mol). The solution was immersed in a thermostated water bath at 30 °C, and portions (2 mL) were removed at time zero, after 220 min, and after 48 h. Each sample was concentrated and then dissolved in $D_2O/H_2O$ for isotopic analysis: $^{13}$C($^1$H) NMR (75.47 MHz, tetra-*n*-butylammonium salt, referenced to ethylbenzene peak at 128 ppm) $\delta$ 72.40 (d, $J_{CP}$ = 6.9 Hz, $^{16}$O isomer $(CH_3)_3COP$), 72.37 (d, $J_{CP}$ = 6.9 Hz, $^{18}$O isomer $(CH_3)_3COP$), 55.63 (s, $NCH_2CH_2CH_2CH_3$), 27.12 (d, $J_{CP}$ = 3.5 Hz, $(CH_3)_3COP$), 20.60 (s, $NCH_2CH_2CH_2CH_3$), 16.60 (s, $NCH_2CH_2CH_2CH_3$), 10.33 (s, $NCH_2CH_2CH_2CH_3$); $^{31}$P($^1$H) NMR (121.5 MHz, tetra-*n*-butylammonium salt) $\delta$ 0.732 (s, $^{16}$O isomer), 0.716 (s, $^{18}$O isomer). By both $^{13}$C and $^{31}$P NMR each sample was shown to contain 65 ± 2% of the $^{18}$O isomer and 35 ± 2% of the $^{16}$O isomer of *tert*-butyl phosphate.

## Results and Discussion

In earlier work, we have demonstrated that the aqueous methanolysis of the dianion of 2,4-dinitrophenyl ($R_p$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate, of the monoanion of phenyl ($R_P$)-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate, and of the zwitterion of ($S_P$)-*N*-[$^{16}$O,$^{17}$O,$^{18}$O]phosphocreatine each proceeds with complete inversion of the configuration at phosphorus.[4] Although the evidence from other work for a dissociative mechanism for these reactions was strong, the observation of phospho group transfer with inversion required a reassessment of the supposed intermediacy of monomeric metaphosphate. All the results could be accommodated by one of two postulates: either the reactions proceed

(22) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1980**, *102*, 6601.

through a loose $S_N2$-like transition state (in what Jencks[7] has called a "preassociative concerted" process) where metaphosphate is not an intermediate, or they involve a metaphosphate ion that is, in the presence of such potent nucleophiles as water and methanol, trapped rapidly in a "preassociative stepwise" reaction.[4,7] In an effort to distinguish between these two possibilities, Skoog and Jencks[23] and Bourne and Williams[24] investigated the linearity of Brønsted plots for the phospho group transfer reactions from *N*-phosphopyridinium (or quinolinium) ions to a series of substituted pyridines (or quinolines) in aqueous solution. The deviation from linearity in the Brønsted plots that is expected for reactions proceeding via a discrete intermediate was not observed, and each group concluded that these reactions are most probably preassociative concerted and involve an exploded $S_N2$-like transition state.

The mechanistic and stereochemical data that relate to experiments in aqueous media do not, therefore, support the earlier suggestions that metaphosphate ion is a reaction intermediate in displacement reactions of phosphate monoesters, and we must ask whether metaphosphate is *ever* a kinetically important species in such processes. One way to resolve this question could be to increase the lifetime of the putative metaphosphate intermediate, either by lowering the concentration of trapping nucleophiles in the medium or by lowering the potency of such nucleophiles by steric or electronic means. Following both of these approaches, Ramirez and Marecek observed that in acetonitrile solution, phospho group transfer to *tert*-butyl alcohol from aryl phosphate dianions proceeds at about the same rate as does transfer to water in this solvent.[9d] This finding contrasts sharply with the results from the solvolysis of aryl phosphates in aqueous solution. Here, even isopropyl phosphate can barely be detected among the solvolysis products in aqueous isopropyl alcohol, although the less hindered alcohols methanol and ethanol do compete very effectively with water.[10] These considerations led Ramirez and Marecek to suggest that the formation of *tert*-butyl phosphate was diagnostic of the intermediacy of monomeric metaphosphate. Herschlag and Jencks have, however, pointed out that the phosphorylation of *tert*-butyl alcohol could proceed through an open transition state without the intermediacy of monomeric metaphosphate[25] and cite the second-order reaction of trimethylamine with *p*-nitrophenyl phosphate dianion as an analogously hindered situation.[26]

To investigate whether metaphosphate is involved in the phosphorylation of *tert*-butyl alcohol by phosphate monoesters, we first studied the stereochemical course of the phospho group transfer from phenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate[27] to *tert*-butyl alcohol in solution in acetonitrile. The phospho group donor was solubilized as its bis(tetra-*n*-butylammonium) salt,[28] and the reaction was performed in 1 M *tert*-butyl alcohol in acetonitrile at 70 °C.[9c] After 6 h, the product *tert*-butyl phosphate and the remaining substrate phenyl phosphate were isolated, and each was subjected to stereochemical analysis. Determination of the absolute configuration at phosphorus in the remaining phenyl phosphate was achieved by a slight modification of our original method, involving the alkaline phosphatase-catalyzed transfer of the chiral phospho group to (*S*)-butane-1,3-diol with retention of configuration, followed by ring closure to the cyclic diester, methylation, and $^{31}P$ NMR analysis.[4,22] This sequence demonstrated that the remaining phenyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate was 81% $R_P$ (Figure 1B) (previous syntheses of this material have yielded material between 80 and 86% $R_P$), thus confirming the configurational stability of the substrate under the reaction conditions. The configuration of the product *tert*-butyl phosphate could not be determined in precisely the same way, since neither of the phosphatases whose stereochemical course was known (alkaline phosphatase from *E. coli*,[21] and human prostatic acid phosphatase[29]) catalyzes the transfer of the phospho group from this hindered ester. We found, however, that one of the isozymes of wheat germ acid phosphatase is capable of effecting the required phospho group transfer, allowing the configurations of *tert*-butyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate to be defined. The resulting $^{31}P$ NMR spectrum is shown in Figure 1A and indicates that the product is largely *racemic* at phosphorus. There is, evidently, a small level (6% enantiomeric excess) of *retention* of the configuration, which is too large to be dismissed as experimental uncertainty. Before we can conclude that the phospho group suffers considerable racemization during its transfer from phenyl phosphate to *tert*-butyl alcohol, several alternative possibilities must be evaluated. Thus it was possible that the observed racemization could have derived either from the configurational instability of *tert*-butyl phosphate under the reaction conditions or from a lack of stereospecificity in one or more of the manipulations of the stereochemical analysis. Indeed, wheat germ acid phosphatase is known to be a mixture of at least three isozymes,[30] and racemization could conceivably (if improbably) result if two isozymes were to catalyze the phospho group transfer by opposite stereochemical routes. To provide answers to both of these uncertainties, authentic *tert*-butyl

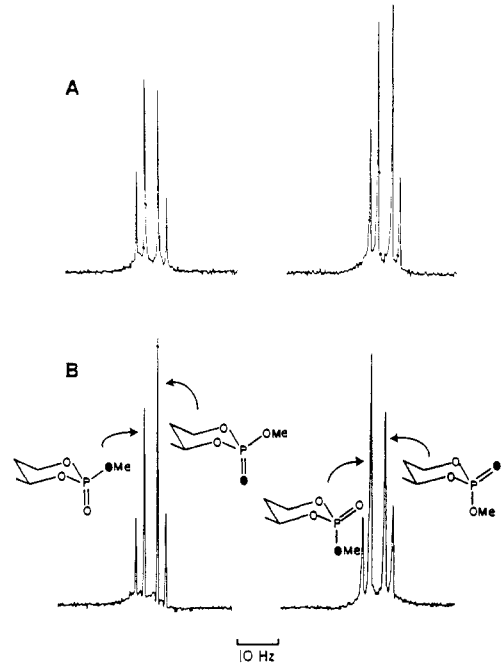
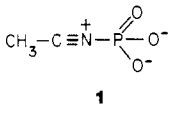



**Figure 1.** $^{31}P$ NMR spectra of the cyclic phosphate triesters derived from stereochemical analysis of (A) the *tert*-butyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate product and (B) the recovered phenyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate substrate from the solvolysis of phenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate in *tert*-butyl alcohol (1 M) in acetonitrile at 70 °C. The spectra were taken on a Bruker WM-300 instrument at 121.5 MHz, Gaussian multiplication with Gaussian broadening, 0.12 Hz, and line broadening, −0.3 Hz. The downfield multiplets (syn isomers) are centered at −4.85 ppm and the upfield multiplets (anti isomers) are centered at −5.85 ppm. The singly $^{18}O$-labeled isotopomers that provide sterochemical information are identified. The downfield signal in each quartet is from the completely unlabeled triester and the upfield signal in each quartet is from the doubly labeled $^{18}O_2$ triester. The remaining substrate [in (B)] is 81% $R_P$, and the product [in (A)] is 53% $R_P$.

(23) Skoog, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597.
(24) Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591.
(25) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 7938.
(26) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209.
(27) The $R_P$ enantiomer of phenyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate was used in our earlier experiments,[11] not the $S_P$ enantiomer stated. The stereochemical consequences of all phospho transfers were, however, correctly described.[11]
(28) In our previous work, the sodium salts of aryl phosphates were used, but to achieve the required solubility in *tert*-butyl alcohol in the present experiments, it was necessary to employ the bis(tetra-*n*-butylammonium) salts. While it has been suggested that changing the cation from metal to non-metal (as here) could increase the dissociative character of the reaction (ref 26; Mildvan, A. S. *Adv. Enzymol.* **1979**, *49*, 103; Benkovic, S. J.; Dunikoski, L. K. *J. Am. Chem. Soc.* **1971**, *93*, 1526; Hall, A. D.; Williams, A. *Biochemistry* **1986**, *25*, 4784), Herschlag and Jencks have recently shown this to be unlikely (Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4665).
(29) Buchwald, S. L.; Saini, M. S.; Knowles, J. R.; Van Etten, R. L. *J. Biol. Chem.* **1984**, *259*, 2208.
(30) Brouillard, J.; Ouellet, L. *Can. J. Biochem.* **1965**, *43*, 1899.

($S_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate was synthesized[18] and subjected to the conditions of the solvolysis reaction. The reisolated *tert*-butyl phosphate was then analyzed and the configuration at phosphorus was found to be 83% $S_P$. This result proved, in one experiment, both that *tert*-butyl phosphate is configurationally stable in acetonitrile at 70 °C and that wheat germ acid phosphatase catalyzes phospho group transfer from *tert*-butyl phosphate stereospecifically with retention of configuration at phosphorus.

The control experiments described above allow us to conclude that the transfer of the phospho group from phenyl phosphate to *tert*-butyl alcohol in acetonitrile proceeds with considerable racemization at phosphorus and that there is a small extent of net *retention* of configuration in the *tert*-butyl phosphate product. At first sight, substantial racemization seems to be good evidence for the intermediacy of a liberated metaphosphate intermediate. However, the solvent acetonitrile is potentially nucleophilic, and our stereochemical result could be accommodated by postulating a sequence of displacements in which acetonitrile attacks the phenyl phosphate donor to give a highly reactive acetonitrile–metaphosphate adduct (**1**), this adduct then suffering many se-



**1**

quential displacements by other acetonitrile molecules before being finally trapped by *tert*-butyl alcohol as *tert*-butyl phosphate. The small amount of net retention observed could then arise from the occasional trapping by *tert*-butyl alcohol of the *first-formed* acetonitrile adduct. In agreement with this proposal, Cullis and Rous have recently observed a similar amount of retention of configuration at phosphorus in the phospho group transfer reaction from adenosine 5′-$\beta$-($S$)-[$^{16}O$,$^{17}O$,$^{18}O$]diphosphate to a primary alcohol in acetonitrile.[31] Even though there is only indirect evidence for the existence of an acetonitrile–metaphosphate adduct in solution, analogy with the behavior of dioxane with sulfur trioxide[32] (which is isoelectronic with metaphosphate) as well as the observation of an acetonitrile–metaphosphate species in the gas phase[33] makes such solvent participation a real possibility. Thus although it is clear that phospho group transfer to *tert*-butyl alcohol in acetonitrile proceeds with extensive racemization, we cannot conclude that free metaphosphate is an intermediate under these conditions.

To eliminate the possibility of nucleophilic participation by acetonitrile, we chose to investigate the phospho group transfer reaction from phenyl phosphate to *tert*-butyl alcohol both in decalin and in the neat alcohol. However, under the conditions required for the reaction (20 h at 80 °C for *tert*-butyl alcohol (1 M) in decalin, and 28 h at 70 °C for neat *tert*-butyl alcohol), the product *tert*-butyl phosphate was found to be configurationally labile. Thus the bis(tetra-*n*-hexylammonium) salt of synthetic *tert*-butyl ($S_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate was subjected to the solvolysis conditions in decalin containing *tert*-butyl alcohol (1 M) at 80 °C for 20 h. On stereochemical analysis, this sample was found to be only 54% $S_P$. That is, *tert*-butyl phosphate largely racemizes under these solvolysis conditions. To confirm this result, bridge-labeled [$^{18}O$]*tert*-butyl phosphate was incubated under similar conditions, and the rate of loss of the $^{18}O$ label from the bridge position of reisolated *tert*-butyl phosphate was found by mass spectrometric analysis to be $(3.3 \pm 0.1) \times 10^{-5}$ s$^{-1}$ ($t_{1/2} \sim$ 5.8 h). This rate constant is consistent with the observed partial racemization of *tert*-butyl ($S_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate described above, providing that each phospho group transfer from *tert*-butyl phosphate to *tert*-butyl alcohol proceeds with racemization at phosphorus. Similarly, the stability of *tert*-b tyl phosphate in neat *tert*-butyl alcohol at 70 °C was probed by using the bridge-labeled [$^{18}O$]*tert*-butyl phosphate, and the loss of $^{18}O$ from the bridge position has a rate constant of $(2.8 \pm 0.2) \times 10^{-5}$ s$^{-1}$ ($t_{1/2} \sim$ 6.9 h).

The instability of *tert*-butyl phosphate came as a surprise for two reasons. First, *tert*-butyl phosphate is configurationally stable in acetonitrile. Second, in both decalin and neat *tert*-butyl alcohol, *tert*-butyl phosphate is *more* labile than phenyl phosphate. This relative reactivity is opposite to that which would be predicted on the basis of the behavior of these molecules in aqueous solution. Thus extrapolation of the Brønsted plot of Kirby and Varvoglis[34] suggests that the hydrolysis of phenyl phosphate dianion is about $10^{11}$ times faster than that of *tert*-butyl phosphate dianion. Whatever the reasons for the change in relative reactivity, however, we are left with the fact that *tert*-butyl phosphate is configurationally labile under these conditions. Clearly, a more reactive phospho group donor was needed that would react with *tert*-butyl alcohol under conditions where *tert*-butyl phosphate is stable. Accordingly, *p*-nitrophenyl phosphate was used, since Ramirez and Marecek have shown that *tert*-butyl alcohol reacts more readily with the dianion of *p*-nitrophenyl phosphate ($t_{1/2}$ = 5 h at 35 °C) than with the dianion of phenyl phosphate ($t_{1/2}$ = 48 h at 70 °C) in neat *tert*-butyl alcohol,[9d] and we were confident that the product *tert*-butyl phosphate would be configurationally stable at 30 °C. [Even though calculation using a reasonable activation energy[35] suggests that the dianion of *tert*-butyl phosphate is stable at 30 °C, a further control is important. It is conceivable that the product of solvolysis of *p*-nitrophenyl phosphate could be the *mono*anion of *tert*-butyl phosphate and *p*-nitrophenoxide. Indeed, even if the phosphate dianion predominates, it is possible that the *tert*-butyl phosphate could react subsequently through a small amount of the monoanion at equilibrium. To show that *tert*-butyl phosphate is configurationally stable under the actual conditions of the solvolysis, 1 equiv of *p*-nitrophenol was included in an incubation of the bis(tetra-*n*-butylammonium) salt of [$^{18}O$] bridge-labeled *tert*-butyl phosphate in neat *tert*-butyl alcohol at 30 °C. The reaction mixture was analyzed at intervals by $^{13}C$ NMR. It was found that the *tert*-butyl phosphate is completely stable under these conditions.]

*p*-Nitrophenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate was synthesized by the route used earlier for 2,4-dinitrophenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate[4] except that *p*-nitrophenol was used in place of lithium 2,4-dinitrophenolate. The reaction of the bis(tetra-*n*-butylammonium) salt of *p*-nitrophenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate was monitored by following the release of *p*-nitrophenolate at 405 nm. After 220 min, half of the ester had decomposed and the components of the reaction mixture were separated by ion-exchange chromatography. The configuration at phosphorus in the isolated *tert*-butyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate product was analyzed as described above and yielded the spectrum shown in Figure 2A. It is clear from this spectrum that the stereochemically informative peaks (the middle pair of each quartet) are of equal intensity, showing that the *tert*-butyl phosphate is *completely racemic*. The donor *p*-nitrophenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate reisolated from the solvolysis reaction was also subjected to stereochemical analysis and found to be 85% $R_P$ (Figure 2B), showing that *p*-nitrophenyl phosphate is configurationally stable under the conditions of the solvolysis reaction.

We can therefore conclude that the phospho group transfer from *p*-nitrophenyl phosphate to *tert*-butyl alcohol proceeds with complete racemization at phosphorus. This observation is consistent with a dissociative reaction that proceeds through a liberated, symmetrically solvated, metaphosphate intermediate.[36]

(31) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 1298.
(32) Sisler, H. H.; Audrieth, L. G. *Inorg. Synth.* **1946**, *2*, 173. Gilbert, E. E. *Chem. Rev.* **1962**, *62*, 549.
(33) Henchman, M.; Viggiano, A. A.; Paulson, J. F.; Freedman, A.; Wormhoudt, J. *J. Am. Chem. Soc.* **1985**, *107*, 1453.
(34) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415.
(35) Bel'skii, V. E. *Russ. Chem. Rev. (Engl. Transl.)* **1977**, *46*, 828.
(36) This conclusion assumes that, once phosphorylated, *tert*-butyl phosphate is stable. Yet, as pointed out by a referee, the first formed *tert*-butyl phosphate would be bridge-protonated, and this species could either (a) lose a proton to form *tert*-butyl phosphate or (b) undergo multiple transfers with *tert*-butyl alcohol similar to those described above for acetonitrile. Our interpretation of the racemization result thus assumes that proton transfer is faster than the diffusive replacement of *p*-nitrophenoxide by *tert*-butyl alcohol and the subsequent reaction of *tert*-butyl alcohol with bridge-protonated *tert*-butyl phosphate.

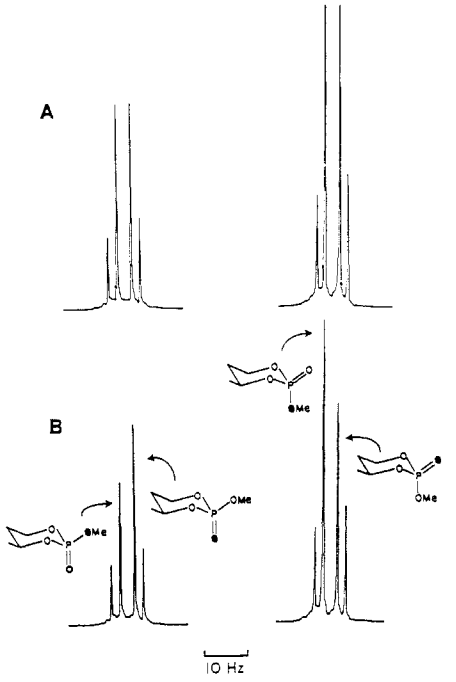



**Figure 2.** $^{31}P$ NMR spectra of the phosphate triesters provided from stereochemical analysis of (A) the *tert*-butyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate product and (B) the recovered *p*-nitrophenyl [$^{16}O$,$^{17}O$,$^{18}O$]phosphate substrate from the solvolysis of *p*-nitrophenyl ($R_P$)-[$^{16}O$,$^{17}O$,$^{18}O$]phosphate in neat *tert*-butyl alcohol at 30 °C. The spectra were taken as described in the legend to Figure 1, except that a Gaussian multiplication with Gaussian broadening of 0.05 Hz was applied. The remaining substrate [in (B)] is 85% $R_P$, and the product [in (A)] is completely racemic.

In a recent investigation, Cullis and Rous have attempted to increase the effective lifetime of monomeric metaphosphate by lowering the concentration of the trapping nucleophile in the medium.[37] The phospho group donor $P^1$-*O*-ethyl-$P^1$-thio-[$P^2$-$^{16}O$,$^{17}O$,$^{18}O$]pyrophosphate was labilized by S methylation in dichloromethane solution containing a primary alcohol. The phospho group transfer proceeded with partial racemization (30% inversion) at phosphorus. This result, while not consistent with a completely free metaphosphate species, does suggest that most of the phospho group transfers in this experiment involve a metaphosphate intermediate. In another study, Cullis and Nicholls have looked at the rate of positional isotope exchange in the reaction of adenosine 5′-[$\alpha,\beta$-$^{18}O$]diphosphate trianion in acetonitrile, in acetonitrile/*tert*-butyl alcohol, and in neat *tert*-butyl alcohol.[38] In each case, some scrambling of the $^{18}O$ label between the "bridge" and "nonbridge" positions was evident in the reisolated starting material, this finding being consistent with the transient formation of monomeric metaphosphate. It should be noted, however, that positional isotope exchange only requires rotation around one P–O bond within the solvent cage and that this reaction could proceed stereospecifically with retention or inversion of the configuration at the terminal (transferred) phospho group. Racemization of the transferred phospho group is, therefore, a more demanding requirement for monomeric metaphosphate. In any case, it is gratifying that the isotope exchange and stereochemical studies are in agreement.

The conclusion from the present work is that metaphosphate *can* be a viable, liberated, intermediate in the solvolytic reactions of phosphate monoesters. If the intermediate is trapped by ambient nucleophiles before the leaving group has diffused away, inversion of the configuration at phosphorus is seen, while if the nucleophilicity of the available nucleophiles is low, racemization of chiral phospho groups can be observed. In these cases, we must conclude that a metaphosphate intermediate is formed and that it survives long enough so that collapse to an acceptor nucleophile is equally probable from each face of the planar species.

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# A Theoretical Treatment of Nucleophilic Reactivity in Additions to Carbonyl Compounds. Role of the Vertical Ionization Energy

**Erwin Buncel,*[1a] Sason S. Shaik,*[1b] Ik-Hwan Um,[1a] and Saul Wolfe*[1a]**

*Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6, and the Department of Chemistry, Ben-Gurion University, Beer-Sheva 84105, Israel.* *Received July 21, 1986*

**Abstract:** In water solvent, experimental $\Delta G^{\ddagger}$ values for attack of X:$^-$ upon several esters correlate with the *vertical* ionization potentials of X:$^-$. Two kinds of nucleophiles are discerned in this way, delocalized nucleophiles ($AcO^-$, $N_3^-$, $NO_2^-$, etc.) exhibiting a larger slope than localized nucleophiles ($F^-$, $HO^-$, $CH_3O^-$, etc.). In terms of the state correlation diagram model, the existence of a $\Delta G^{\ddagger}$ versus IP(X$^-$)* correlation implies that an important aspect of the activation process is the single electron switch from X:$^-$ to the substrate that occurs during the nucleophilic attack.

The rationalization of nucleophilic reactivities has been a longstanding goal in physical organic chemistry.[2-4] Theoretical approaches to this problem have been found to be increasingly fruitful, but these have been largely limited to nucleophilic sub-

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