Novel Functionalized Acylphosphonates as Phosphonoformate Analogs

Alan R. Glabe, Katherine L. Sturgeon, Sally B. Ghizzoni, W. Kenneth Musker,* and Joyce N. Takahashi*,[1]

Department of Chemistry, University of California, Davis, California 95616

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Introduction

In 1978, B. Oberg reported that phosphonoformic acid ($O_2CPO_3^{3-}$, PFA) specifically inhibited cell-free DNA polymerase from herpes simplex virus (HSV). Since that time PFA and acylphosphonates have been shown to be polymerase inhibitors in avian myeloblastosis (AMS) and human immunodeficiency virus type 1 (HIV-1). PFA, **1**, is a potent inhibitor of the reverse transcriptase of HIV-1 and is the active ingredient in Foscarnet.[2] However, PFA's clinical value is limited by its toxic side effects which may arise from its high ionic character at physiologic pH.[3] Most modifications to PFA, such as the esterification of either the carboxylate or the phosphonate groups, have been designed to decrease PFA's highly ionic nature, but these changes did not produce a more potent antiviral agent.[4] We have used two other approaches which utilize hydroxamic acid derivatives of PFA[5] and acylphosphonic acids which resemble PFA homologs in that they bear an oxygen-containing group on the α-carbon atom. In this work we describe the synthesis of four novel α-substituted acylphosphonates (α-hydroxyacetyl)-phosphonate, **2**, (*trans*-2,3-epoxybutanoyl)phosphonate, **3**, (2,3-epoxypropanoyl)phosphonate, **4**, and (*cis*-2,3-epoxybutanoyl)phosphonate, **5**, designed to be less ionic than PFA while retaining the acylphosphonate moiety (Figure 1). Additionally, the three (epoxyacyl)phosphonates (**3**−**5**) were designed to be affinity labels for reverse transcriptase, the enzyme responsible for replicating HIV-1.[6] Since our preliminary report on *N*-hydroxyphosphonoformamide, **6**, the synthesis of this compound has been improved.[5]

Preparation of the functionalized acylphosphonates required three different synthetic schemes with the key and yield-limiting step in each scheme being the formation of the carbon−phosphorus bond. For this transformation we used a modified Michaelis−Arbuzov reaction,[7] the scope and utility of which we necessarily explored. In this modified version of the Michaelis−Arbuzov reaction, acylphosphonates result from the combination of a phosphorus-containing nucleophile and an acyl halide. α-Substituted acyl chlorides with bromo-, tributylsilyloxy-, or *trans*-epoxide substituents were successfully transformed, while those with *cis*-epoxides or mono substituted epoxide substituents were not. While PFA is stable as the trisodium salt, most acylphosphonic acids are most stable at about pH 6 and are isolated as the amine salt[8] and then, if necessary, converted to the sodium or potassium salt by ion exchange.

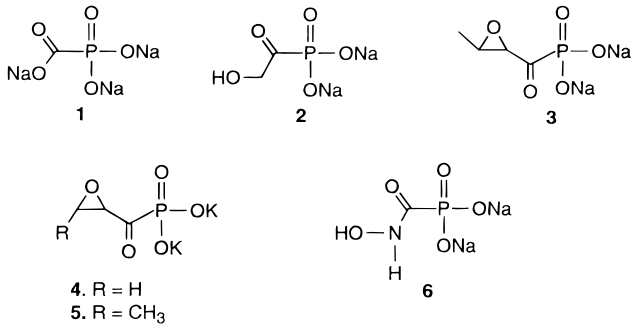

Figure 1.

Results and Discussion

The structure of (α-hydroxyacetyl)phosphonate, **2**, is a combination of glycolic acid and phosphonic acid. Since this transformation could not be accomplished directly by condensation, the hydroxy groups were protected by treating glycolic acid with a solution of 2 equiv of *tert*-butyldimethylsilyl chloride (TBDMSCl), imidazole (4 equiv), and DMF (Scheme 1).[9] After workup and removal of the volatiles under reduced pressure, *tert*-butyldimethylsilyl (*tert*-butyldimethylsilyloxy)acetate was obtained in 91% yield and converted to the acyl chloride by treatment with oxalyl chloride. The acyl chloride was then combined with tris(trimethylsilyl) phosphite to yield bis(trimethylsilyl) [(*tert*-butyldimethylsilyloxy)acetyl]-phosphonate. Deesterification of the silyl ester was achieved by dissolution in a methanol−pyridine solution. The *tert*-butyldimethylsilyl protecting group was removed by passing the pyridinium salt through H^+ Dowex and purified by passing pyridinium (α-hydroxyacetyl)phosphonate through Sephadex (Na^+) (67%).[10]

Since Breuer had already developed a protocol for the preparation of numerous acylphosphonates, including α,β-unsaturated acylphosphonates, the most facile route to **3** seemed to involve the epoxidation of dimethyl (*E*)-but-2-enoylphosphonate followed by deesterification.[8b] However, attempts to epoxidize the unsaturated acylphos-

(1) aka Joyce Takahashi Doi.

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(3) Seidel, E. A.; Koenig, S.; Polis, M. A. *AIDS* **1993**, *7*, 941−945.

(4) (a) Iyer, R. P.; Phillips, L. R.; Biddle, J. A.; Thakker, D. R.; Egan, W. *Tetrahedron Lett.* **1989**, *30*, 7141−7144. (b) Neto, C. C.; Stein, J. M.; Sarin, P. S.; Sun, D. K.; Bhongle, N. N.; Piralta, R. K.; Turcotte, J. G. *Biochem. Biophys. Res. Commun.* **1990**, *1*, 458−463.

(5) Doi, J. T.; Ma, Q.-F.; Rowley, G. L.; Kenyon, G. L. *Med. Chem. Res.* **1991**, *1*, 226−229. Correction in the RT assay solution reported in this paper: The oligo$(dT)_{12-18}$ primer-template concentration should be nM instead of mM.

(6) (a) Larder, B. A.; Purifoy, D. J. M.; Powell, K. L.; Darby, G. *Nature* **1987**, *327*, 716−717. (b) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and Co.: New York, 1985; pp 252−253. (c) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and Co.: New York, 1985; pp 422−423.

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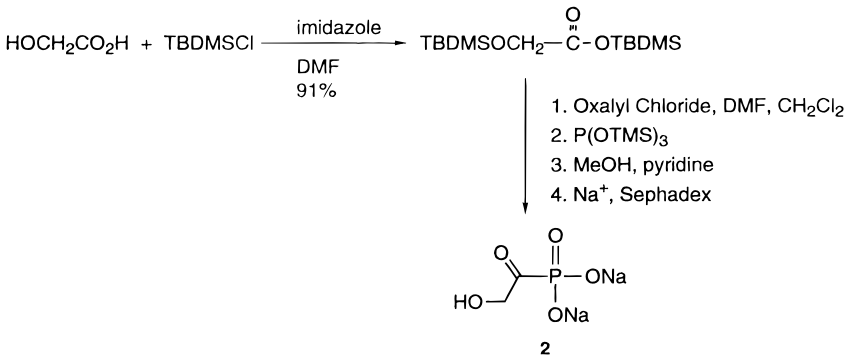
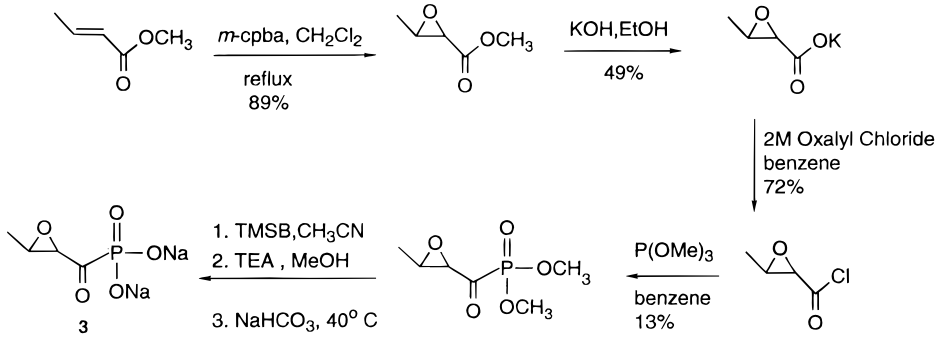
Scheme 1



Scheme 2



phonate with *m*-CPBA resulted in the formation of dimethyl (*E*)-but-2-enoyl phosphate presumably via a Baeyer−Villiger type rearrangement.[11] Since the use of other oxidants such as dimethyldioxirane[12] also proved to be ineffectual, an alternate scheme was devised.

The preparation of **3** began with the epoxidation of methyl crotonate (Scheme 2). Methyl *trans*-2,3-epoxybutanoate was obtained in 89% yield by refluxing methyl crotonate and *m*-CPBA in CH_2Cl_2 overnight. The ester was saponified with alcoholic KOH to produce the potassium salt in 49% yield.[13] The low yield probably resulted from the opening of the oxirane by hydroxide to either initiate polymerization or diol formation. After the salt was converted to the acyl chloride (72%) by treatment with oxalyl chloride, formation of dimethyl (*trans*-epoxybutanoyl)phosphonate was accomplished by adding 1 equiv of trimethyl phosphite to a solution of the acyl chloride in benzene. Purified phosphonate ester was obtained in low yield by Kugelrohr distillation (13%). A significant competing reaction to acylphosphonate formation was nucleophilic attack by trimethyl phosphite on the oxirane. This resulted in the production of dimethyl (*E*)-but-2-enoylphosphonate and trimethyl phosphate as suggested by the ^1H and ^{31}P NMR spectra of the crude product. The enoylphosphonate and trimethyl phosphate presumably arise from a mechanism similar to that of olefin and phosphine oxide formation in Wittig reactions. We attempted to reduce the amount of side product due to epoxide opening by using phosphites with increased bulk and found that triisopropyl and tris(trimethylsilyl) phosphites resulted in cleaner looking ^{31}P NMR spectra, but we were unable to further purify the corresponding acylphosphonates. Dimethyl acylphosphonates are readily deesterified by treatment with 2 equiv of trimethylsilyl bromide (TMSB).[14] However, the presence of an epoxide required the use of an additional 1 equiv of TMSB. The silicon of TMSB behaves as a Lewis acid which coordinates to the oxirane oxygen and further polarizes the C−O bonds to create a partial positive charge on the ring carbons to facilitate ring opening by bromide ion. Treatment of the demethylation product, bis(trimethylsilyl) [3-bromo-2-(trimethylsilyloxy)butanoyl]phosphonate, with a methanol−triethylamine solution produced triethylammonium (3-bromo-2-hydroxybutanoyl)phosphonate which was then converted without purification to **3** by stirring in a 40 °C aqueous solution of $NaHCO_3$ and NaOH (pH 9) for 40 min.[15] Because both the epoxide opening and reformation steps occur with inversion of configuration, the net result is retention of configuration. Since the epoxidation of methyl crotonate produces a racemic mixture, the final product, **3**, is racemic.

Initially, the protocol used for **3** was used for the preparation of **4** and **5**. However, when the epoxy acyl chlorides required for the syntheses of **4** and **5** (2,3-epoxypropanoyl chloride and *cis*-2,3-epoxybutanoyl chloride, respectively) were treated with either trimethyl phosphite or tris(trimethylsilyl) phosphite, no epoxy acylphosphonates were detected. Since the less stable *cis* and monosubstituted epoxides apparently do not withstand the Arbuzov reaction intact, the syntheses of **4** and **5** required a different approach (Scheme 3). (2*S*,3*R*)-2-Bromo-3-hydroxybutanoic acid was prepared in 72% yield by the nitrous acid deamination of L-threonine

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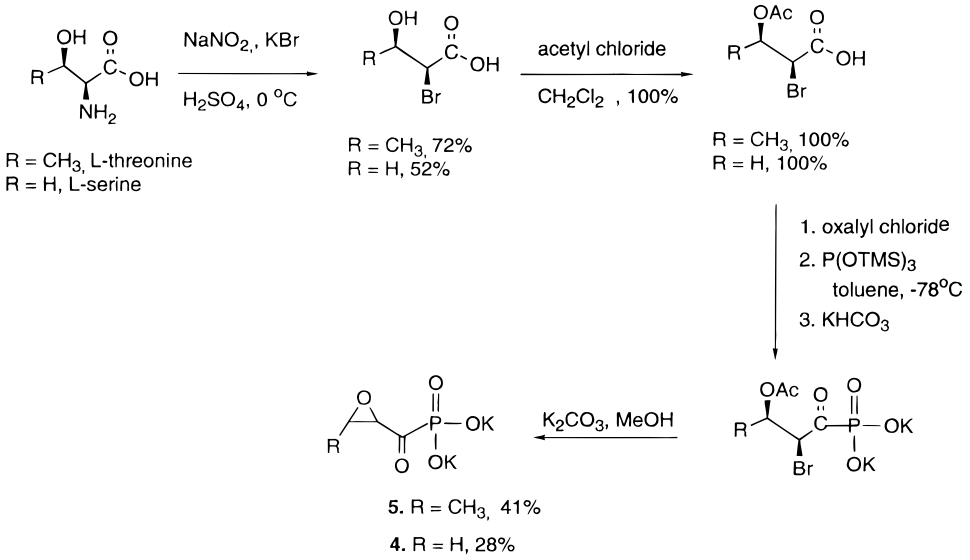
Scheme 3



using a solution of KBr, $NaNO_2$, and H_2SO_4.[16] While the nitrous acid deamination may also be accomplished using $NaNO_2$ and concd HCl to give (2*S*,3*R*)-2-chloro-3-hydroxybutanoic acid, higher yields of the bromo compound were obtained. Before preparing the acyl chloride, the hydroxyl group was protected as the acetate ester by treating the acid with a slight excess of acetyl chloride at ambient temperature. Removal of the volatiles under reduced pressure yielded (2*S*,3*R*)-2-bromo-3-(ethanoyloxy)butanoic acid in quantitative yield. Formation of the acyl chloride was affected by treating the acid with oxalyl chloride and removing the volatiles *in vacuo*. For the formation of the acylphosphonates, phosphites with varying steric bulk and nucleophilicity were tried before relying upon tris(trimethylsilyl) phosphite. Tris(trimethylsilyl) phosphite was added to the acyl chloride to form bis(trimethylsilyl) (2*S*,3*R*)-[2-bromo-3-(ethanoyloxy)butanoyl]phosphonate which was deesterified without isolation by treatment with $KHCO_3$ (aq) to give 40% potassium (2*S*,3*R*)-[2-bromo-3-(ethanoyloxy)butanoyl]-phosphonate. The final transformations, removal of the protecting group and epoxide formation, were completed in one step by use of a solution of water, methanol, and 1.5 equiv of K_2CO_3 at ambient temperature for 1 h to yield **5**.

The preparation of **4** was accomplished starting with L-serine and using the same methodology. The yields for the corresponding nitrous acid deamination (52%) of L-serine and the formation of potassium (*S*)-[2-bromo-3-(ethanoyloxy)propanoyl]phosphonate (21%) were lower than those obtained for similar steps in the preparation of **5**.

Compounds **3**−**5** all contain one or two chiral centers. While the method used to prepare **3** results in a racemic mixture, the method used to prepare **4** and **5** is stereoselective. By using Scheme 3 and starting with enantiomerically pure amino acids, it is possible to prepare any one of the stereoisomers of **3**−**5**.

We improved upon the method previously used by Doi to prepare **6**.[5] As in Doi's synthesis, we started by forming the carbon−phosphorus bond via a Michaelis−Arbuzov reaction. In the previous synthesis, this was accomplished by adding phenyl chlorothioformate to a cooled solution of bis(trimethylsilyl) phosphite, triethylamine, and anhydrous ethyl ether. Because the product, bis(trimethylsilyl) [(phenylthio)carbonyl]phosphonate, proved to be a potent allergen and vessicant, it was used without further purification. This led to decreased yields of the ensuing products. However, the use of methyl chlorothioformate in this synthesis resulted in bis(trimethylsilyl) [(methylthio)carbonyl]phosphonate, an easily handled, vacuum distillable oil which was obtained in high purity in 41% yield.[17] The hydroxamic acid was formed by adding a solution of *O*-(trimethylsilyl)hydroxylamine (1 equiv) and ethyl ether dropwise to bis(trimethylsilyl) [(methylthio)carbonyl]phosphonate dissolved in ethyl ether. Removal of the volatiles under reduced pressure yielded an oil which was then treated with an ice cold methanol−pyridine solution to remove the trimethylsilyl groups to give the pyridinium salt of **6** which was purified by passing the pyridinium salt through (Na^+) Sephadex.

All of the compounds are more effective inhibitors of recombinant HIV-1 RT P-66 from the yeast, *Saccharomyces cerevisiae*, than pyrophosphate, but less effective than PFA.[1] The IC_{50} values are **1**:**4**:**3**:**2**:**5**:fosfomycin = 0.3:170:200:240:700:>5000 μM. The antibiotic fosfomycin, (−)-(1*R*,2*S*)-(1,2-epoxypropyl)phosphonic acid, is included to show that the acyl group is a necessary part of more effective structures. The IC_{50} values were determined by use of solutions of varying concentrations of inhibitors in the enzyme assay.[5] Although these pyrophosphate analogs appear to be less effective than phosphonoformate as an inhibitor of HIV-1 RT P66, they may be effective inhibitors of other viruses.

Experimental Section

Negative ion fast atom bombardment (−FAB) high resolution mass spectra (HRMS) were obtained from Dr. Dan Jones at the Facility for Advanced Instrumentation, University of California, Davis. Acetonitrile and methylene chloride were distilled from CaH_2 and stored under N_2. Diethyl ether, benzene, and toluene were distilled from Na and stored under N_2. Bis(trimethylsilyl) phosphite was prepared by the method of Sekine, Okimoto,

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Yamada, and Hata.[18] All other reagents were available from commercial sources and used without further purification. Cation exchange column chromatography was performed using SP SEPHADEX C25 (washed with NaCl and deionized water) and DOWEX 50× 8-100 (washed with ethanol and deionized water).

Sodium α-Hydroxyacetylphosphonate (2). *tert*-Butyldimethylsilyl (*tert*-butyldimethylsilyloxy)acetate was prepared by the method of Wissner and Grudzinskas.[9] Glycolic acid (4.19 g, 0.055 mol) and *tert*-butyldimethylchlorosilane (17.72 g, 0.0117 mol) were stirred in 40 mL of dry DMF. Imidazole (15.62 g, 0.2295 mol) was added to the mixture and stirred under N_2 for 18 h. The mixture was poured into deionized water (approximately 250 mL) and extracted with petroleum ether (3 × 100 mL). The organic fractions were combined, washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to give 15.28 g (91%) of **2** as a white solid. This solid product was further dried over P_2O_5 under reduced pressure for 2 days prior to use. ^{1}H NMR ($CDCl_3$) δ 4.169 (s, 2H,OCH_2C), 0.898 (s, 18H, 2 $C(CH_3)_3$), 0.261 (s, 6H, $C(O)OSi(CH_3)_2$), 0.082 (s, 6H, $Si(CH_3)_2O$); (lit.[9] ^{1}H NMR ($CDCl_3$) δ 4.14 (s, 2), 0.87 (s, 18), 0.22 (s, 6), 0.04 (s, 6)). *tert*-Butyldimethylsilyl (*tert*-butyldimethylsilyloxy)acetate (2.01 g, 0.0066 mol) was dissolved in 10 mL of dry CH_2Cl_2 containing 4 drops of DMF. A solution of 4.09 mL 2 M oxalyl chloride/methylene chloride and 5.0 mL of dry methylene chloride was added dropwise under N_2 for a period of 40 min.[9] After stirring the solution at ambient temperature for 1 h, the volatiles (unreacted oxalyl chloride) were removed in vacuo to yield a yellow oily residue. The residue was mixed with 8.0 mL of dry benzene and cooled <0 °C before the dropwise addition of tris(trimethylsilyl)phosphite (2.2 mL, 0.0066 mol). After 15 min, the ice bath was removed and the mixture was stirred for a total of 1 h. The mixture was concentrated under vacuum, and then a solution of (0.53 mL, 0.0066 mol) pyridine and (1.34 mL, 0.033 mol) methanol was added dropwise to form a gooey white precipitate. This material was passed through a column of DOWEX (H^+) using cold deionized water as the eluent.[10] The resultant (hydroxyacetyl)-phosphonic acid was neutralized with pyridine before chromatographing on a Sephadex Na^+ column with deionized water. The eluent was lyophilized to give 0.24 g of **2** (67%). ^{1}H NMR (D_2O) δ 3.880 (t, J = 11 Hz), OCH_2); ^{31} P NMR (D_2O) δ 17.057 (t, J = 11 Hz); HRMS (−FAB) for $C_2H_4O_5P$, calcd 138.9784, obs 138.9784.

Sodium (*trans*-2,3-Epoxybutanoyl)phosphonate (3). Methyl *trans*-2,3-epoxybutanoate was prepared by the method of Danishefsky.[19] A solution of methyl crotonate (9.4 g, 94 mmol), 80% *m*-chloroperoxybenzoic acid (22.4 g, 104 mmol), and CH_2Cl_2 (70 mL) was refluxed overnight. The reaction mixture was suction filtered, and the solid (*m*-chlorobenzoic acid) was washed with cold CH_2Cl_2. The mother liquor was washed successively with 5% Na_2CO_3, water, and saturated NaCl, dried (Na_2SO_4), and concentrated *in vacuo* to give methyl *trans*-2,3-epoxybutanoate (9.7 g, 89%) as a liquid. ^{1}H NMR ($CDCl_3$) δ 1.40 (d, J = 5.1 Hz, 3 H, CH_3), 3.21 (d, J = 1.9 Hz, 1 H, CH), 3.25 (qd, J = 5.1, 1.9 Hz, 1 H, CH), 3.78 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 17.42, 52.65, 54.12, 54.83, 169.97.

Potassium *trans*-2,3-epoxybutanoate was prepared by a modification of the method of Kagan.[13] A cold solution of 85% KOH (4.7 g, 72 mmol) and ethanol (75 mL) was added in two parts to a 0 °C solution of methyl *trans*-2,3-epoxybutanoate (8.3 g, 72 mmol) and ethanol (25 mL), and the solution was stirred at room temperature overnight. The potassium salt was suction filtered, washed with cold ethanol, and recrystallized from ethanol to give potassium *trans*-2,3-epoxybutanoate (4.9 g, 49%) as a white solid. IR (KBr pellet) υ 1603 cm^{-1} (C=O str); ^{1}H NMR (D_2O) δ 1.35 (d, J = 5.1 Hz, 3 H, CH_3), 3.11 (qd, J = 5.1 Hz, 2.4 Hz, 1 H, CH), 3.17 (d, J = 2.4 Hz, 1 H, CH); ^{13}C NMR (D_2O) δ 16.63, 54.77, 56.77, 176.57.

trans-2,3-Epoxybutanoyl chloride was prepared by a modification of the method of Miyano.[20] A 2 M solution of oxalyl chloride (11.6 mL, 23 mmol) in CH_2Cl_2 was slowly added to a suspension of potassium *trans*-2,3-epoxybutanoate (3.0 g, 21 mmol) and benzene (50 mL) at room temperature. The mixture was stirred at room temperature for an additional 3.5 h after which the potassium chloride was removed by suction filtration and washed with cold benzene. Concentration of the filtrate *in vacuo* gave *trans*-2,3-epoxybutanoyl chloride (1.8 g, 72%) as a pale yellow liquid. IR (neat) υ 1816 cm^{-1} (C=O str); ^{1}H NMR ($CDCl_3$) δ 1.45 (d, J = 4.8 Hz, 3 H, CH_3), 3.34 (m, 2 H, CH); ^{13}C NMR ($CDCl_3$) δ 17.70, 54.34, 56.43, 165.41.

Trimethyl phosphite (4.6 mL, 40 mmol) was added dropwise under nitrogen atmosphere to a room temperature solution of *trans*-2,3-epoxybutanoyl chloride (6.1 g, 40 mmol) and benzene over a 30 min period. The solution was stirred at ambient temperature overnight. After the volatiles were removed under reduced pressure, dimethyl (*trans*-2,3-epoxybutanoyl)phosphonate was purified by vacuum distillation (Kugelrohr). Yield 13%, bp 50−100 °C/0.05 Torr; IR (neat) υ 1750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.6 Hz, 3 H, CH_3), 3.17 (m, 1 H, CH), 3.21 (m, 1 H, CH), 3.78 (d, J = 11.1 Hz, 6 H, $POCH_3$); ^{13}C NMR ($CDCl_3$) δ 17.86, 54.48, 55.07, 55.23, 171.86; ^{31}P NMR ($CDCl_3$) δ 3.62 (m), 10.8 Hz).

Trimethylsilyl bromide (930 mg, 6.2 mmol), 2-methyl-2-butene (2 drops), and dry acetonitrile (5 mL) were combined and swirled in an ice bath for 20 min before being slowly added (15 min) to a mixture of dimethyl (*trans*-2,3-epoxybutanoyl)phosphonate (374 mg, 1.9 mmol) and dry acetonitrile (3 mL). The resulting mixture was stirred for 3 h at room temperature while under nitrogen. After concentrating the reaction mixture *in vacuo*, a cold solution of methanol (10 mL) and triethylamine (2 mL) was added and swirled for 15 min. Concentration under reduced pressure yielded a light yellow residue, triethylammonium (3-bromo-2-hydroxybutanoyl)phosphonate (1.105 g) which was used without further purification. ^{31}P NMR (D_2O) δ 0.90, singlet.

Crude triethylammonium (3-bromo-2-hydroxybutanoyl)phosphonate (1.105 g) was dissolved in saturated sodium bicarbonate (2 mL). Enough 6 N sodium hydroxide was added to increase the pH to 9.0. The mixture was stirred in a 40 °C water bath for 40 min, acidified with 6 N HCl to pH 6, reduced i*n vacuo* to ca. 30% of its original volume, and chilled in an ice bath. A few drops of cold acetone were added to the cold solution, and the large amount of white crystalline solid that precipitated out of solution was removed by suction filtration. The aqueous filtrate was lyophilized to give **3** (310 mg, 97%) as a white solid. IR (KBr) υ 1625 cm^{-1} (C=O str); ^{1}H NMR (D_2O) δ 1.36 (d, J = 5.1 Hz, 3H, CH_3), 3.12 (qd, J = 5.1, 2.1 Hz, 1H, epoxy), 3.17 (d, J = 2.1 Hz, 1H, epoxy); ^{13}C NMR (D_2O) δ 16.68, 54.85, 56.86, 160.90; ^{31}P NMR (D_2O) δ 3.14 s; HRMS (−FAB) for $C_4H_6PO_5$, calcd 164.9953, obs 164.9980.

Potassium (*cis*-2,3-Epoxybutanoyl)phosphonate (5). (2*S*,3*R*)-2-Bromo-3-hydroxybutanoic acid was synthesized by a modification of the method of Izumiya.[21] Sodium nitrite (9.2 g, 133 mmol) was added in portions to a 0 °C solution of L-threonine (10.0 g, 84 mmol), KBr (35.0 g, 294 mmol), and 2.5 N H_2SO_4 (180 mL) at a rate such that the reaction temperature did not exceed 5 °C (1 h). The solution was stirred for an additional 2.5 h at 0 °C and extracted with diethyl ether (5 × 50 mL). The ether layer was dried over Na_2SO_4(s), concentrated in vacuo, and further dried under high vacuum for 24 h to give (2*S*,3*R*)-2-bromo-3-hydroxybutanoic acid as a yellow liquid (11.1 g, 72%). ^{1}H NMR (DMSO-d_6) δ 1.14 (d, J = 6.3 Hz, 3H, CH_3), 3.90 (quintet, J = 6.3 Hz, 1H, C*H*OH), 4.22 (d, J = 6.3 Hz, 1H, CHBr); ^{13}C NMR (DMSO-d_6) δ 19.57, 52.03, 67.01, 172.29.

Acetyl chloride (4.4 g, 55 mmol) was added in one portion to a cold solution of (2*S*,3*R*)-2-bromo-3-hydroxybutanoic acid (6.3 g, 45 mmol) and CH_2Cl_2 (40 mL). The ice bath was removed, and the solution was stirred at ambient temperature overnight while under nitrogen. The mixture was concentrated *in vacuo* for 24 h to give pure (2*S*,3*R*)-2-bromo-3-(ethanoyloxy)butanoic acid. Yield 100%; ^{1}H NMR ($CDCl_3$) δ 1.43 (d, J = 6.6 Hz, 3H, CH_3), 2.13 (s, 3H, $C(O)CH_3$), 4.39 (d, J = 6.3 Hz, 1H, CHBr), 5.35 (quintet, J = 6.3 Hz, 1H, C(O)CH), 11.01 (s, 1H, C(O)OH); ^{13}C NMR ($CDCl_3$) δ 17.48, 20.59, 47.70, 69.74, 170.47, 170.97.

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General Procedure for the Preparation of Potassium 2-Bromo-3-(ethanoyloxy)acyl]phosphonates. A 2 M solution of oxalyl chloride in CH_2Cl_2 (27 mL, 54 mmol) was added dropwise over 1.5 h to a stirred solution of 2-bromo-3-ethanoyloxy butanoic acid (45 mmol) and CH_2Cl_2 (50 mL) at ambient temperature under nitrogen. The mixture was refluxed overnight. Removal of the volatiles *in vacuo* (ca. 100 mmHg) yielded the acyl chloride as a yellow liquid. Toluene (100 mL) was added to the crude acyl chloride and the solution cooled to −78 °C. Tris-(trimethylsilyl) phosphite (12.0 mL 36 mmol) was added to the cold solution via dropping funnel over 2 h. The cold bath was removed, and a solution of $KHCO_3$ (11.3 g, 113 mmol) and water (40 mL) was slowly added with stirring. The layers were separated, and the organic layer was extracted with 20 mL of 5% $KHCO_3$. The aqueous fractions were combined, and if needed, adjusted to pH 6 with 6 N HCl, and lyophilized to give the crude product as a yellow solid. Purification was effected by triturating the crude product in methanol (0.2 g crude/mL methanol) for 30 min, removing the solid by gravity filtration, and concentrating the filtrate *in vacuo*.

Potassium (2*S*,3*R*)-[2-bromo-3-(ethanoyloxy)butanoyl]phosphonate was produced in 40% yield from the reaction of (2*S*,3*R*)-2-bromo-3-(ethanoyloxy)butanoic acid with oxalyl chloride followed by reaction of the acid chloride with tris(trimethylsilyl) phosphite. ^{1}H NMR (D_2O) δ 1.34 (d, J = 6.3 Hz, 3H, CH_3), 2.11 (s, 3H, C(O)CH_3), 4.35 (d, J = 5.7 Hz, 1H, CHBr), 5.27 (quintet, J = 6.1 Hz, 1H, C(O)OCH); ^{13}C NMR (D_2O) δ 16.93, 20.85, 48.76, 70.43, 171.05, 172.14; ^{31}P NMR (D_2O) δ 0.90, (s).

Potassium (2*S*,3*R*)-[2-bromo-3-(ethanoyloxy)butanoyl]phosphonate (3.0 g, 10.3 mmol) was dissolved in methanol (15 mL), and water (10 mL). Potassium carbonate (2.1 g, 15.5 mmol) was added, and the solution was stirred for 1 h at room temperature. The solution was acidified to pH 6 with 6 N HCl and concentrated under reduced pressure to give crude **5** as a yellow solid. The crude solid was triturated with methanol for 30 min and suction filtered to remove the solid. The filtrate was concentrated in vacuo to yield **5** as a pale yellow solid (692 mg, 41%): IR (KBr) υ 1629 cm^{-1} (C=O str); ^{1}H NMR (D_2O) δ 1.26 (d, J = 5.4 Hz, 3H, CH_3), 3.31 (quintet, J = 5.3 Hz, 1H, epoxy), 3.51 (d, J = 5.1 Hz, 1H, epoxy); ^{13}C NMR (D_2O) δ 16.92, 55.46, 57.15, 161.20; ^{31}P NMR (D_2O) δ 3.70, (s) ; HRMS (−FAB) for $C_4H_6PO_5$, calcd 164.9953, obs 164.9943.

Potassium (2,3-Epoxypropanoyl)phosphonate (4). (*S*)-2-Bromo-3-hydroxypropanoic acid was prepared by the same method as (2*S*,3*R*)-2-bromo-3-hydroxybutanoic acid using L-serine as starting material in a modification of the method of Izumiya.[21] Yield, 52%; ^{1}H NMR ($CDCl_3$) δ 3.92 (dd, J = 5.7, 11.3 Hz, 1H, C*H*OH), 4.02 (dd, J = 7.8, 11.3 Hz, 1H, C*H*OH), 4.33 (dd, J = 5.7, 7.8 Hz, 1H, C*H*Br); ^{13}C NMR ($CDCl_3$) δ 44.91, 63.04, 170.30.

(*S*)-2-Bromo-3-(ethanoyloxy)propanoic acid was prepared by the same method as (2*S*,3*R*)-2-bromo-3-(ethanoyloxy)butanoic acid by use of acetyl chloride on (*S*)-2-bromo-3-hydroxypropanoic acid. Yield 100%; ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3H, CH_3), 4.52 (m, 3H), 11.62 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 20.26, 40.18, 64.18, 170.62, 171.45.

Potassium (*S*)-[2-bromo-3-(ethanoyloxy)propanoyl]phosphonate was prepared in 21% yield from (*S*)-2-bromo-3-(ethanoyloxy)propanoic acid, oxalyl chloride, and tris(trimethylsilyl) phosphite, using the general procedure. ^{1}H NMR (D_2O) δ 2.149 (s, 3H, C(O)CH_3), 4.521 (m, 3H); ^{13}C NMR (D_2O) δ 20.09, 44.75, 65.89, 172.90, 173.06; ^{31}P NMR (D_2O) δ 1.45, (s).

Potassium (2,3-epoxypropanoyl)phosphonate, **4**, was prepared from potassium (*S*)-[2-bromo-3-(ethanoyloxy)propanoyl]phosphonate in the same manner as **5** to give **4** as a yellow solid (28%). IR (KBr) υ 1632 cm^{-1} (C=O str); ^{1}H NMR (D_2O) δ 2.81 (dd, J = 2.9, 5.7 Hz, 1H, epoxy), 2.97 (dd, J = 5.1, 5.7 Hz, 1H, epoxy), 3.40 (dd, J = 2.9, 5.7 Hz, 1H, epoxy); ^{13}C NMR (D_2O) δ 46.38, 49.80, 161.65; ^{31}P NMR (D_2O) δ 3.60, s. HRMS (−FAB) for $C_3H_4PO_5$, calcd 150.9796; obs 150.9794.

***N*-Hydroxyphosphonoformamide** (**6**) was prepared by a variation of method of Doi.[5] Bis(trimethylsilyl) [(methylthio)-carbonyl]phosphonate was prepared by the method of Chrzanowski, Han, and McIntosh.[17] Bis(trimethylsilyl)phosphonate (4.42 g, 0.0196 mol) was dissolved in 20 mL of anhydrous diethyl ether and cooled to <20 °C in an ice bath. Triethylamine (2.8 mL, 0.0201 mol) was added dropwise to the solution with stirring. Methyl chlorothioformate (1.52 mL, 0.0177 mol) was then added dropwise to the solution at <10 °C. After complete addition, the solution was allowed to come to room temperature and stirred for 16.5 h. Triethylamine hydrochloride was removed by filtration under N_2 and the filtrate concentrated under reduced pressure. The product was distilled under reduced pressure to afford 2.17 g (41%), bp 102−124 °C (2.25 mmHg) (lit.[17] bp 100−103 °C (0.25 mmHg)). ^{1}H NMR (C_6D_6) δ 2.217 (s, 3H, SCH_3), 0.392 (s, 18H, $2Si(CH_3)_3$); ^{31}P NMR ($CDCl_3$) δ −20.959 (s, 1). The [(methylthio)carbonyl]phosphonate (0.20 g, 6.7×10^{-4} mol) was dissolved in 5.0 mL of dry ethyl ether.

O-(Trimethylsilyl)hydroxylamine (0.050 mL, 6.7×10^{-4} mol) was mixed with 2 mL of dry diethyl ether and added dropwise to the [(methylthio)carbonyl]phosphonate under N_2 <20 °C and then stirred for 6 h. After concentrating the solution under reduced pressure to 1/3 original volume, the precipitate/liquid was centrifuged and separated. Further concentration of the liquid *in vacuo* afforded an oil to which a cold solution of pyridine (0.0247 mL, 3.08×10^{-4} mol) in 5 mL of methanol was added dropwise. The resulting solid/liquid was separated and the solid washed with 2 × 5 mL of methanol. Concentration of the methanol solution afforded an oily product which produced a solid upon trituration with diethyl ether (20 mL). The solid was chromatographed on Sephadex (Na^+) in a cold room (1.7 °C) with deionized water as the eluent. The first six fractions (approximately 5.5 mL/fraction) which contained product were combined and lyophilized to give 0.07 g (23%) of product. ^{31}P NMR (D_2O) δ −1.165 (s, 1) (lit.[5] ^{31}P (D_2O) δ −3.7). The ^{31}P chemical shift of the acyl phosphonates are dependent on the pH of the solution, e.g. the chemical shift of **6** was −0.926 ppm at pH 6.5 and −1.439 ppm at pH 5.

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