Supporting information for

The Reaction of HppE with Substrate Analogues: Evidence for Carbon-Phosphorus Bond Cleavage by a Carbocation Rearrangement

Wei-chen Chang, Steven O. Mansoorabadi, and Hung-wen Liu*

CONTENTS

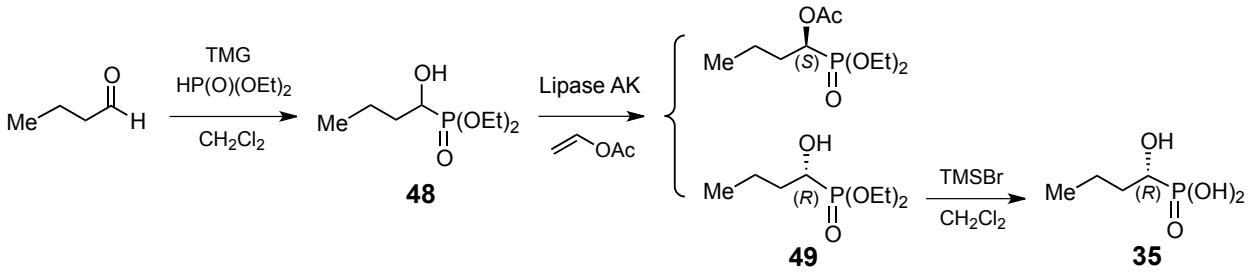
1. General

NMR spectra were recorded on Varian 400, 500 or 600 MHz spectrometers at the Nuclear Magnetic Resonance Facility of the Department of Chemistry and Biochemistry, The University of Texas at Austin. Chemical shifts (δ in ppm) are given relative to that of solvent ($CDCl_3$, DMSO, or D_2O), with coupling constants reported in Hertz (Hz). Analytical thin layer chromatography (TLC) was carried out on pre-coated TLC aluminum plates (silica gel, grade 60, F_{254}, 0.25 mm layer thickness) acquired from EMD Chemicals. Flash column chromatography was performed using silica gel (230-400 mesh, grade 60) obtained from Sorbent Technologies. Ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2•6H_2O$) and other reagents were purchased from Sigma-Aldrich. Lipase AK was obtained from Amano Enzyme Inc. All reagents and solvents were used directly as obtained from commercial sources unless otherwise noted. HppE was purified as previously described (*1*). Protein concentrations were determined by the Bradford assay (*2*) using bovine serum albumin as the standard.

2. Preparation of 1-Hydroxybutylphosphonic Acids

2.1.Preparation of (*R*)-(1-hydroxybutyl)phosphonic acid (35)

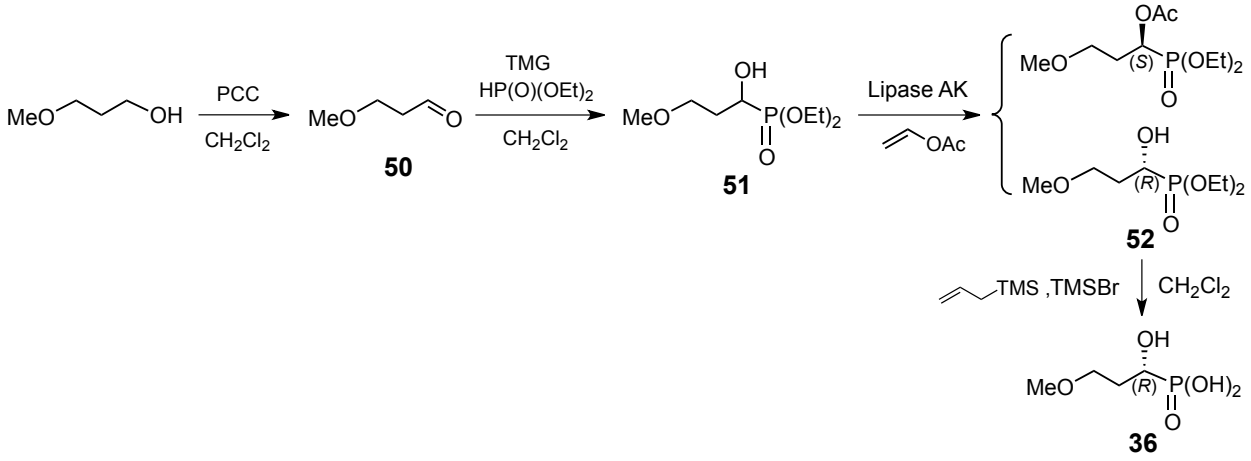
Diethyl (1-hydroxybutyl)phosphonate (48). Diethylphosphite (3.5 mL, 27 mmol) and a catalytic amount of tetramethylguanidine (TMG) were added to a solution of 1-butanal (2.7 mL, 30 mmol) in CH_2Cl_2 (30 mL) at room temperature (*3*). After the reaction was complete (~ 48 hr), the solvent and TMG were removed under reduced pressure to afford **48** as a colorless oil in 90% yield. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 6.8 Hz, 3H), 1.33 (t, J = 6.8 Hz, 6H), 1.40-1.47 (m, 1H), 1.62-1.74 (m, 3H), 3.85-3.88 (m, 1H), 4.14-4.18 (m, 4H); ^{31}P NMR ($CDCl_3$) δ 25.4.



(*R*)-Diethyl (1-hydroxybutyl)phosphonate (49). Lipase AK (3.5 g) and vinyl acetate (70 mL) were added to a solution of **48** (2.5 g, 12 mmol) in 70 mL diisopropyl ether. The reaction was stirred at room temperature and monitored by ^{1}H and ^{31}P NMR. Once the reaction reached roughly 50% conversion (~120 hr), the reaction mixture was filtered through a celite pad. Concentration under reduced pressure gave an oily residue that was purified by flash chromatography on silica gel using hexanes/ethyl acetate/triethyl amine (20:20:1) as the eluting solvent to give (*S*)-1-(diethoxyphosphoryl)butyl acetate (48%) and **49** (47%) as a colorless oil. The enantiomeric excess (e.e.) of **49** was determined to be 96% (*4-5*). The ^{1}H and ^{31}P NMR spectra are identical to those of **48**.

(*R*)-(1-Hydroxybutyl)phosphonic acid (35). TMSBr (1.3 g, 10 mmol) was added to a solution of **49** (420 mg, 2 mmol) in CH_2Cl_2 (20 mL) at room temperature, and the solution was stirred overnight (~14 hr). Solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL) and extracted with a 0.2 M NH_4OAc solution. The aqueous layer was collected and lyophilized to afford **35** as a white solid (84%). ^{1}H NMR (D_2O) δ 0.79 (t, J = 7.2 Hz, 3H), 1.21-1.29 (m, 1H), 1.30-1.49 (m, 2H), 1.54-1.58 (m, 1H), 3.54-3.58 (m, 1H); ^{31}P NMR (D_2O) δ 21.8; ^{13}C NMR (D_2O) δ 13.0, 18.7 (d, J = 13.2 Hz), 33.4, 68.4 (d, J = 156.2 Hz).

2.2. Synthesis of (*R*)-(1-hydroxy-3-methoxypropyl)phosphonic acid (36)



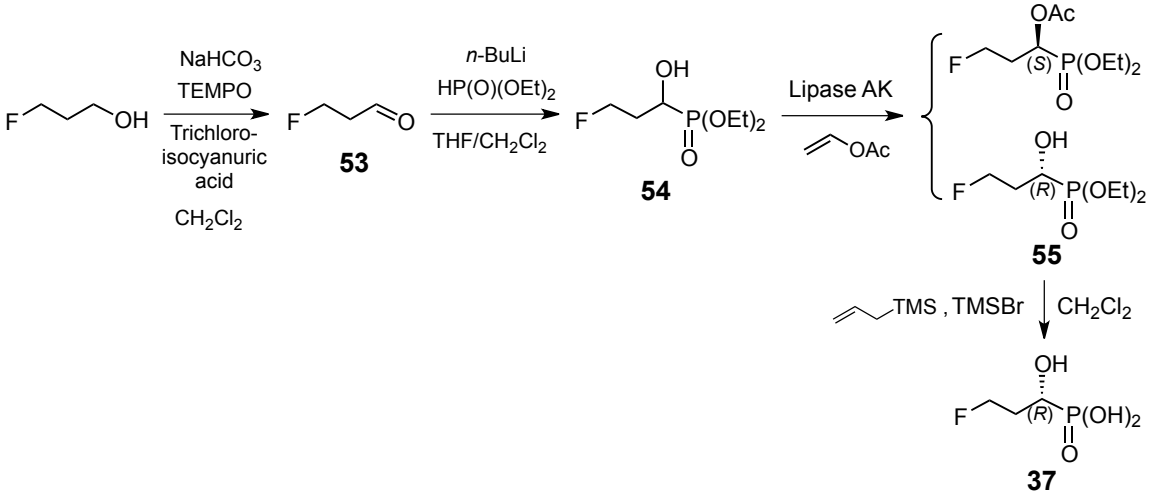
Diethyl (1-hydroxy-3-methoxypropyl)phosphonate (51). Pyridinium chlorochromate (1.4 g, 6.5 mmol) was added to a solution of 3-methoxypropanol (450 mg, 5.0 mmol) in CH_2Cl_2. The reaction was stirred at room temperature for 14 hr, and then the reaction mixture was filtered through a short silica pad. The combined filtrates were concentrated and the crude **50** was directly used in the next step (*6*). Diethylphosphite (5.5 mmol, 700 μL) and a catalytic amount of TMG were added to a solution of **50** in CH_2Cl_2 (10 mL) (*3*). After reaction was

complete (~ 48 hr), the solvent was removed under reduced pressure and the residue was subjected to flash silica gel chromatography using hexanes/ethyl acetate (1:2), and then pure ethyl acetate with 1% Et_3N as the eluents. The solvent of the collected fractions was removed to afford **51** as a colorless oil (40%, over two steps). ^{1}H NMR ($CDCl_3$) δ 1.29 (t, *J* = 7.2 Hz, 6H), 1.86-1.94 (m, 1H), 1.98-2.04 (m, 1H), 3.30 (s, 3H), 3.49-3.55 (m, 1H), 3.62-3.67 (m, 1H), 4.01-4.06 (m, 1H), 4.12 (dq, *J* = 1.2, 7.2 Hz, 4H); ^{31}P NMR ($CDCl_3$) δ 24.6; ^{13}C NMR ($CDCl_3$) δ 16.4 (d, *J* = 5.6 Hz) , 31.1, 58.6, 62.5 (d, *J* = 6.9 Hz), 65.6 (d, *J*= 164.2 Hz); 69.5 (d, *J* = 14.7 Hz).

(*R*)-Diethyl (1-hydroxy-3-methoxypropyl)phosphonate (52). Lipase AK (1.2 g) and vinyl acetate (30 mL) were added to a solution of **51** (1.0 g, 4.4 mmol) in 30 mL diisopropyl ether. The reaction was stirred at room temperature and the progress of the reaction was monitored by ^{1}H and ^{31}P NMR. Once the reaction reached roughly 50% conversion (~ 72 hr), the mixture was filtered through a silica pad. Concentration under reduced pressure gave an oily residue that was purified by flash chromatography on silica gel, eluting with hexanes/ethyl acetate (1:1) and then ethyl acetate/MeOH (20:1) with 1% Et_3N to give (*S*)-1-(diethoxyphosphoryl)-3-methoxy-propyl acetate (40%) and **52** (43%) as a colorless oil. The enantiomeric excess (e.e.) of **52** was determined to be 97% (*4-5*). The ^{1}H NMR and ^{31}P NMR spectra of **52** are the same as **51**.

(*R*)-(1-Hydroxy-3-methoxypropyl)phosphonic acid (36). To a solution of **52** (226 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added allylTMS (915 mg, 8 mmol) followed by TMSBr (650 mg, 5 mmol) at room temperature. The solution was stirred overnight. Solvent was removed under reduced pressure at the end of the reaction. The residue was dissolved in $CHCl_3$ (10 mL) and extracted with a 0.2 M NH_4OAc solution. The aqueous layer was collected and lyophilized to afford **36** as a white solid (85%). ^{1}H NMR (D_2O) δ 1.55-1.64 (m, 1H), 1.84-1.92 (m, 1H), 3.20 (s, 3H), 3.43-3.48 (m, 3H); ^{31}P NMR (D_2O) δ 18.6; ^{13}C NMR (D_2O) δ 31.9, 57.9, 67.4 (d, *J* = 150.5 Hz), 70.1 (d, *J* = 14.0 Hz).

2.3. Preparation of (*R*)-(3-fluoro-1-hydroxypropyl)phosphonic acid (37)



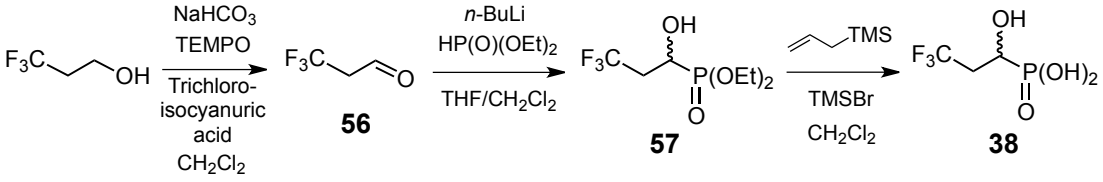
Diethyl (3-fluoro-1-hydroxypropyl)phosphonate (54). Trichloroisocyanuric acid (1.7 g, 7.3 mmol) was added to a solution of 3-fluoropropanol (1.6 g, 20 mmol), 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) (catalytic amount) and $NaHCO_3$ (1.7 g, 20 mmol) in CH_2Cl_2/water (30 mL/1 mL) at room temperature. The reaction was

stirred until the color changed from orange to pale yellow. The organic layer of the reaction mixture, which contained product **53**, was separated from the aqueous layer, dried over $MgSO_4$, and directly used in the next step without further purification (*7*). *n*-BuLi (2.5 M, 8.0 mL) was added to a solution of diethylphosphite (2.58 mL, 20 mmol) in THF (50 mL) at -78 ^oC. After 30 min, crude **53** (in 30 mL of CH_2Cl_2) was added to the reaction. After stirring at -78 ^oC for 30 min, the reaction mixture was quenched by adding brine (30 mL) and extracted with CH_2Cl_2 (20 mL × 5). The combined organic layers were concentrated and subjected to silica gel chromatography, eluting with hexanes/ethyl acetate (1:1), and then hexanes/ethyl acetate (1:2) containing 1% Et_3N. The solvent of the desired fractions was removed to afford **54** as a colorless oil (45%, over two steps). ^1H NMR ($CDCl_3$) δ 1.34 (t, *J* = 7.2 Hz, 6H), 1.91-2.00 (m, 1H), 2.11-2.24 (m, 1H), 4.06 (m, 1H), 4.17 (m, 4H), 4.51-4.80 (m, 2H); ^{31}P NMR ($CDCl_3$) δ 24.5; ^{19}F NMR ($CDCl_3$) δ -222.1 – -222.5 (m).

(*R*)-Diethyl (3-fluoro-1-hydroxypropyl)phosphonate (55). Lipase AK (1.2 g) and vinyl acetate (30 mL) were added to a solution of **54** (1.0 g, 4.4 mmol) in 30 mL diisopropyl ether. The reaction was stirred at room temperature and monitored using ^1H and ^{31}P NMR. Once the reaction reached roughly 50% conversion (~36 hr), the reaction mixture was filtered through a silica pad. Concentration under reduced pressure gave an oily residue that was purified by flash chromatography on silica gel with hexanes/ethyl acetate (1:1) containing 1% Et_3N as the eluting solvent to give (*S*)-1-(diethoxyphosphoryl)-3-fluoropropyl acetate (43%) and **55** (44%) as a colorless oil. The enantiomeric excess (e.e.) of **55** was determined to be 96% (*4-5*). The ^1H, ^{31}P, and ^{19}F NMR spectra are identical to those of **54**.

(*R*)-(3-Fluoro-1-hydroxypropyl)phosphonic acid (37). Compound **37** was obtained from **55** following the same procedure for the synthesis of **36** in 80% yield. ^1H NMR (D_2O) δ 1.69-1.80 (m, 1H), 2.01-2.09 (m, 1H), 3.65-3.38 (m, 1H); 4.48-4.50 (m, 1H), 4.55-4.58 (m, 1H); ^{31}P NMR (D_2O) δ 18.9; ^{19}F NMR (D_2O) δ -219.8 – -220.0 (m); ^{13}C NMR (D_2O) δ 32.5 (dd, *J* = 2.4, 19.2 Hz), 65.2 (d, *J* = 155.3 Hz); 82.0 (dd, *J* = 14.4, 157.8 Hz).

2.4. Preparation of (3,3,3-trifluoro-1-hydroxypropyl)phosphonic acid (38)

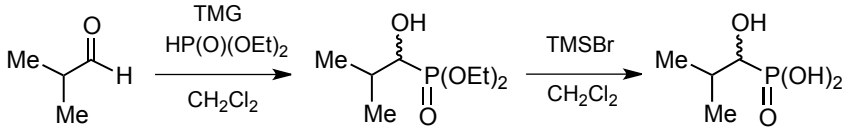


Diethyl (3,3,3-trifluoro-1-hydroxypropyl)phosphonate (57). Trichloroisocyanuric acid (1.7 g, 7.3 mmol) was added to a solution of 3,3,3-trifluoropropanol (2.3 g, 20 mmol), TEMPO (catalytic amount), and $NaHCO_3$ (1.7 g, 20 mmol) in CH_2Cl_2/water (30 mL/1 mL) at room temperature. The reaction was stirred until the color changed from orange to pale yellow, and then the organic layer containing product **56** was separated from the aqueous layer (*7*). The crude product **56** in CH_2Cl_2 was dried over $MgSO_4$ and used directly in the next step. *n*-BuLi (2.5 M, 8.0 mL) was added to a solution of diethylphosphite (2.58 mL , 20 mmol) in THF (60 mL) at -78

^{o}C. After 30 min, crude **56** (in 30 mL of CH_2Cl_2) was added to the reaction mixture. After stirring at -78 ^{o}C for 30 min, the reaction was quenched by the addition of brine (30 mL) and extracted with ethyl acetate (20 mL × 4). The combined organic layers were evaporated and subjected to silica gel chromatography using hexanes/ethyl acetate (1:1) containing 1% Et_3N as the eluent. The solvent of the pooled fractions was removed to afford **57** as a colorless oil (55% yield, over two steps).^{1}H NMR ($CDCl_3$) δ 1.31 (dt, *J* = 4.2, 7.2 Hz, 6H), 2.46-2.54 (m, 2H), 4.13-4.21 (m, 5H), 5.09 (broad s, exchangeable, 1H); ^{31}P NMR ($CDCl_3$) δ 21.6 (q, *J* = 3.2 Hz); ^{19}F NMR ($CDCl_3$) δ -64.1 (dt, *J* = 3.2, 10.7 Hz); ^{13}C NMR ($CDCl_3$) δ 16.3 (d, *J* = 5.4 Hz), 36.0 (dq, *J* = 3.8, 28.8 Hz), 62.3 (dq, *J* = 168.8, 2.9 Hz), 63.1 (d, 7.2 Hz), 63.4 (d, 7.1 Hz), 126.1 (dq, *J* = 23.7, 276.0 Hz).

(3,3,3-Trifluoro-1-hydroxypropyl)phosphonic acid (38). To a solution of **57** (106 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) was added allylTMS (510 μL, 3.2 mmol) followed by TMSBr (262 μL, 2 mmol) at room temperature, and the solution was stirred overnight. Solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (5 mL) and extracted with a 0.2 M NH_4OAc solution. The aqueous layer was collected and lyophilized to afford **38** as a white solid (85%).^{1}H NMR (D_2O) δ 2.30-2.39 (m ,1H), 2.44-2.51 (m, 1H), 3.89 (dt, *J* = 1.2, 10.8 Hz, 1H); ^{31}P NMR (D_2O) δ 16.6; ^{19}F NMR (D_2O) δ -63.8 (dt, *J* = 2.8, 11.3 Hz); ^{13}C NMR (D_2O) δ 35.9 (dq, *J* = 4.4, 27.3 Hz), 63.5 (d, *J* = 156.9 Hz); 126.8 (dq, *J* =25.7, 275.4 Hz).

2.5. Preparation of (1-hydroxy-2-methylpropyl)phosphonic acid (43)



58 **43**

Diethyl (1-hydroxyethyl)phosphonate (58). Compound **58** was synthesized using the same procedure for the preparation of **48**, except that isopropyl aldehyde was used as the starting material. The yield of the reaction was 92%. ^{1}H NMR ($CDCl_3$) δ 1.05 (d, *J* = 6.8 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 1.32 (t, *J* = 6.8 Hz, 6H), 2.04-2.11 (m, 1H), 3.63 (dd, *J* = 6.0, 6.4 Hz, 1H), 4.13-4.17 (m, 4H); ^{31}P NMR ($CDCl_3$) δ 25.1.

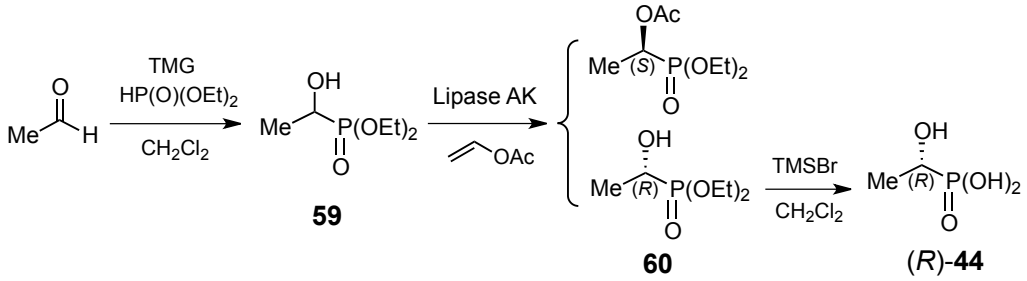
(1-Hydroxy-2-methylpropyl)phosphonic acid (43). Following the same procedure for the synthesis of **35**, compound **43** was obtained in 88% yield using **58** as the starting material. ^{1}H NMR (D_2O) δ 0.83 (d, *J* = 6.6 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 3H), 1.79-1.86 (m, 1H), 3.23 (dd, *J* = 6.0, 8.4 Hz, 1H) ; ^{31}P NMR (D_2O) δ 19.6; ^{13}C NMR (D_2O) δ 17.5 (d, *J* = 6.9 Hz), 20.0 (d, *J* = 8.6 Hz), 29.9 (d, *J* = 2.1 Hz), 75.3 (d, *J* = 148.4 Hz).

2.6. Preparation of (*R*)-(1-hydroxyethyl)phosphonic acid ((*R*)-44)

Diethyl (1-hydroxyethyl)phosphonate (59). Following the same procedure for the synthesis of **48,** compound **59** was obtained as a colorless oil (90%). ^{1}H NMR ($CDCl_3$) δ 1.33 (dt, *J* = 2.0, 6.8 Hz, 6H), 1.43 (dd, *J* = 6.8, 17.6 Hz, 3H), 3.08 (broad s, 1H, exchangeable), 4.00-4.06 (m, 1H), 4.13-4.22 (m, 4H); ^{31}P NMR ($CDCl_3$) δ 25.7.

(*R*)-Diethyl (1-hydroxyethyl)phosphonate (60). Lipase AK (3.5 g) and vinyl acetate (70 mL) were added to a solution of **59** (2.40 g, 13.2 mmol) in 70 mL diisopropyl ether. The reaction was stirred at room temperature and monitored by ^{1}H and ^{31}P NMR. Once the reaction reached roughly 50% conversion (~ 96 hr), the reaction mixture

was filtered through a celite pad and the filtrate concentrated under reduced pressure. The oily residue was purified by flash chromatography on silica gel with hexanes/ethyl acetate/triethyl amine (20:20:1) as the eluting solvent to give (*S*)-1-(diethoxyphosphoryl)ethyl acetate (45%) and **60** (45%) as colorless oil. The enantiomeric excess (e.e.) of **60** was determined to be 95% (*4-5*). The ^{1}H and ^{31}P NMR spectra of **60** are identical to those of **59**.



(*R*)-(1-Hydroxyethyl)phosphonic acid ((*R*)-44). Following the same procedure for the synthesis of **35**, compound (*R*)-**44** was obtained from **60** in 80% yield as a white solid. ^{1}H NMR (D_2O) δ 1.12 (dd, J = 7.2, 14.4 Hz, 3H), 3.53-3.58 (m, 1H); ^{31}P NMR (D_2O) δ 19.6; ^{13}C NMR (D_2O) δ 17.7, 66.3 (d, J = 150.2 Hz).

3. NMR Studies of the Reactions of HppE with 1-Hydroxyalkylphosphonic Acid Derivatives

Reaction mixtures containing 0.25 mM HppE, 0.25 mM $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, 7.5 mM FMN, 25 mM substrate analogue (**35**, **36**, **37**, **38**, **43** or **44**), and 25 mM NADH in 700 μL of 20 mM Tris buffer (pH 7.5) were prepared and subjected to ^{1}H NMR analysis (using a 500 or 600 MHz spectrometer). The NMR spectra were recorded using selective pre-saturation of the water signal with a 2 s pre-saturation interval. The lock signal was dimethyl-d_6 sulfoxide (DMSO-d_6, 30 μL). Chemical shifts were calibrated according to the DMSO-d_6 signal at δ 2.49. The enzymatic reactions were initiated by the addition of reconstituted HppE. The ^{1}H NMR spectra were recorded after aeration of the reaction mixture by bubbling of air through the solution with gentle pipetting. Samples derived from **37** and **38** were also subjected to ^{19}F NMR analysis.

4. Density Functional Theory Calculations

DFT calculations were performed to obtain estimates for the C2-H bond dissociation energies (BDEs) of the (*R*)-1-HPP substrate analogues and the ionization energies (IEs) of the corresponding substrate radicals. Geometry optimizations were performed using Becke-style three-parameter DFT with the Lee-Yang-Parr correlation functional (B3LYP) and Pople's diffuse polarized triple-ζ 6-311+G(d,p) basis set (as implemented in Gaussian 98 with the Opt=Tight and Int=Ultrafine keywords) (*8*), starting from the geometry of (*R*)-1-HPP bound to HppE (*9*). Vibrational frequency calculations were then performed on the optimized geometries at 25 °C and 1 atm, using the same level of theory and a scale factor of 0.9877 to correct zero-point vibrational energies (*10*). The IEs were obtained as the difference in electronic energy of the cation and radical species via single-point energy

calculations using the optimized geometries of the radicals.

5. References

1. Liu, P. *et al.* "Biochemical and spectroscopic studies on (*S*)-2-hydroxypropylphosphonic acid epoxidase: a novel mononuclear non-heme iron enzyme." *Biochemistry* **2003**, *42*, 11577-11586.
2. Bradford, M. M. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." *Anal. Biochem.* **1976**, *72*, 248-254.
3. Simoni, D.; Invidiata, F. P.; Manferdini, M.; Lampronti, I.; Rondanin, R.; Roberti, M.; Pollini, G. P. "Tetramethylguanidine (TMG)-catalyzed addition of dialkyl phosphites to alpha,beta-unsaturated carbonyl compounds, alkenenitriles, aldehydes, ketones and imines." *Tetrahedron Lett.* **1998**, *39*, 7615-7618.
4. Żymańczyk-Duda, E.; Skwarczyński, M.; Lejczak, B.; Kafarski, P. "Accurate assay of enantiopurity of 1-hydroxy- and 2-hydroxyalkylphosphonate esters." *Tetrahedron-Asymmetry* **1996**, *7*, 1277-1280.
5. Skwarczyński, M.; Lejczak, B.; Kafarski, P. "Enantioselective hydrolysis of 1-butyryloxyalkylphosphonates by lipolytic microorganisms: *Pseudomonas fluorescens* and *Penicillium citrinum*." *Chirality* **1999**, *11*, 109-114.
6. Juric, S.; Kronja, O. "Reactivity of some tertiary chlorides with olefinic and methoxy neighboring groups: case of extended *pi*, *n*-participation." *J. Phys. Org. Chem.* **2002**, *15*, 556-560.
7. Ashley, G. W.; Burlingame, M.; Desai, R.; Fu, H.; Leaf, T.; Licari, P. J.; Tran, C.; Abbanat, D.; Bush, K.; Macielag, M. "Preparation of erythromycin analogs having functional groups at C-15." *J. Antibiot. (Tokyo)* **2006**, *59*, 392-401.
8. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc. Pittsburgh, PA: 1998.
9. Chang, W.-c.; Dey, M.; Liu, P.; Mansoorabadi, S. O.; Moon, S.-J.; Zhao, K. Z.; Drennan, C. L.; Liu, H.-w. "Mechanistic studies of an unprecedented enzyme-catalyzed 1,2-phosphono migration reaction." *Nature* **2013**, *496*, 114-118.
10. Andersson M. P.; Uvdal P. "New scale factors for harmonic vibrational frequencies using the B3LYP density functional method with the triple-zeta basis set 6-311+G(d,p)." *J. Phys. Chem. A* **2005**, *109*, 2937–2941.

6. Figures S1 and S2

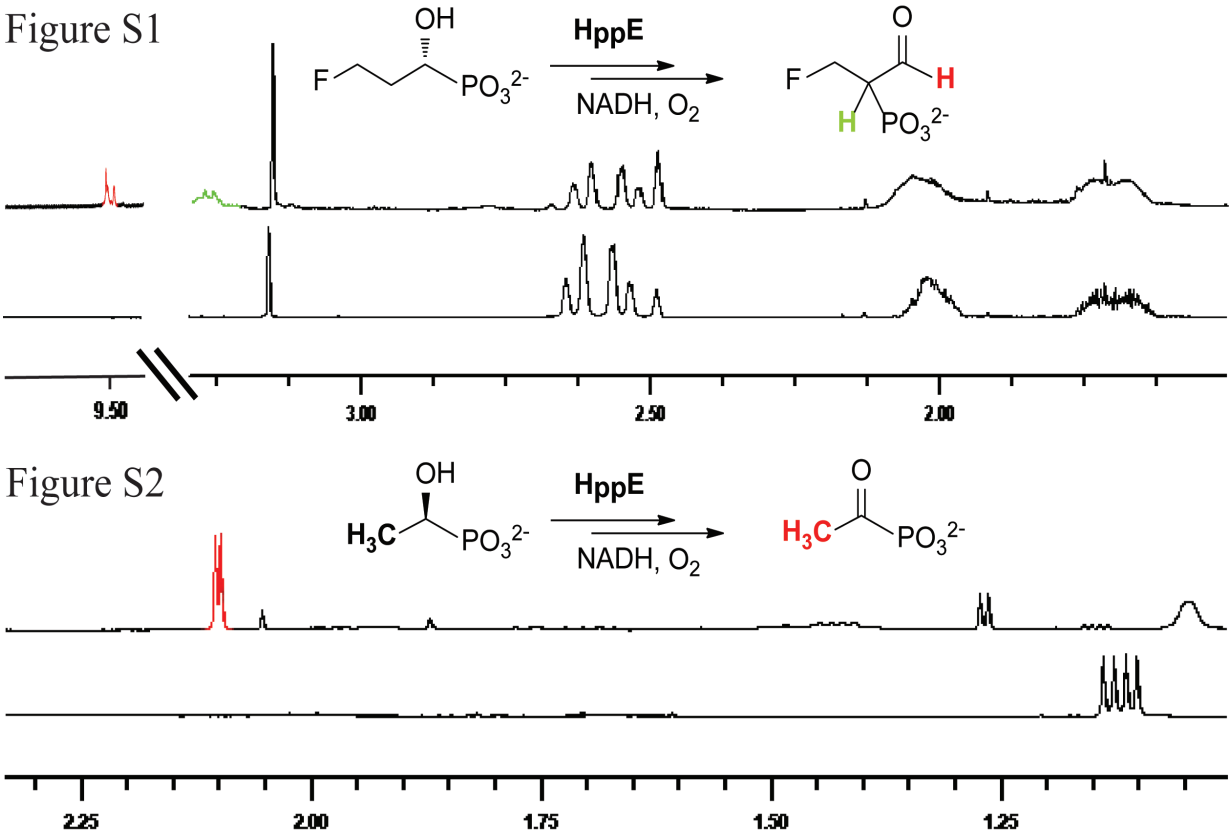


Figure S1 and S2. ^{1}H NMR assays of HppE with substrate analogues: (S1) **37**, and (S2) **(*S*)-44**. The bottom trace is the spectrum taken 3 min after mixing HppE with the substrate analogue, and the top trace is taken 27 min after mixing. The peak at δ 2.49 is from DMSO-d_6, and those centered between δ 2.5-2.7 are from NADH. The peak at δ ~3.1 is the residual peak of Tris buffer. The NMR signals and the contributing proton(s) are color-coded.

Figure S3

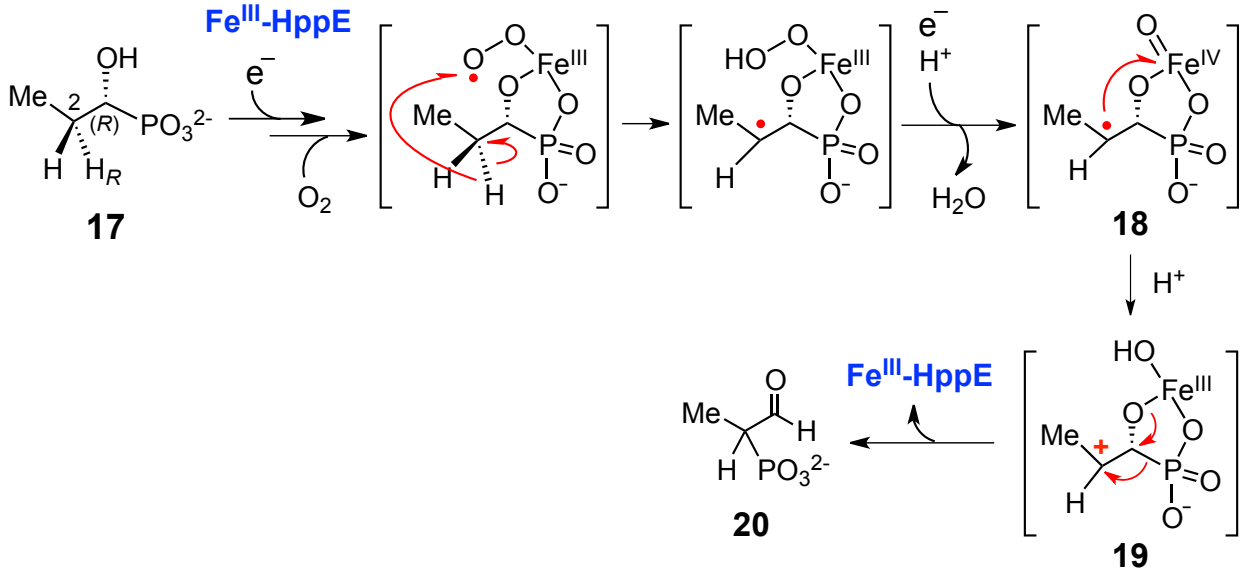


Figure S3. Proposed mechanism for the HppE-catalyzed 1,2-phosphono migration reaction with (*R*)-1-HPP (**17**).