

## SUPPORTING INFORMATION

### Evidence for Radical-Mediated Catalysis by HppE – A Study Using Cyclopropyl and Methylenecyclopropyl Substrate Analogues

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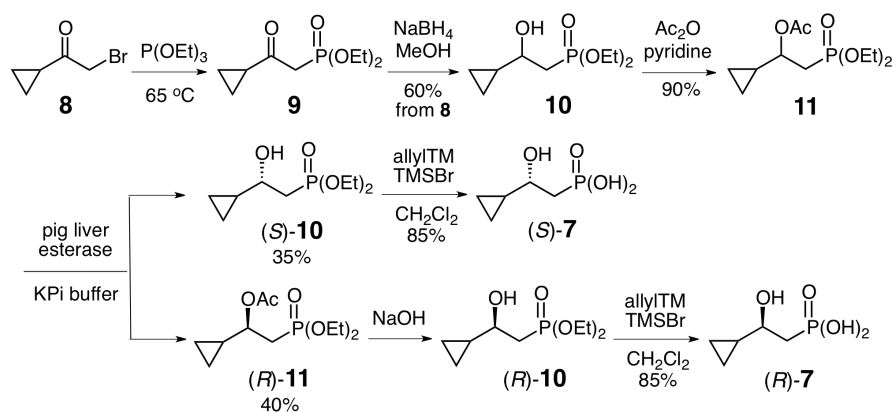
#### **1. General information**

Apo-HppE was expressed and purified as previously described.<sup>1</sup> The enzyme was reconstituted with one equivalent of Fe(II)(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O before use in assays.

Compounds **8**<sup>2</sup> and **17**<sup>3</sup> were prepared as previously described. Tetrahydrofuran (THF) and dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) were distilled under a N<sub>2</sub> atmosphere from Na/benzophenone and CaH<sub>2</sub>, respectively, prior to use. Other dry solvents were purchased from commercial suppliers without further purification. All reagents were used directly as obtained commercially unless otherwise noted. NMR spectra of the synthetic samples were recorded on Varian NMR spectrometers operating at 300, 400, 500, or 600 MHz in the NMR facility of the Department of Chemistry & Biochemistry of the University of Texas at Austin. Chemical shifts (δ in ppm) are reported relative to that of the solvent peak (CDCl<sub>3</sub> or D<sub>2</sub>O), with coupling constants given in Hertz (Hz). High-resolution mass spectral (HRMS) analyses of all synthesized compounds were carried out at the Mass Spectrometry Facility (MSF) of the Department of Chemistry & Biochemistry of the University of Texas at Austin. High-resolution mass spectrometry (HRMS) analyses of all enzyme reaction products were carried out using a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR-MS) at the Department of Chemistry, Texas A&M University.

## 2. Chemical syntheses of compounds 7, 15, 16, and 24.

### 2.1. Chemical synthesis of compounds (*S*)- and (*R*)-7.



**Scheme S1.** Synthetic scheme for the preparation of (*S*)- and (*R*)-7.

**Diethyl (2-cyclopropyl-2-hydroxyethyl)phosphonate (10).**<sup>4</sup> A mixture of **8** (728 mg, 4.5 mmol) and P(OEt)<sub>3</sub> (830 mg, 5.0 mmol) was stirred under argon at 65 °C for 12 h. The mixture was subjected to high vacuum evaporation using an oil pump to remove the unreacted P(OEt)<sub>3</sub>. The resulting colorless oil containing mostly compound **9** was used directly for the next reaction without further purification.

To a stirred solution of **9** (850 mg, 3.86 mmol) in MeOH (20 mL) was added NaBH<sub>4</sub> (160 mg, 4.2 mmol) at room temperature, and the resulting mixture was stirred for 1 h. After drop-wise addition of acetone (2 mL) to quench the reaction, water (10 mL) and ethyl acetate (40 mL) were added. The organic layer was separated, washed with brine (20 mL), dried over MgSO<sub>4</sub>, and concentrated to dryness. The crude residue was re-dissolved in ethyl acetate and purified using flash column chromatography (ethyl acetate) to afford **10** as a colorless oil (600 mg, 60% from **8**). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.10-4.15 (m, 4H), 3.27-3.35 (m, 1H), 2.10-2.16 (m, 2H), 1.33 (t, *J* = 6.8 Hz, 6H), 0.97-1.01 (m, 1H), 0.40-0.57 (m, 3H), 0.23 (m, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 70.5, 61.5 (d, *J* = 6.5 Hz), 61.4 (d, *J* = 6.5 Hz), 33.3 (d, *J* = 137.0 Hz), 18.1 (d, *J* = 18.8 Hz), 16.0 (d, *J* = 5.6 Hz), 3.0, 1.9; <sup>31</sup>P NMR (161.8 MHz, CDCl<sub>3</sub>) δ 30.1.

**1-Cyclopropyl-2-(diethoxyphosphoryl)ethyl acetate (11).** To a stirred solution of **10** (4.4 g, 20.0 mmol) in pyridine (10 mL) was added acetic anhydride (10 mL) at room temperature. Stirring was continued for 14 h, then ice-water (100 mL) was added in one portion to quench the reaction. After stirring for another 30 min, the reaction mixture was extracted with ethyl acetate (30 mL × 5). The combined organic layers were washed with brine (20 mL), dried over MgSO<sub>4</sub>, and concentrated to dryness. The crude residue was redissolved in ethyl acetate and purified using flash column chromatography (hexanes : ethyl acetate : Et<sub>3</sub>N = 1 : 1 : 0.05) to afford **11** as a colorless liquid (4.75 g, 90%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.50-4.59 (m, 1H), 4.05-4.14 (m, 4H), 2.15-2.30 (m, 2H), 2.06 (s, 3H), 1.32 (t, *J* = 7.2 Hz, 6H), 1.12-1.18 (m, 1H), 0.45-0.61 (m, 3H), 0.31-0.36 (m, 1H); <sup>13</sup>C NMR (100 MHz,

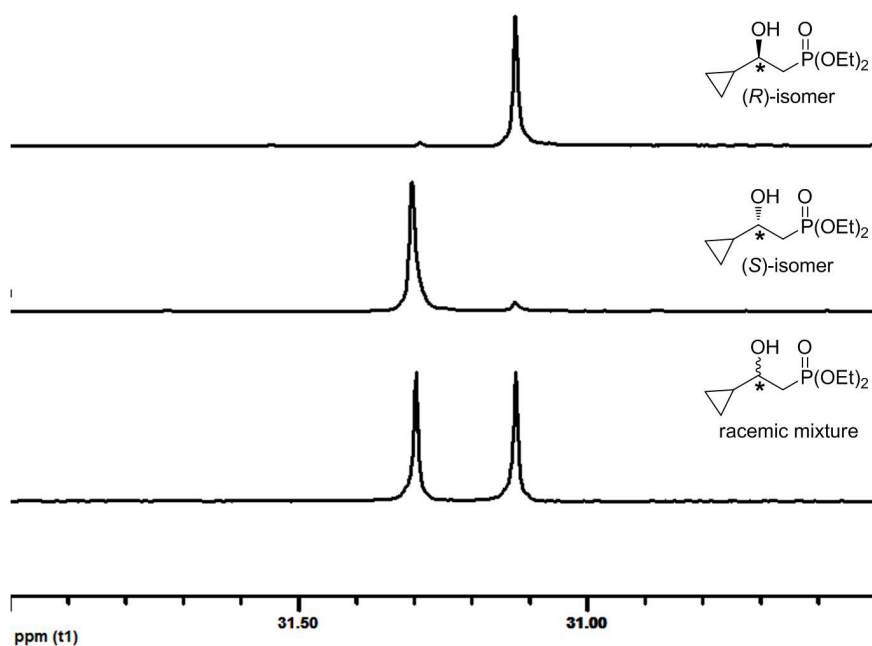
CDCl<sub>3</sub>) δ 170.1, 73.0, 61.7 (d, *J* = 6.5 Hz), 61.6 (d, *J* = 6.5 Hz), 31.5 (d, *J* = 140.5 Hz), 16.4 (d, *J* = 5.9 Hz), 16.2 (d, *J* = 12.5 Hz), 3.8, 3.4; <sup>31</sup>P NMR (161.8 MHz, CDCl<sub>3</sub>) δ 27.8; HRMS-Cl calc. for C<sub>11</sub>H<sub>22</sub>O<sub>5</sub>P (M+H)<sup>+</sup> 265.1205, found 265.1208.

**(*R*)-1-Cyclopropyl-2-(diethoxyphosphoryl)ethyl acetate ((*R*)-11) and (*S*)-diethyl (2-cyclopropyl-2-hydroxyethyl)phosphonate ((*S*)-10).** To a solution of **10** (1.0 g, 3.85 mmol) in potassium phosphate buffer (100 mL, 20 mM, pH 7.0) was added pig liver esterase (PLE, E.C. 3.1.1.1, Sigma-Aldrich, 200 mg). The mixture was stirred at room temperature. The progress of the reaction was followed using <sup>1</sup>H NMR and TLC until ~50% **10** was converted (~ 18 h). NaCl was then added to the reaction solution to saturation, and the resulting mixture was extracted with ethyl acetate (30 mL × 8). The combined organic layers were washed with brine (20 mL), dried over MgSO<sub>4</sub>, and concentrated to dryness. The crude residue was redissolved in ethyl acetate and purified using flash column chromatography (hexanes : ethyl acetate : Et<sub>3</sub>N = 1 : 1 : 0.05) to afford pure **10** and **11**. The above procedure was repeated three times to give (*S*)-**10** (0.30 g, 84% *ee*) in 35% yield, and (*R*)-**11** (0.40 g) in 40% yield. The <sup>1</sup>H and <sup>31</sup>P-NMR spectra of (*S*)-**10** and (*R*)-**11** are identical to those of (*rac*)-**10** and (*rac*)-**11**, respectively<sup>4,5</sup>.

**(*R*)-Diethyl (2-cyclopropyl-2-hydroxyethyl)phosphonate ((*R*)-10).** To a stirring solution of (*R*)-**11** (530 mg, 2.0 mmol) in water (10 mL) was added NaOH (3.0 mL, 1.0 M) at 0 °C. The reaction mixture was allowed to warm to room temperature slowly and stirred for 24 h. NaCl was added to the reaction solution until saturation and the mixture was extracted with ethyl acetate (10 mL × 5). The combined organic layers were washed with brine (20 mL), dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. Purification using flash column chromatography (hexanes : ethyl acetate : Et<sub>3</sub>N = 1 : 1 : 0.05) afforded (*R*)-**10** as a colorless oil (377 mg, 85%, >90% *ee*). The <sup>1</sup>H and <sup>31</sup>P NMR spectra of (*R*)-**10** are identical to those of (*rac*)-**10**.

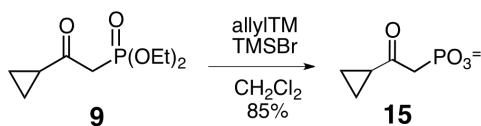
**(*S*)-(2-Cyclopropyl-2-hydroxyethyl)phosphonic acid ((*S*)-7).** To a stirred solution of (*S*)-**10** (444 mg, 2.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added allylTMS (2.5 mL, 16.0 mmol) and TMSBr (1.3 mL, 10.0 mmol). The reaction was stirred at room temperature for 12 h. The reaction mixture was then concentrated under reduced pressure. The residue was redissolved in CHCl<sub>3</sub> (6 mL) and extracted with 0.5 M NH<sub>4</sub>HCO<sub>3</sub> (8 mL × 2). The aqueous extracts were pooled and lyophilized to afford (*S*)-**7** as a white solid (225 mg, 85%). <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 2.92-2.98 (m, 1H); 1.60-1.80 (m, 2H), 0.70-0.76 (m, 1H), 0.25-0.32 (m, 2H), 0.02-0.08 (m, 2H); <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O) δ 70.9, 33.3 (d, *J* = 129.5 Hz), 15.6 (d, *J* = 14.7 Hz), 0.8, 0.0; <sup>31</sup>P NMR (161.8 MHz, D<sub>2</sub>O) δ 22.5; HRMS-Cl calc. for C<sub>5</sub>H<sub>12</sub>O<sub>4</sub>P (M+H)<sup>+</sup> 167.0474, found 167.0473.

**(*R*)-(2-Cyclopropyl-2-hydroxyethyl)phosphonic acid ((*R*)-7).** Using the same procedure for the synthesis of (*S*)-**7**, compound (*R*)-**7** was obtained from (*R*)-**10** in 85% yield. The spectral data of (*R*)-**7** are identical to those of (*S*)-**7**.



**Figure S2.1-1.**  $^{31}\text{P}$  NMR spectra (161.8 MHz,  $\text{CDCl}_3$ ) of chemically synthesized (*R*)-**10** (top spectrum), (*S*)-**10** (middle spectrum), and racemic-**10** (bottom spectrum). Quinine was added as chiral shifting reagent. Each sample contains 40 mg of quinine and 10 mg of substrate dissolved in 0.65 mL of  $\text{CDCl}_3$ .

## 2.2. Chemical synthesis of compound 15.



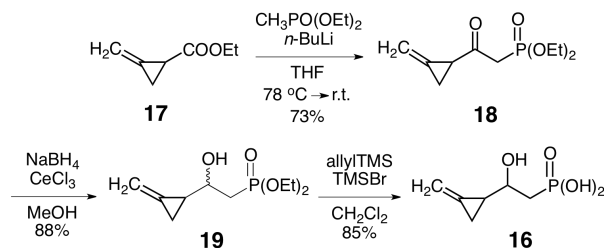
**Scheme S2.** Synthetic scheme for the preparation of **15**.

**(2-Cyclopropyl-2-oxoethyl)phosphonic acid (15).** To a stirred solution of **9** (439 mg, 2.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) was added allylTMS (2.5 mL, 16.0 mmol) and TMSBr (1.3 mL, 10.0 mmol). The reaction was stirred at room temperature for 12 h, and the reaction mixture was concentrated under reduced pressure. The residue was dissolved in  $\text{CHCl}_3$  (6 mL) and extracted with 0.5 M  $\text{NH}_4\text{HCO}_3$  (8 mL  $\times$  2). The aqueous extracts were collected and lyophilized to afford **15** as a white solid (220 mg, 85%).  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  2.97 (d,  $J = 22.0$  Hz, 2H), 2.07-2.14 (m, 2H), 0.88-0.92 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  211.8, 46.3 (d,  $J = 112.7$  Hz), 22.0, 12.5;  $^{31}\text{P}$  NMR (161.8 MHz,  $\text{D}_2\text{O}$ )  $\delta$  13.3; HRMS-Cl calc. for  $\text{C}_5\text{H}_{12}\text{O}_4\text{P}$  ( $\text{M}+\text{H}$ ) $^+$  165.0311, found 165.0311.

## 2.3. Chemical synthesis of compound 16.

**Diethyl (2-oxo-2-methylenecyclopropyl)ethylphosphonate (18).** To a stirred solution of diethyl methylphosphonate (400 mg, 2.62 mmol) in THF (15 mL) was added *n*-BuLi (1.1 mL, 2.5 M solution in hexanes, 2.65 mmol) at  $-78$   $^\circ\text{C}$ . After stirring at the same temperature for 20 min, the lithiated reagent was transferred via a

cannula needle to a solution of **17** (315 mg, 2.50 mmol) in THF (15 mL) at  $-78\text{ }^{\circ}\text{C}$ . The resulting mixture was stirred at  $-78\text{ }^{\circ}\text{C}$  for 1 h and then slowly warmed to room temperature. After stirring for an additional 2 h, the reaction was quenched with saturated  $\text{NH}_4\text{Cl}$  (8 mL) and extracted with ethyl acetate (30 mL  $\times$  5). The organic extracts were combined, washed with brine, and dried over  $\text{Na}_2\text{SO}_4$ . The solvent was removed under reduced pressure, and the resulting residue was purified using flash column chromatography (ethyl acetate) to give the desired compound **18** as a colorless oil (423 mg, 73%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.41 (d,  $J = 1.5$  Hz, 2H), 4.02-4.11 (m, 4H), 2.91-3.11 (m, 2H), 2.64-2.68 (m, 1H), 1.87-1.92 (m, 1H), 1.65 (tt,  $J = 2.1, 6.3$  Hz, 1H), 1.24 (dt,  $J = 0.6, 5.7$  Hz, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  199.4 (d,  $J = 3.0$  Hz), 132.0, 104.8, 62.8 (dd,  $J = 3.8, 6.5$  Hz), 40.4 (d,  $J = 127.5$  Hz), 27.6, 16.6 (d,  $J = 6.0$  Hz), 13.3;  $^{31}\text{P}$  NMR (121.5 MHz,  $\text{CDCl}_3$ )  $\delta$  20.9; HRMS-ESI ( $\text{M}+\text{H}$ ) $^+$  calc. for  $\text{C}_{10}\text{H}_{18}\text{O}_4\text{P}$  233.0937, found 233.0940.



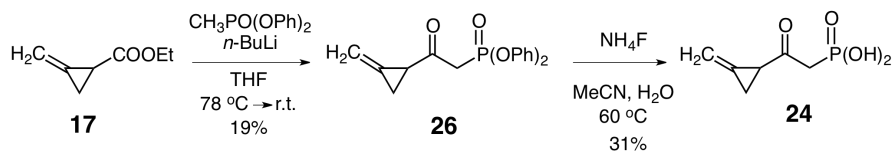
**Scheme S3.** Synthetic scheme for the preparation of **16**.

**Diethyl (2-hydroxy-2-methylenecyclopropyl)ethylphosphonate (19).** To a stirred solution of **18** (350 mg, 1.51 mmol) and  $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$  (555 mg, 1.51 mmol) in methanol (20 mL) was added  $\text{NaBH}_4$  (58 mg, 1.52 mmol) at room temperature. The resulting mixture was stirred for 20 min before quenching with a mixture of 1 N HCl (3 mL, 3 mmol) and water (10 mL). The resulting solution was extracted with ethyl acetate (20 mL  $\times$  5). The organic extracts were combined, washed with brine (20 mL  $\times$  2), and dried over  $\text{Na}_2\text{SO}_4$ . The solvent was removed under reduced pressure, and the resulting residue was purified using flash column chromatography (ethyl acetate) to give the desired compound **19** as a mixture of inseparable diastereomers (colorless oil, 311 mg, 88%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.45-5.47 (m), 5.32-5.36 (m), 3.97-4.07 (m), 3.78 (d,  $J = 3.2$  Hz), 3.56-3.63 (m), 3.40-3.49 (m), 1.91-2.06 (m), 1.58-1.88 (m), 1.15-1.27 (m), 0.92-1.04 (m), 0.90-0.95 (m);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  132.6, 132.0, 104.1, 104.0, 69.0 (d,  $J = 5.2$  Hz), 68.4 (d,  $J = 4.4$  Hz), 61.7, 61.6, 61.52, 61.48, 33.3 (d,  $J = 137.7$  Hz), 32.6 (d,  $J = 135.7$  Hz), 22.2 (d,  $J = 19.4$  Hz), 21.7 (d,  $J = 18.6$  Hz), 16.1 (d,  $J = 6.0$  Hz), 8.2, 6.7;  $^{31}\text{P}$  NMR (161.8 MHz,  $\text{CDCl}_3$ )  $\delta$  29.6, 29.5; HRMS-ESI calc. for  $\text{NaC}_{10}\text{H}_9\text{O}_4\text{P}$  ( $\text{M}+\text{Na}$ ) $^+$  257.0913, found 257.0915.

**2-Hydroxy-2-(methylenecyclopropyl)-ethylphosphonic acid (16).** To a stirred solution of **19** (159 mg, 0.68 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added allylTMS (542 mg, 4.76 mmol) and TMSBr (420 mg, 2.75 mmol). The mixture was stirred at room temperature for 12 h and the solvent was then removed under reduced pressure. The residue was dissolved in  $\text{CHCl}_3$  (6 mL) and extracted with 0.2 M  $\text{NH}_4\text{HCO}_3$  (8 mL  $\times$  2). The aqueous extracts were collected and lyophilized to afford **16** as a white solid (102 mg, 85%).  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ , pre-saturated)  $\delta$

5.40 (s, 1H), 5.29 (s, 1H), 3.30-3.42 (m, 1H), 1.72-1.90 (m, 2H), 1.58 (br, 1H), 1.85 (t,  $J = 9$  Hz), 0.89-0.93 (m, 1H);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ )  $\delta$  133.8, 132.8, 104.0, 103.7, 70.5 (d,  $J = 2.4$  Hz), 70.3 (d,  $J = 3.4$  Hz), 35.3 (d,  $J = 130.1$  Hz), 35.0 (d,  $J = 130.4$  Hz), 22.0 (d,  $J = 15.1$  Hz), 21.5 (d,  $J = 14.4$  Hz), 7.4, 7.1;  $^{31}\text{P}$  NMR (242.8 MHz,  $\text{D}_2\text{O}$ )  $\delta$  21.4; HRMS-ESI cal. for  $\text{C}_6\text{H}_{10}\text{O}_4\text{P}$  (M-H) $^-$ , 177.0317, found 177.0322.

## 2.4. Chemical synthesis of compound 24.



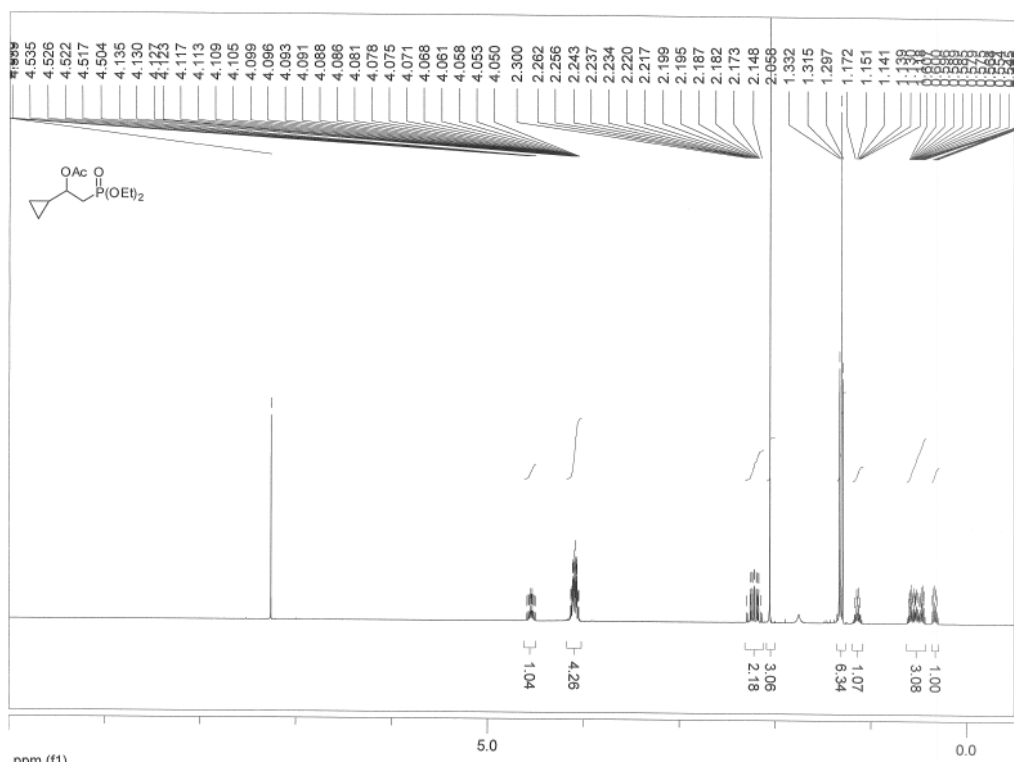
**Scheme S4.** Synthetic scheme for the preparation of **24**.

**Diphenyl (2-oxo-2-methylenecyclopropyl)ethylphosphonate (26).** To a solution of diphenyl methylphosphonate (650 mg, 2.62 mmol) in THF (15 mL) was added dropwise  $n\text{-BuLi}$  (1.1 mL, 2.5 M solution in hexanes, 2.65 mmol) at  $-78\text{ }^\circ\text{C}$ . After stirring at the same temperature for 3 min, a solution of **17** (315 mg, 2.50 mmol) in THF (15 mL) was added to the lithiated reagent in one portion. The resulting mixture was stirred at  $-78\text{ }^\circ\text{C}$  for 15 min and then quenched with saturated  $\text{NH}_4\text{Cl}$  (8 mL). The mixture was warmed to room temperature and extracted with ethyl acetate (30 mL  $\times$  5). The organic extracts were combined, washed with brine, and dried over  $\text{Na}_2\text{SO}_4$ . The solvent was removed under reduced pressure, and the resulting residue was purified using flash column chromatography (hexanes : ethyl acetate = 3:2 ~ 0:1) to give the desired compound **26** as a colorless oil (155 mg, 19%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.29-7.35 (m, 4H), 7.15-7.28 (m, 6H), 5.46-5.52 (m, 2H), 3.30-3.49 (m, 2H), 2.80-2.86 (m, 1H), 1.98-2.04 (m, 1H), 1.77 (tt,  $J = 2.7, 8.7$  Hz, 1H);  $^{31}\text{P}$  NMR (121.5 MHz,  $\text{CDCl}_3$ )  $\delta$  14.0;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  198.2 (d,  $J = 6.5$  Hz), 150.4 (d,  $J = 8.2$  Hz), 131.8, 130.2, 125.9, 121.1 (d,  $J = 4.3$  Hz), 105.4, 41.1 (d,  $J = 130$  Hz), 28.0 (d,  $J = 16.5$  Hz), 13.9, 13.3; HRMS-ESI cal. for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{P}$  (M+H) $^+$  329.0943, found 329.0937.

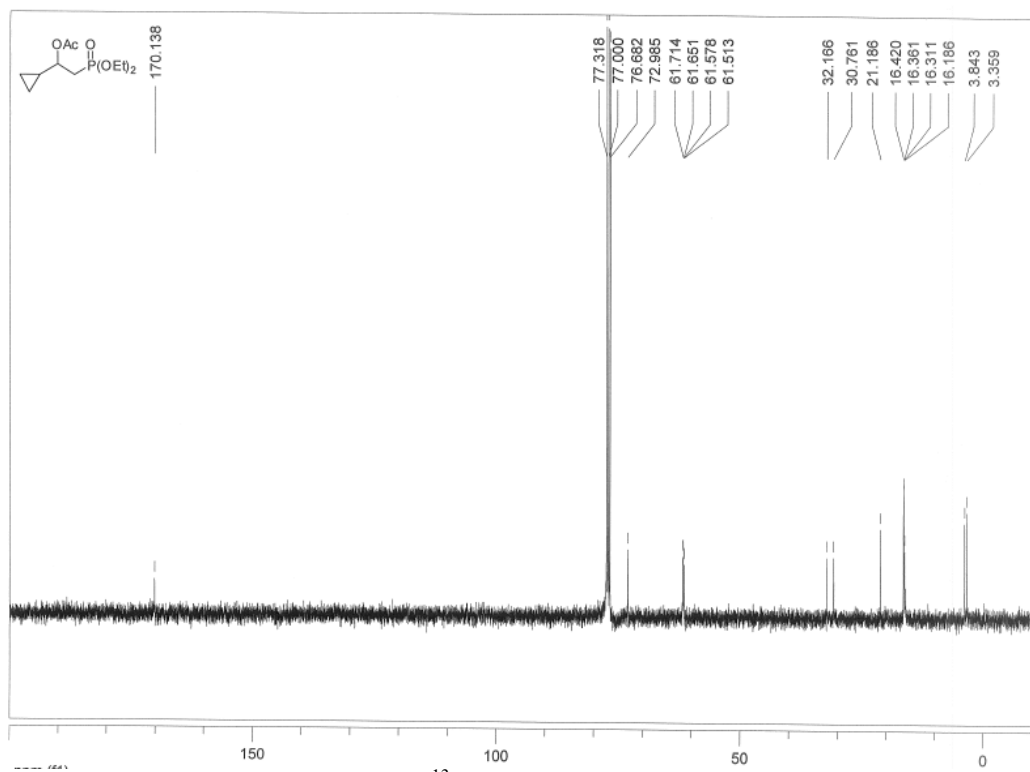
**(2-Oxo-2-methylenecyclopropyl)ethylphosphonate (24).** Compound **26** (100 mg, 0.30 mmol) and  $\text{NH}_4\text{F}$  (111 mg, 3.0 mmol) were dissolved in a 1:1 mixture of  $\text{CH}_3\text{CN} : \text{H}_2\text{O}$  (14 mL), and the reaction mixture was stirred at  $60\text{ }^\circ\text{C}$  for 3 h.<sup>4</sup> After most of the organic solvent was removed under reduced pressure, the mixture was subjected to ion-exchange chromatography using DEAE-Sephadex ( $\text{HCO}_3^-$ ). The column was first washed with  $\text{H}_2\text{O}$  and the crude product was eluted with 100 mM  $\text{NH}_4\text{HCO}_3$ . The eluate was concentrated via lyophilization to 1 mL and further purified by HPLC using a DIONEX CarboPac PA1 column. A 0 to 100% gradient of  $\text{H}_2\text{O}$  and 200 mM  $\text{NH}_4\text{HCO}_3$  was applied to elute the product. Fractions containing the desired product were combined and lyophilized to give **24** as a white solid (16.5 mg, 31%), which slowly decomposed to a red solid.  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ )  $\delta$  5.40 (m, 2H), 2.82-2.96 (m, 1H), 2.81 (t,  $J = 6$  Hz, 1H), 2.00-2.11 (m, 1H), 1.72-1.75 (m, 2H);  $^{13}\text{C}$

NMR (125 MHz, CDCl<sub>3</sub>) δ 209.6, 132.5, 103.6, 47.9, 26.4, 13.8; <sup>31</sup>P NMR (202.3 MHz, CDCl<sub>3</sub>) δ 11.7; HRMS-ESI cal. for C<sub>6</sub>H<sub>10</sub>O<sub>4</sub>P (M+H)<sup>+</sup> 177.0311 found 177.0313.

### 3. NMR spectra of compounds 7, 11, 15, 16, 18, 19, 24, and 26.

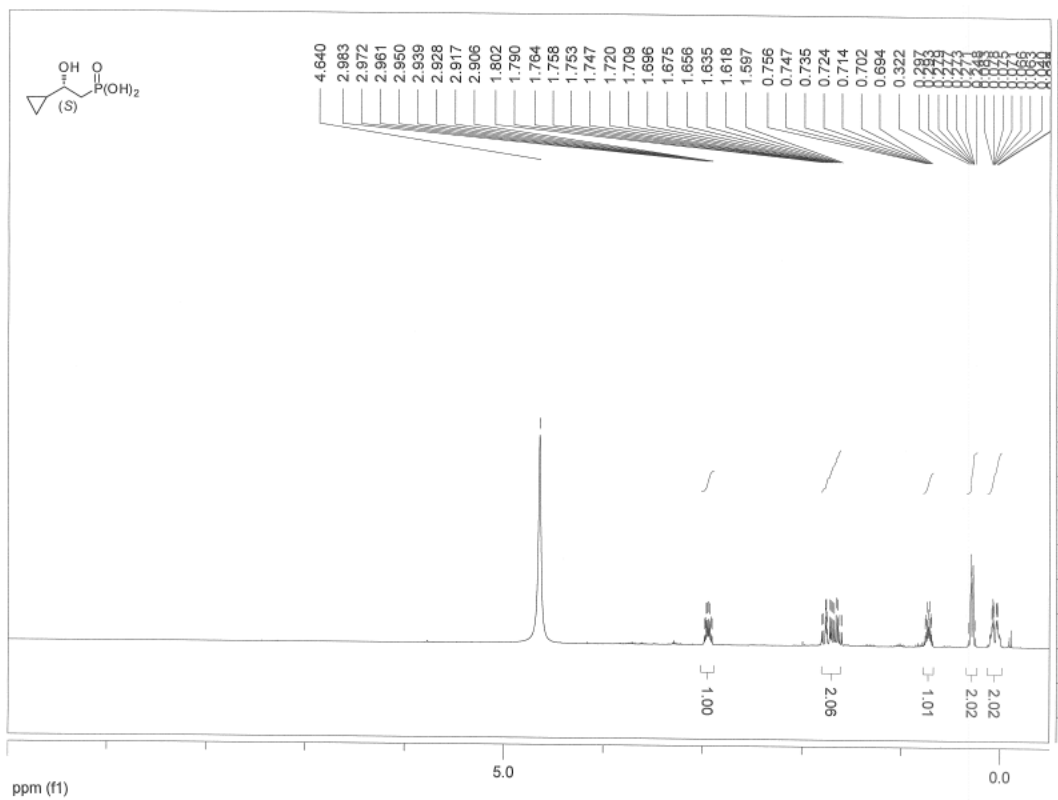


<sup>1</sup>H NMR of 11

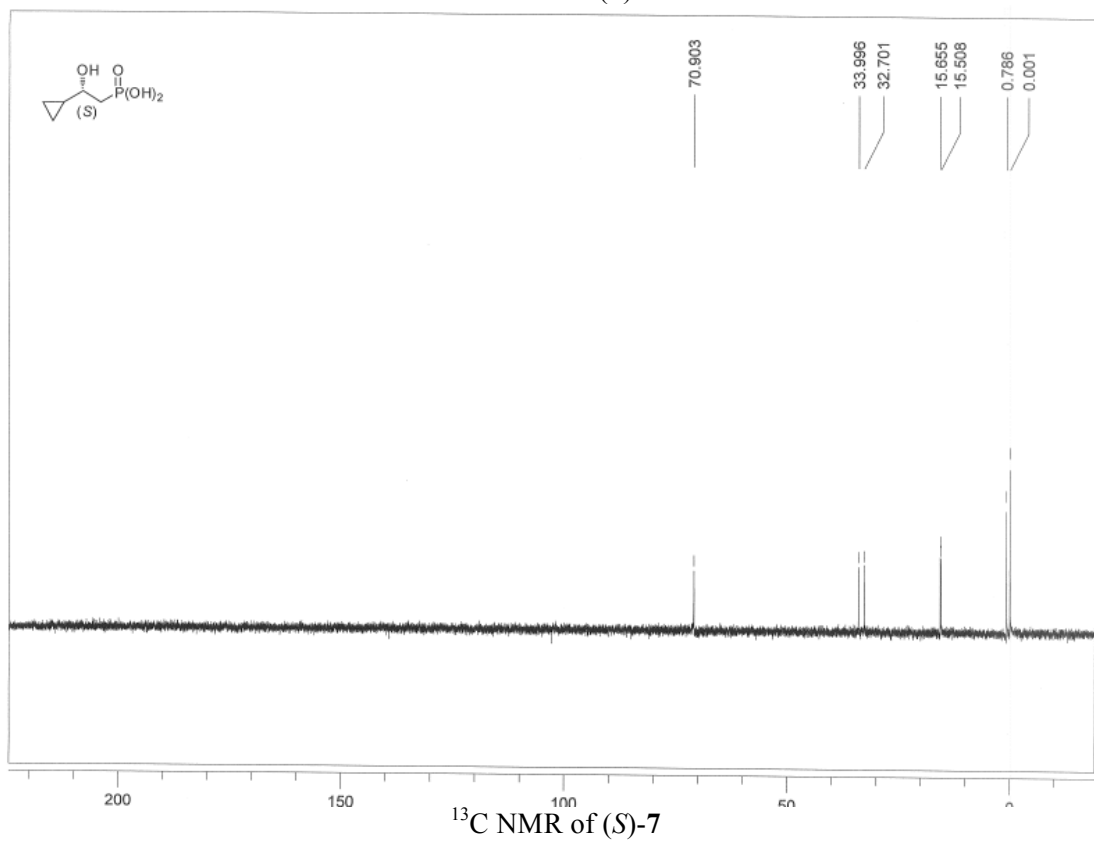


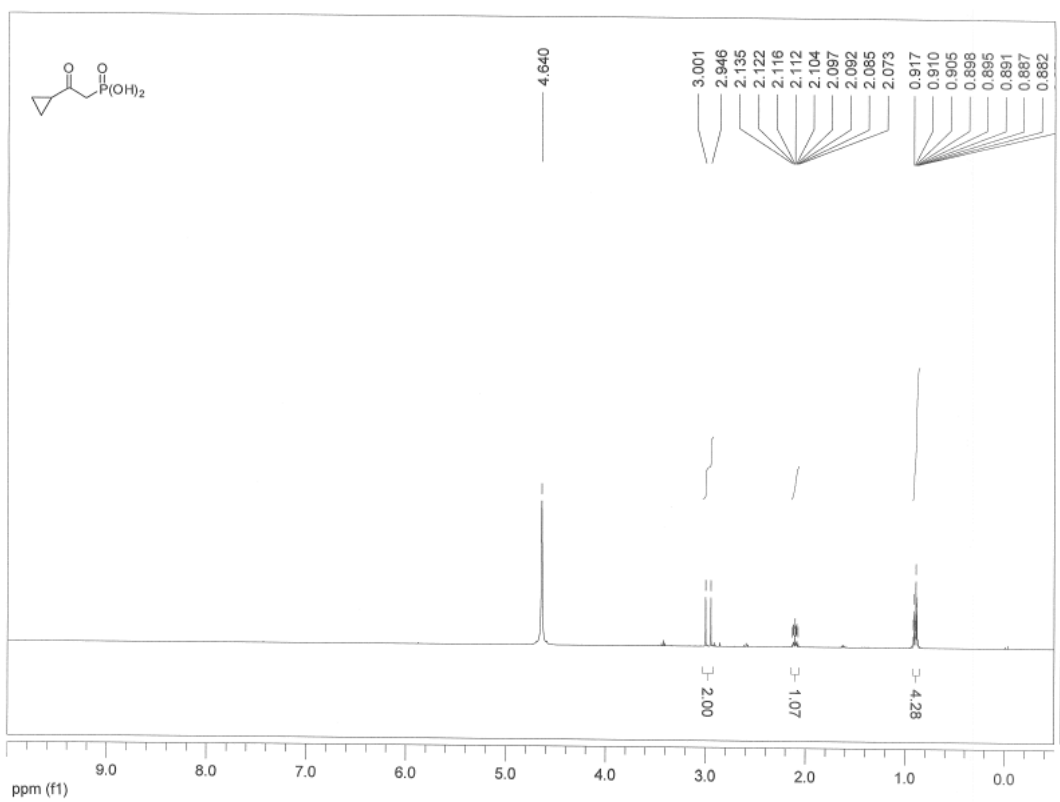
<sup>13</sup>C NMR of 11



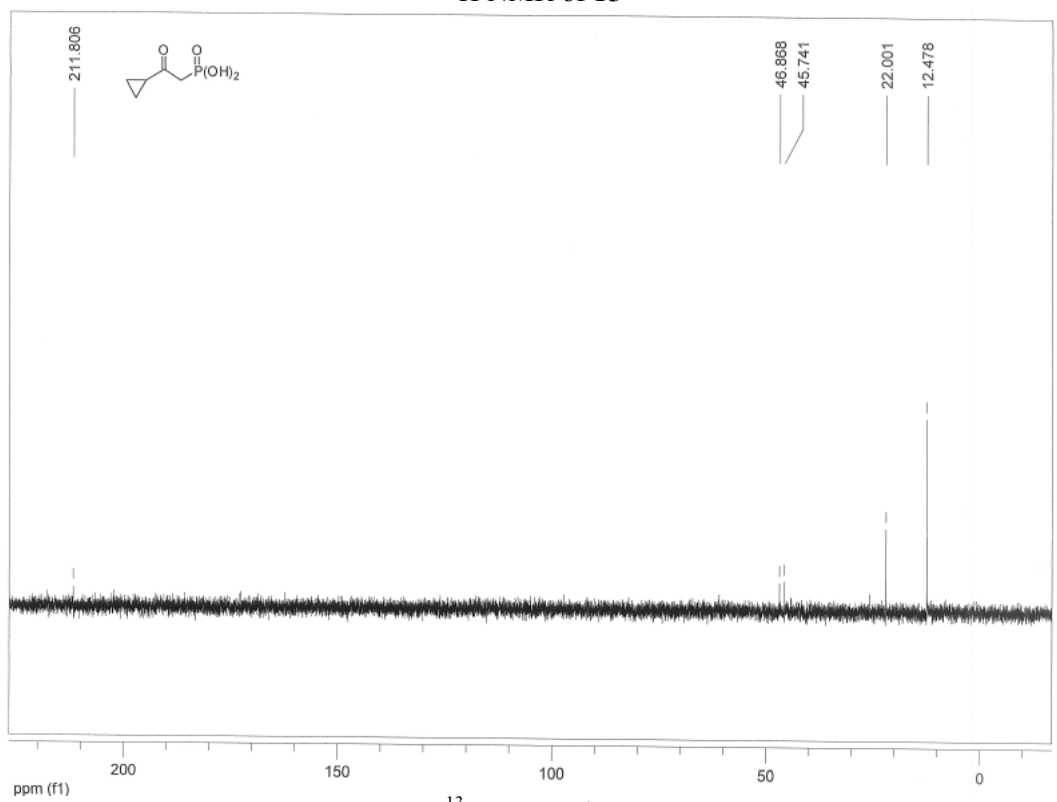


<sup>1</sup>H NMR of (S)-7





$^1\text{H NMR}$  of 15

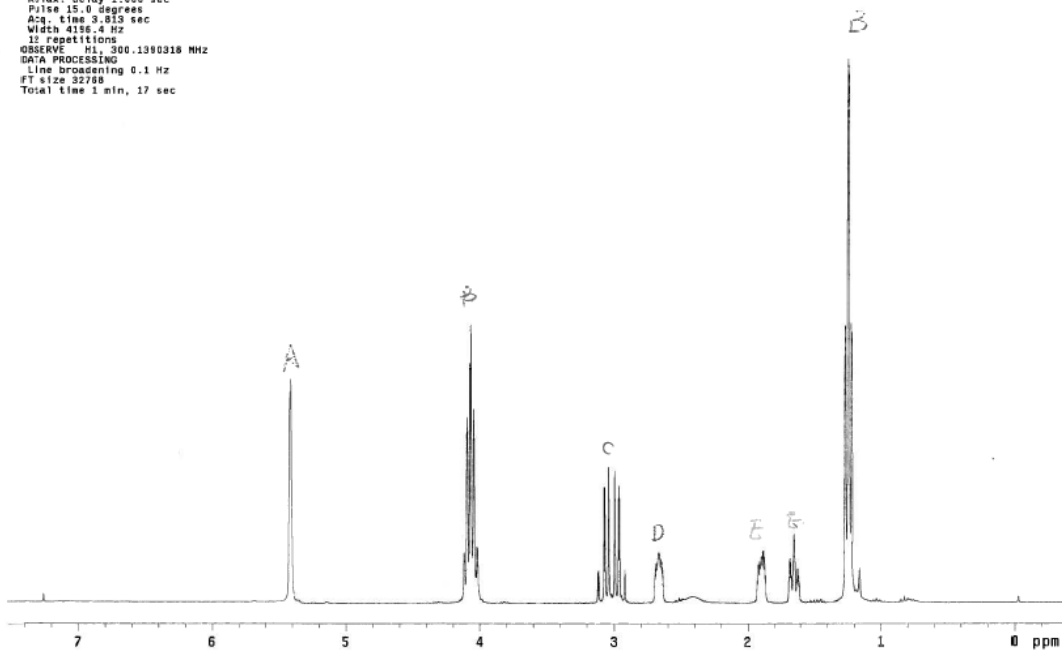
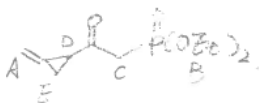


$^{13}\text{C NMR}$  of 15

STANDARD 1H OBSERVE

Pulse Sequence: s2pu1  
Solvent: CDCl3  
Ambient temperature  
UNITYplus-300 "nmr2"

Relax. delay 1.000 sec  
Pulse 15.0 degrees  
Acq. time 3.813 sec  
Width 4156.4 Hz  
12 repetitions  
OBSERVE H1, 300.1390318 MHz  
DATA PROCESSING  
Line broadening 0.1 Hz  
FT size 32768  
Total time 1 min, 17 sec

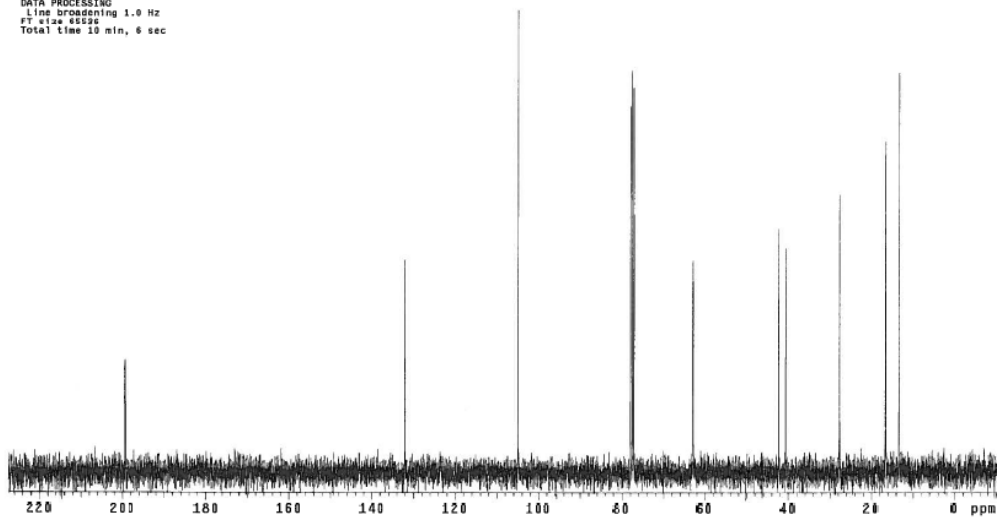


<sup>1</sup>H NMR of 18

13C OBSERVE

Pulse Sequence: s2pu1  
Solvent: CDCl3  
Ambient temperature  
UNITYplus-300 "nmr2"

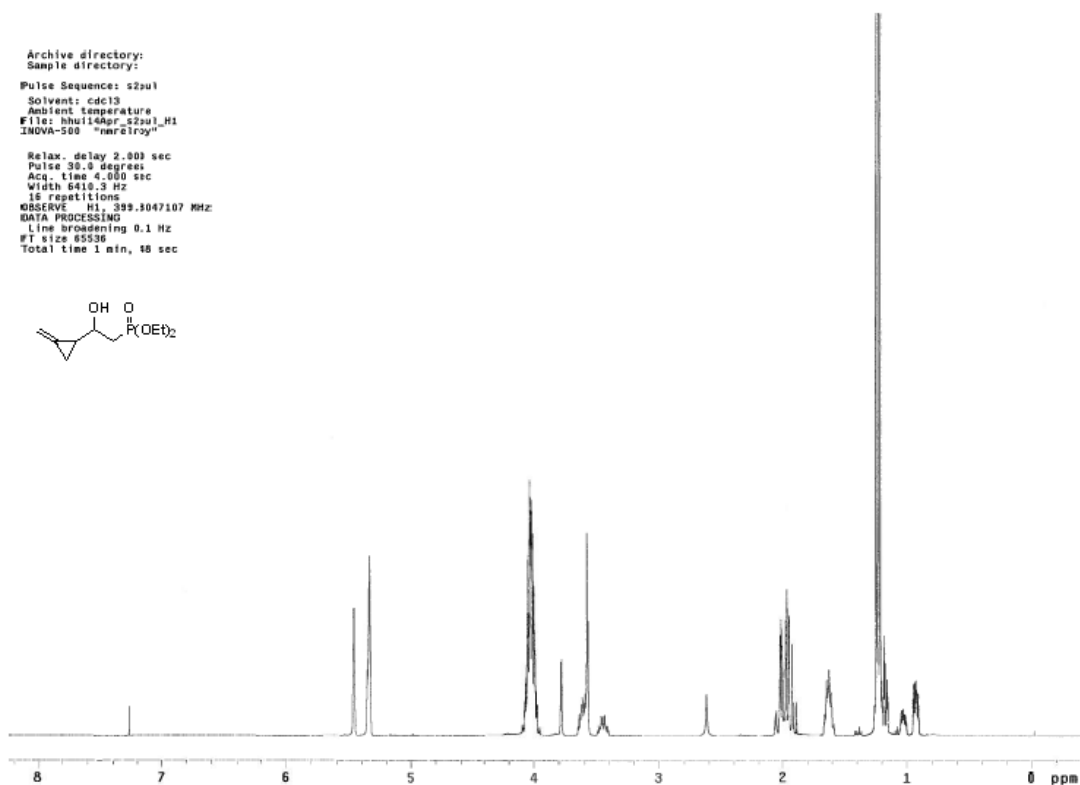
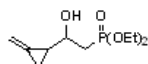
Relax. delay 2.000 sec  
Pulse 35.0 degrees  
Acq. time 1.777 sec  
Width 18095.3 Hz  
32 repetitions  
OBSERVE C13, 75.4699921 MHz  
DECUPLE H1, 300.1409259 MHz  
Power 40 dB  
continuously on  
WALTZ-16 modulated  
Single precision data  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 48526  
Total time 10 min, 6 sec



<sup>13</sup>C NMR of 18

Archive directory:  
 Sample directory:  
 Pulse Sequence: s2pu1  
 Solvent: cdCl3  
 Ambient temperature  
 File: hhu14Apr\_s2pu1\_H1  
 INOVA-500 "nuc1roy"

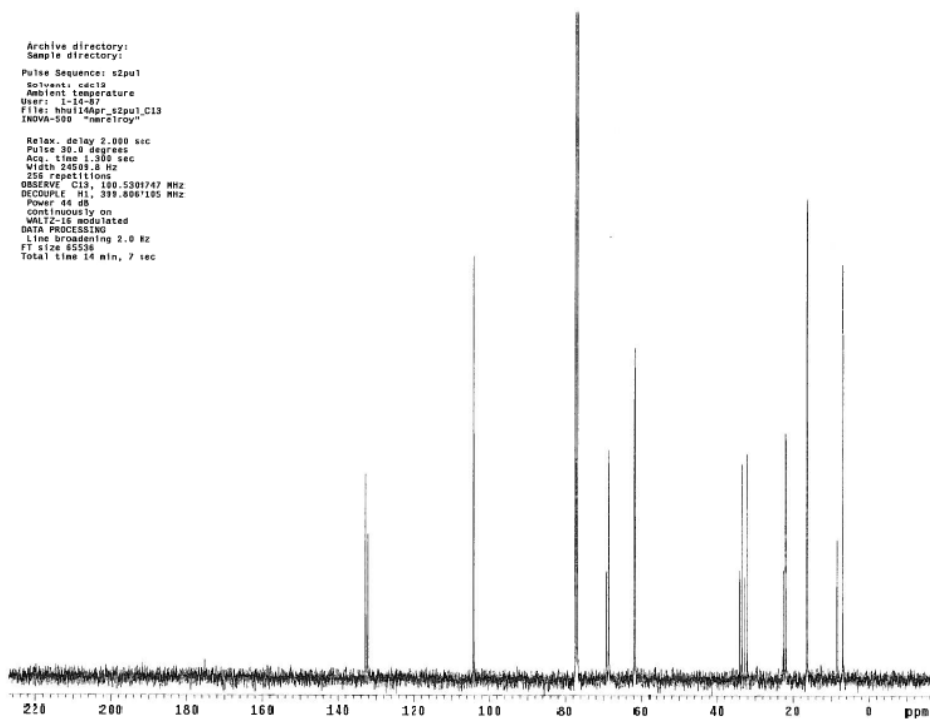
Relax. delay 2.000 sec  
 Pulse 30.0 degree  
 Acq. time 4.000 sec  
 Width 6410.3 Hz  
 16 Reptitions  
 OBSERVE H1, 399.3047107 MHz  
 DATA PROCESSING  
 Line broadening 0.1 Hz  
 FT size 65536  
 Total time 1 min, 48 sec



<sup>1</sup>H NMR of 19

Archive directory:  
 Sample directory:  
 Pulse Sequence: s2pu1  
 Solvent: cdCl3  
 Ambient temperature  
 User: i-14-87  
 File: hhu14Apr\_s2pu1\_C13  
 INOVA-500 "nuc1roy"

Relax. delay 2.000 sec  
 Pulse 30.0 degree  
 Acq. time 1.300 sec  
 Width 24503.8 Hz  
 328 Reptitions  
 OBSERVE C13, 100.5301747 MHz  
 DECOUPLE H1, 399.8061195 MHz  
 Power 16 dB  
 Continuously on  
 WALTZ-16 isolated  
 DATA PROCESSING  
 Line broadening 2.0 Hz  
 FT size 65536  
 Total time 14 min, 7 sec

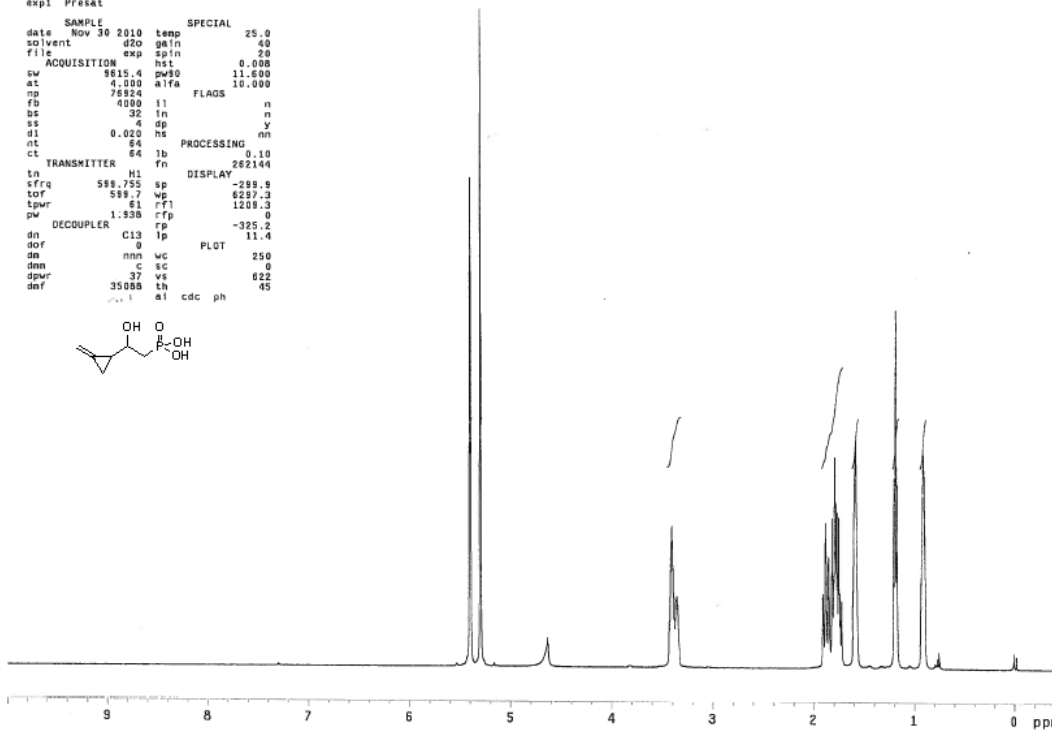
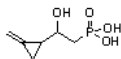


<sup>13</sup>C NMR of 19

```

exp1 Presat
SAMPLE
date Nov 30 2010 temp SPECIAL 25.0
solvent d2o gain 40
file exp spin 20
ACQUISITION exp spin 0.008
sw 9615.4 pw90 11.600
at 0.000 alfa 10.000
np 76924 FLAGS
fb 4000 l1 n
bs 32 l1 n
ss 4 dp y
dl 0.020 hs nn
nt 64 PROCESSING 0.10
ct 1b fn 262144
TRANSMITTER H1 DISPLAY
tn H1
sfrq 599.755 sp -289.9
tof 599.7 wp 6237.3
tpwr 61 rF1 1209.3
pw 1.938 rfp 0
DECOUPLER rp -325.2
dn C13 lp 11.4
dof 9 PLOT
da nnn wc 250
dnn c sc 0
dpr 37 vs 622
dnf 35088 th 45
ai cdc ph

```

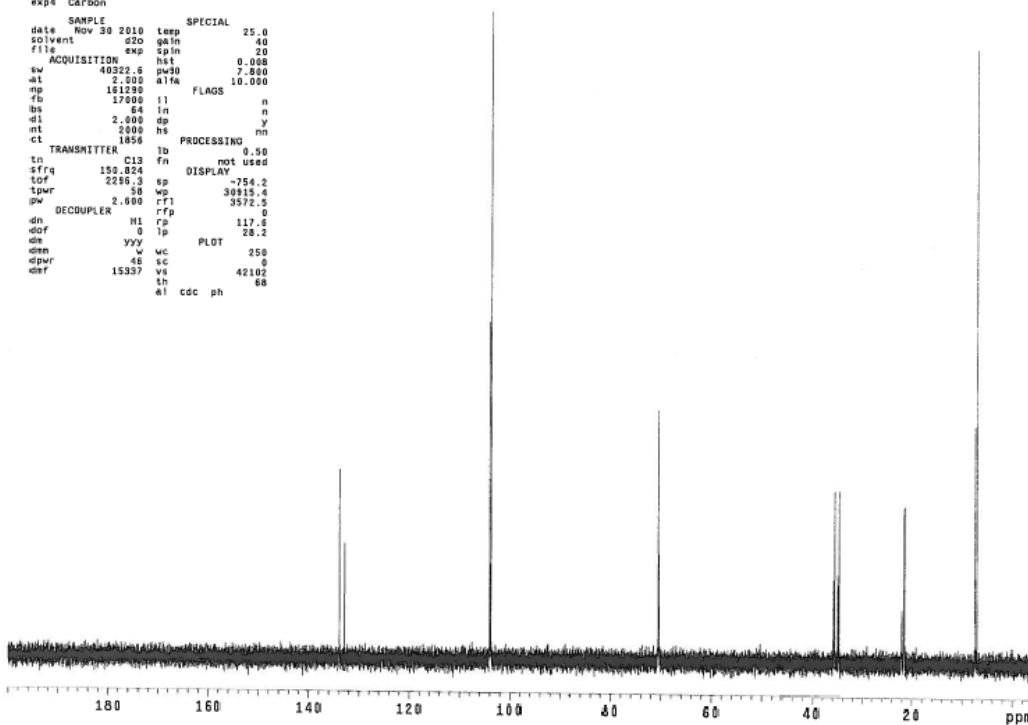


<sup>1</sup>H NMR of 16

```

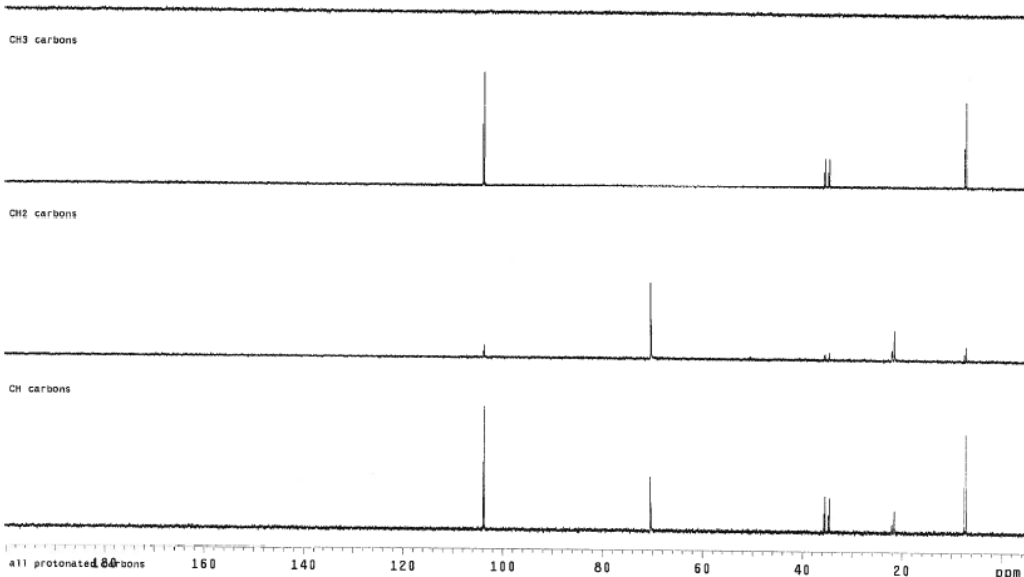
exp4 Carbon
SAMPLE
date Nov 30 2010 temp SPECIAL 25.0
solvent d2o gain 40
file exp spin 20
ACQUISITION exp spin 0.008
sw 40322.6 pw90 7.800
at 161290 alfa 10.000
np 17000 l1 FLAGS
fb 64 l1 n
bs 2.000 dp y
nt 2000 hs nm
ct 1856 PROCESSING 0.50
tn C13 fn not used
sfrq 150.824 DISPLAY -754.2
tof 2286.3 sp 38915.4
tpwr 50 wp 3572.5
pw 2.000 rF1 0
DECOUPLER H1 rfp 117.6
dn 0 lp 28.2
dof yyy wc PLOT
da 46 sc 250
dnn w sc 0
dpr 15337 vs 42102
dnf ai cdc ph 68

```



<sup>13</sup>C NMR of 16

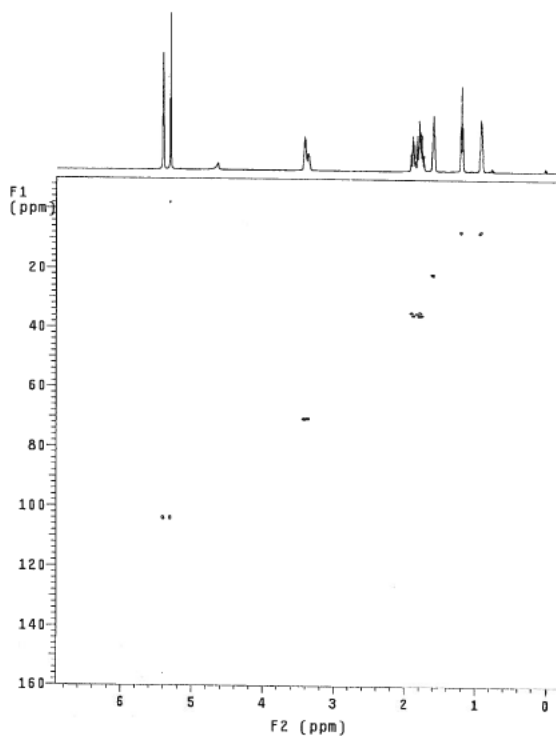
Sample Name:  
 Data Collected on:  
 Archive directory:  
 Sample directory:  
 FldFile: DEPT  
 Pulse Sequence: DEPT  
 Solvent: d2o  
 Data collected on: Nov 30 2010



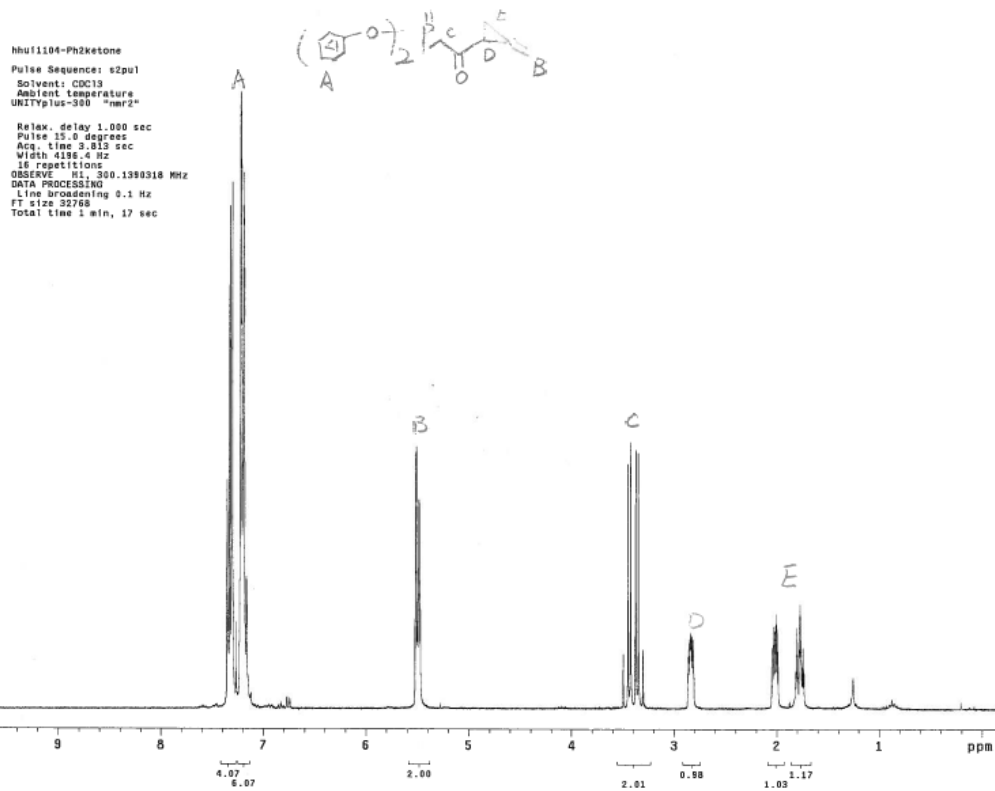
<sup>13</sup>C NMR DEPT of 16

```

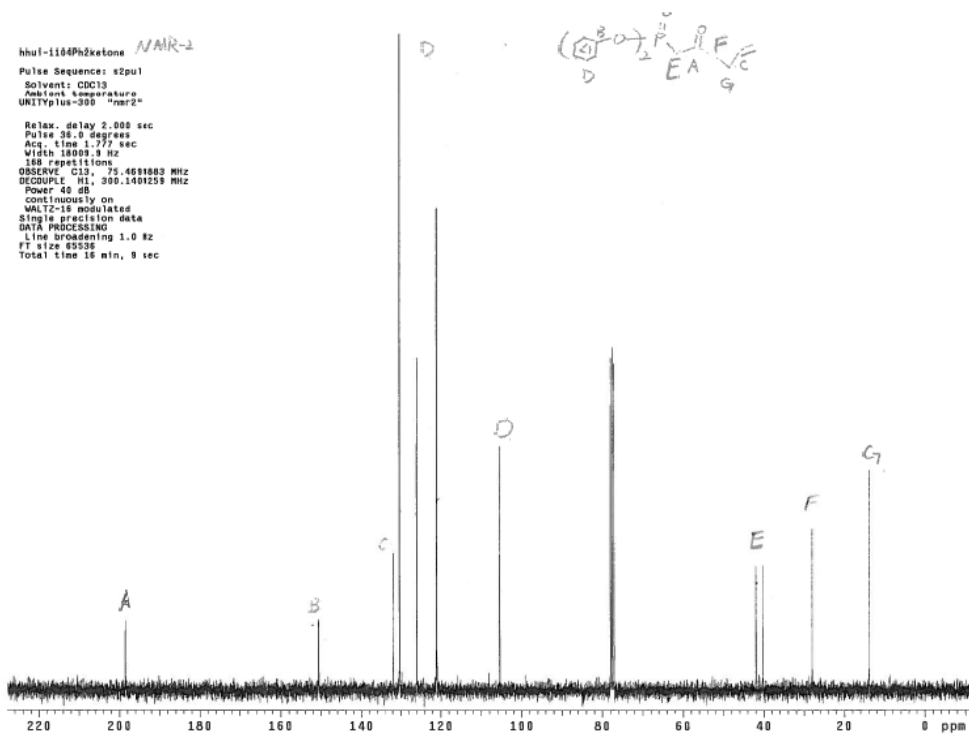
exp6 Ghsqc
SAMPLE          FLAGS      ACQUISITION ARRAYS
date Nov 30 2010 hs          n array phase
solvent d2o    sspul  y arraydim 512
sample PGDF1g  y
ACQUISITION    hsglv1  6696  1  phase
sw 4280.8      SPECIAL 25.0  1  1
at 0.198      Temp   16  2
np 1706       gain   0
fb 4000       spIn   0
ss 32         GRADIENTS
dl 0          g2lv11 8928
nt 4          gtl 0.001000
2D ACQUISITION g2lv13 4089
swl 25641.0   g13 0.000500
nl 256       g1tab 0.000500
phase arrayed F2 PROCESSING
PRESATURATION  gf 0.092
satmode yn    gfa not used
satdly 2.000  fn 4096
satfrq -221.4  F1 PROCESSING
satpur -13    gfl 0.009
TRANSMITTER H1  prc1 not used
tn          H1  prc1 lp
sfreq 599.753 fml 2048
tof -999.3    DISPLAY
tpwr 81       sp -138.9
pw 11.600    wp 4228.7
DECOUPLER c13 wp1 -1484.9
dn -2381.9   wp1 25616.0
dof -2381.9  rf1 141.0
dm nny       rfp 0
dmf 35008    rf11 1509.9
dprw 37      rfp1 0
pwxlv1 57     PLOT
pwx HSOC     wc 116.0
jlxh 140.0   wc2 116.0
mult1 y      sc2 0
mult 2       vs 69503
          th 2
          at cdc ph
  
```



HSQC NMR of 16



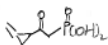
<sup>1</sup>H NMR of 26



<sup>13</sup>C NMR of 26

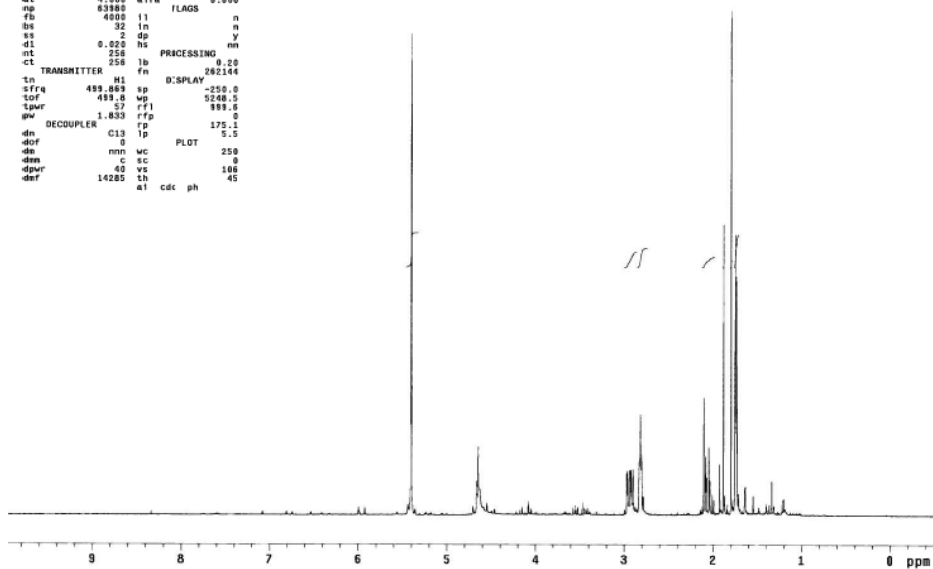
500 MHz nmr0

hhu1117\_h1



exp1 Presat

```
SAMPLE SPECIAL
date Nov 17 2011 temp 27.0
solvent d2o gain not used
file exp spin 20
ACQUISITION hst 0.000
sw 737.6 hfa 11.000
at 4.000 alpha 6.600
pa 63800
fb 4000 f1 FLAGS n
bs 32 in n
ss 2 dp y
dl 0.020 hs
nt 258 PRCESSING nm
ct 258 fb 0.20
TRANSMITTER fn 282104
tn H1 DISPLAY -250.0
rfq 489.863 sp
tof 489.8 wp 5280.5
tpwr 37 rfp 393.5
pw 1.833 rfp 0
DECOUPLER rf 175.1
dn C13 fp 5.5
dof 0 PLOT 250
ds nnn wc 0
dss c sc 0
dpr 40 vs 180
def 14285 th 45
al cdc ph
```



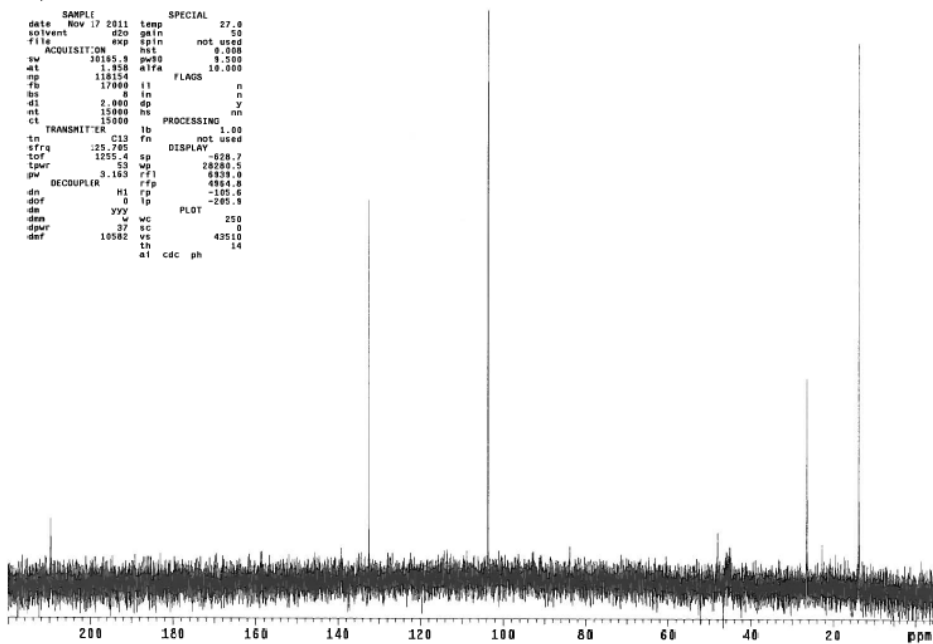
<sup>1</sup>H NMR of 24

500 MHz nmr0

hhu1117\_c13

exp4 Carbon

```
SAMPLE SPECIAL
date Nov 17 2011 temp 27.0
solvent d2o gain 50 not used
file exp spin 20
ACQUISITION hst 0.000
sw 10185.9 hfa 10.000
at 1.358 alpha
pa 118154
fb 17000 f1 FLAGS n
bs 8 in n
ss 2 dp y
dl 2.000 hs
nt 15000 PRCESSING nm
ct 15000 fb 1.00
TRANSMITTER C13 fo not used
tn C13 DISPLAY -520.7
rfq 125.745 sp
tof 125.4 wp 28280.5
tpwr 53 rfp 8839.9
pw 3.163 rfp 4854.8
DECOUPLER H1 fp -105.6
dn 0 fp
dof 392 PLOT 250
ds nnn wc 0
dss c sc 0
dpr 37 vs 42510
def 10502 th 14
al cdc ph
```

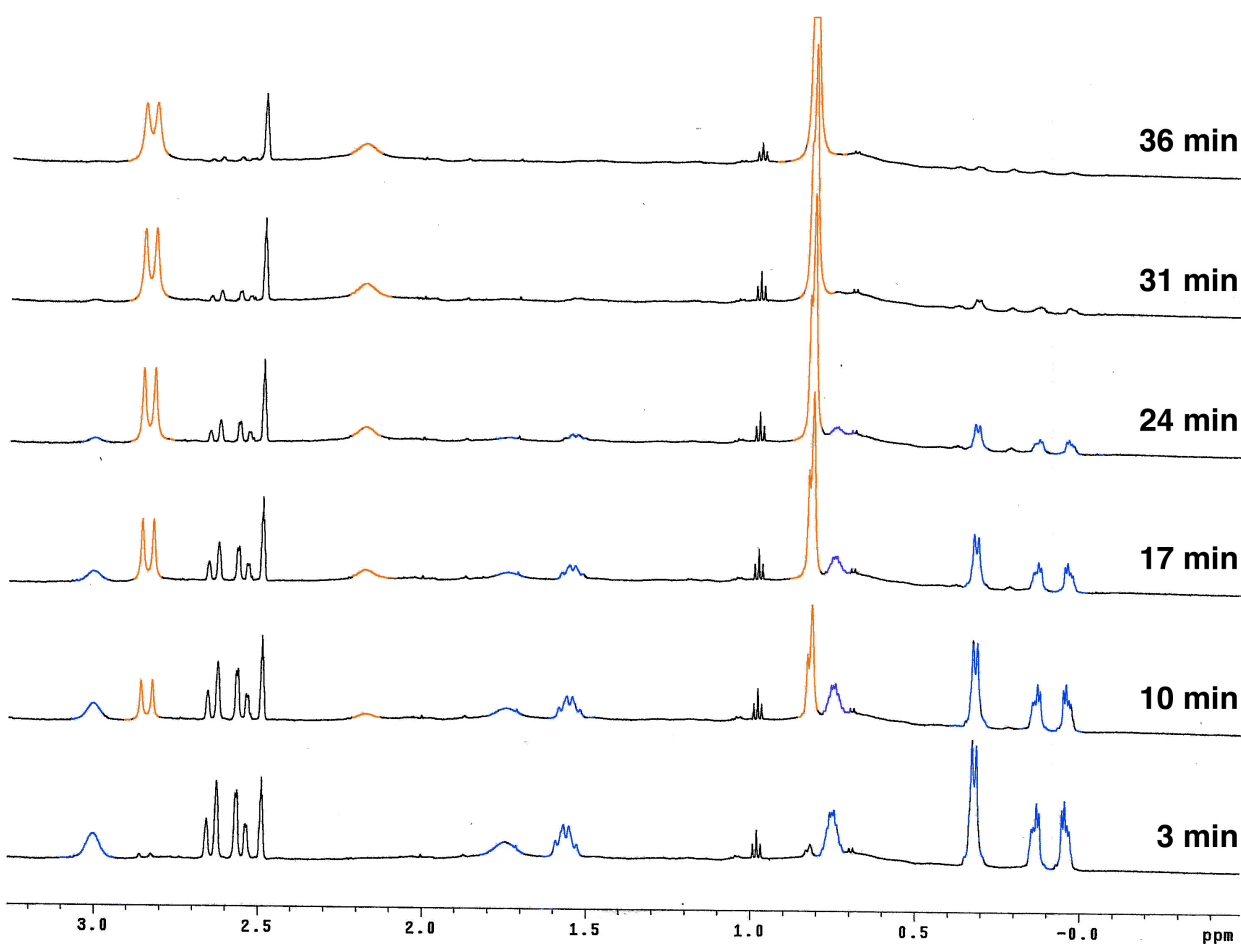


<sup>13</sup>C NMR of 24

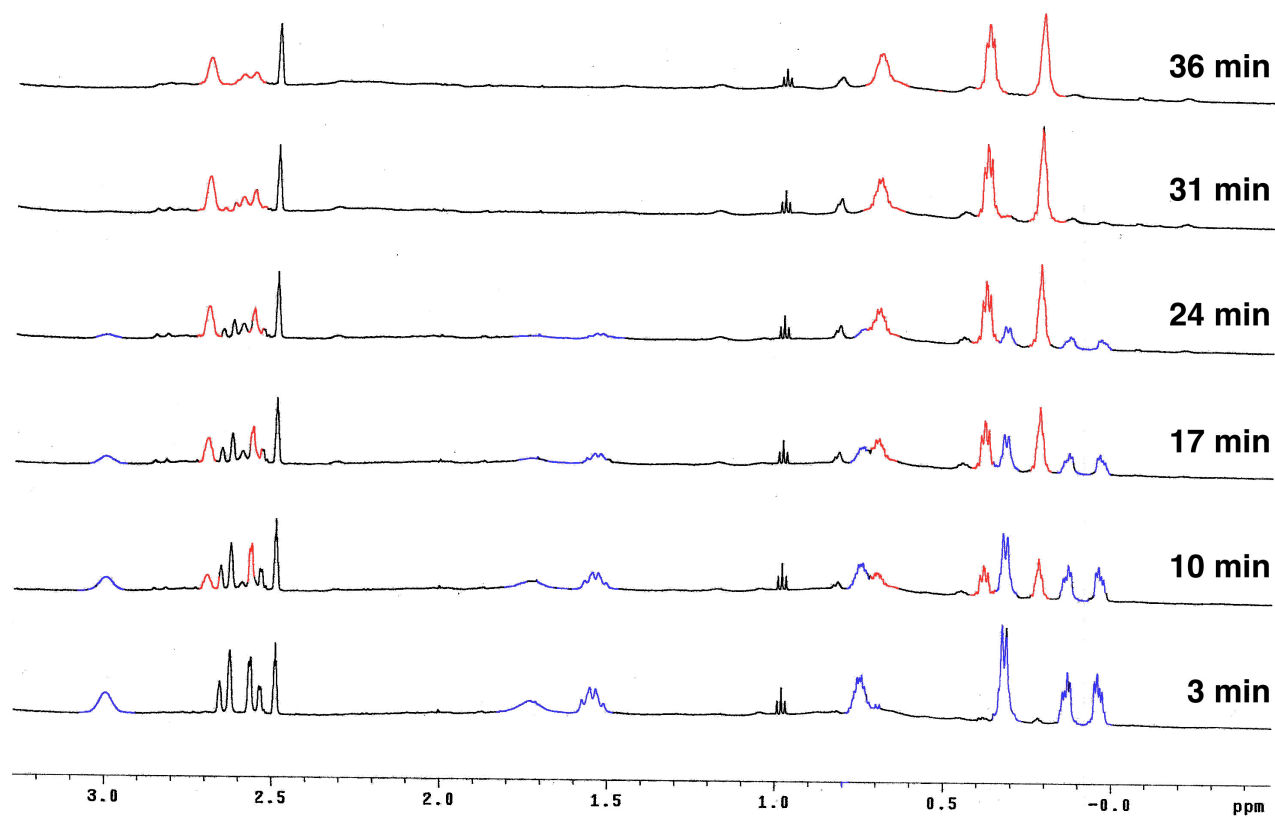


#### 4. Using $^1\text{H}$ NMR to monitor the enzymatic conversion of (*S*)-7, (*R*)-7, 16 and 24 by HppE.

A freshly prepared reaction solution containing 0.25 mM HppE, 7.5 mM FMN, 10 mM NADH, and 5 mM of the test substrate analogue in 650  $\mu\text{L}$  of 50 mM Tris buffer (pH 7.5) was analyzed using  $^1\text{H}$ -NMR (Varian DirectDrive 600 MHz NMR spectrometer) with  $\text{DMSO-d}_6$  (30  $\mu\text{L}$ ) as the internal standard. The reaction was initiated by adding the reconstituted HppE to the pre-mixed solution containing all other reaction reagents. The progress of the conversion was followed by recording the  $^1\text{H}$ -NMR spectra at different time points.



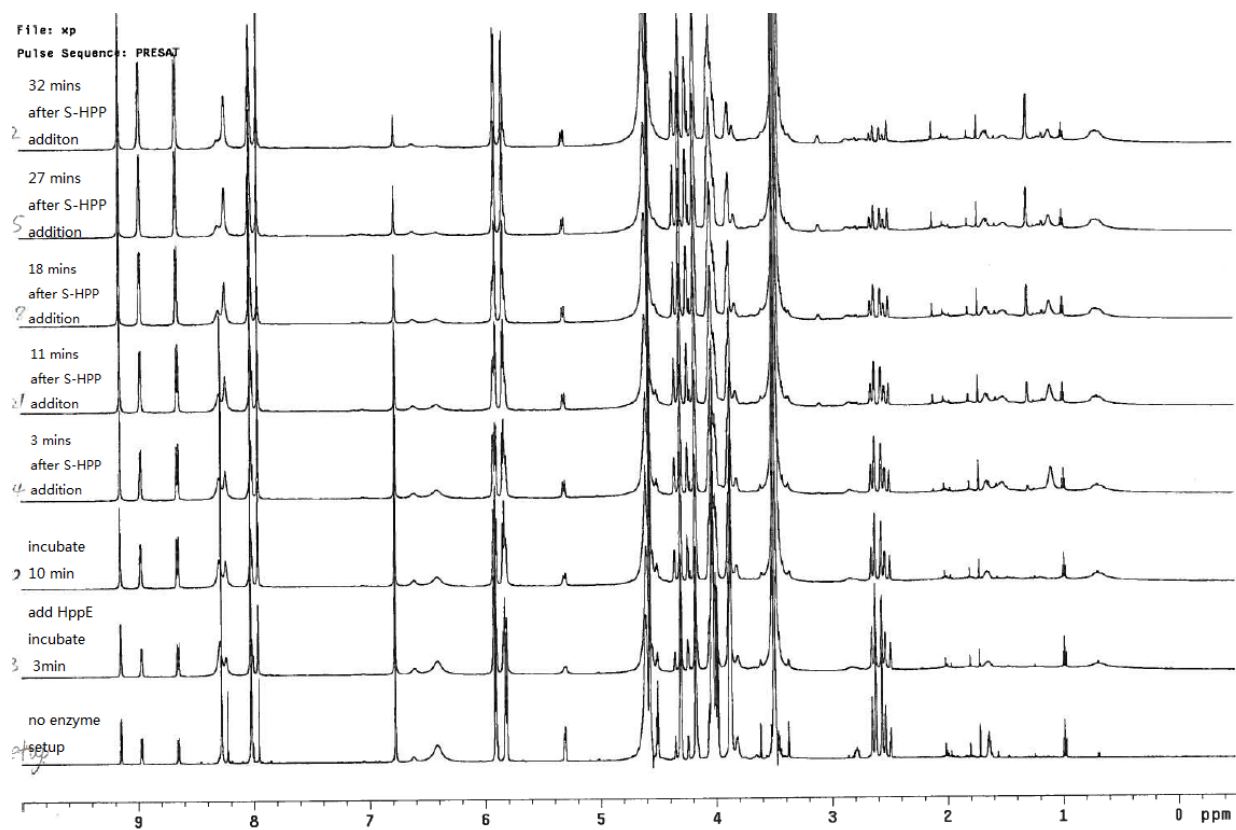
**Figure S4-1.**  $^1\text{H}$  NMR spectra of HppE-catalyzed conversion of (*R*)-7 (blue) to 15 (orange).



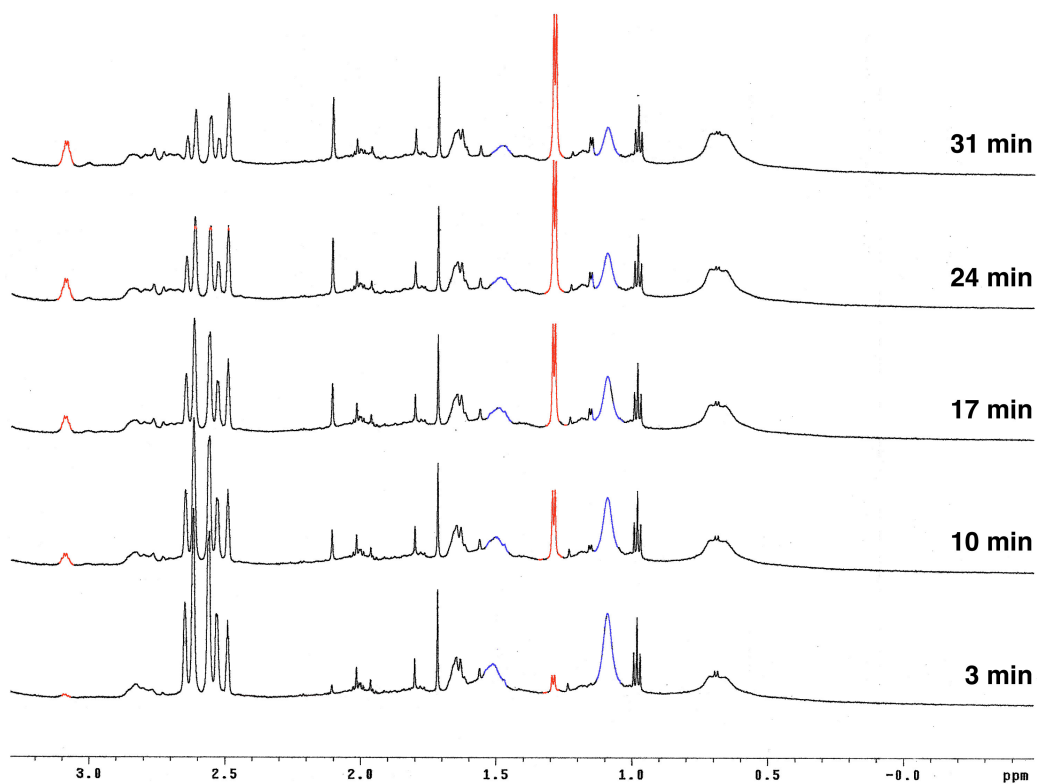
**Figure S4-2.** <sup>1</sup>H NMR spectra of HppE-catalyzed conversion of (S)-7 (blue) to 13 (red).

**Enzymatic reaction between (S)-HPP and HppE that was pretreated with compound 24.**

A freshly prepared solution of 0.35 mM HppE, 7.5 mM FMN, 10 mM NADH, and 8 mM **24** in 600  $\mu$ L of 50 mM Tris buffer (pH 7.5) was pre-incubated for 10 min and then (S)-HPP was added to the reaction mixture.



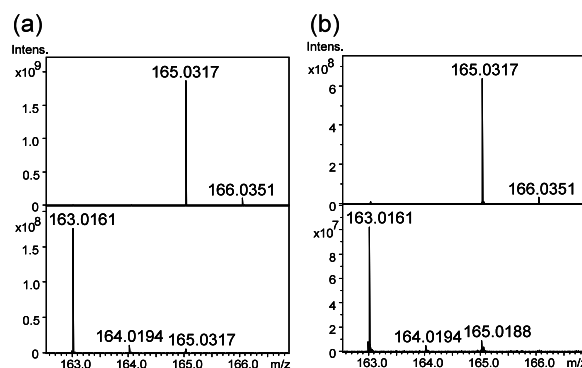
**Figure S4-3.**  $^1\text{H}$  NMR spectra of the enzymatic reaction between (*S*)-HPP (blue) and HppE preincubated with 24. The high-field region is shown below with peaks corresponding to the fosfomycin product labeled in red.



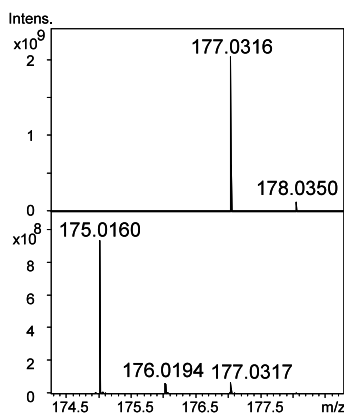
## 5. Analysis of enzymatic reactions by high resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS).

A freshly solution of 5 mM FMN, 20 mM NADH, and 2 mM (*R*)-7, (*S*)-7, or **16** in 20 mM Tris buffer (pH 7.5) was prepared and used as the control sample for each experiment. For the enzymatic reaction samples, reconstituted HppE was added to the above solution prepared in parallel, and the enzyme versus substrate ratio was 1 : 4 for (*R*)-7 and (*S*)-7, and 1.1 : 1 for **16**. Each reaction was allowed to proceed at room temperature for 1 h, and then subjected to centrifugal filtration to remove the protein. The filtrate was collected for MS analysis.

The reaction and control samples were each diluted to 1  $\mu$ M of substrate concentration with an electrospray ionization (ESI) solution of 50% isopropanol immediately prior to MS analysis. All MS analyses were performed on a Solarix 9.4 T: hybrid quadrupole-FTICR mass spectrometer (Burker Daltonik GmbH, Bremen, Germany) equipped with a nano-ESI source, and acquired in negative ion mode ( $m/z$  100-1000) using electrospray voltage of -1300 V. All MS spectra were obtained by quadrupole mass selection of  $m/z$  110 to 210 and accumulation of 20 spectra. The mass accuracy and resolution were about 3 ppm and 200,000, respectively, for  $[M - H]^{-1}$  of cyclopropane-containing substrate analogue (*S*)-7 at  $m/z$  165.0317.



**Figure S5-1.** High resolution mass spectra of HppE-catalyzed reaction products (bottom) and the control experiments (top) obtained with cyclopropyl-containing substrate analogues (a) (*S*)-7 to **13**, and (b) (*R*)-7 to **15**.



**Figure S5-2.** High resolution mass spectra of HppE-catalyzed reaction products (bottom) and the control experiment (top) obtained with methylenecyclopropyl-containing substrate analogue, **16**.

## 6. References.

1. (a) Yan, F.; Munos, J. W.; Liu, P.; Liu, H.-w. *Biochemistry* **2006**, *45*, 11473-11481. (b) Munos, J. W.; Moon, S.-J.; Mansoorabadi, S. O.; Chang, W.; Hong, L.; Yan, F.; Liu, A.; Liu, H.-w. *Biochemistry* **2008**, *47*, 8726-8735.
2. Bradley, P. A.; de Koning, P. D.; Gibson, K. R.; Lecouturier, Y. C.; MacKenny, M. C.; Morao, I.; Poinard, C.; Underwood, T. J. *Syntlett*, **2010**, 873-876.
3. Lai, M. T.; Liu, L. D.; Liu, H.-w. *J. Am. Chem. Soc.* **1991**, *113*, 7388-7397.
4. Yuan, C.; Xie, R. *Phosphorus, Sulfur Silicon Relat. Elem.* **1994**, *90*, 47 – 51.
5. For reference spectra data of (*R*)- and (*S*)-**11**, see: (a) Isabelle Gautier, I.; Ratovelomanana-Vidal, V.; Savignac, P.; Genêt, J.-P. *Tetrahedron Lett.* **1996**, *37*, 7721-7724. (b) Ratovelomanana-Vidal, V.; Genêt, J.-P. *J. Organomet. Chem.* **1998**, *567*, 163-171.
6. For examples of enantiomeric excess (*ee*) determination of hydroxyalkylphosphonate esters, see: Żymańczyk-Duda, E.; Skwarczyński, M.; Lejczak, B.; Kafarski, P. *Tetrahedron Asymm.* **1996**, *7*, 1277-1280.
7. For examples of NH<sub>4</sub>F catalyzed hydrolysis of diphenyl phosphonate esters to free phosphonic acids under mild condition, see: (a) Kim, B.-S.; Kim, B.-T.; Hwang, K.-J. *Bull. Korean Chem. Soc.* **2009**, *30*, 1391-1393. (b) Kim, B.-S.; Kim, B.-T.; Hwang, K.-J. *Bull. Korean. Chem. Soc.* **2010**, *32*, 1643-1648.