Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry. This journal is © The Royal Society of Chemistry 2020

Supporting Information Appendix

Access to chiral α-substituted-β-hydroxy arylphosphonates enabled by biocatalytic dynamic reductive kinetic resolution

Zexu Wang,^[a] Yiping Zeng,^[b] Xiaofan Wu,^[c] Zihan Li,^[a] Yuan Tao,^[a] Xiaomin Yu,^[b] Zedu Huang,^{*[a]} and Fener Chen^{*[a]}

 [a] Department of Chemistry, Engineering Center of Catalysis and Synthesis for Chiral Molecules, Fudan University,
 Shanghai Engineering Research Center of Industrial Asymmetric Catalysis of Chiral drugs, 220 Handan Road, Shanghai, 200433, P. R. China

E-mail: huangzedu@fudan.edu.cn, rfchen@fudan.edu.cn

[b] FAFU-UCR Joint Center for Horticultural Biology and Metabolomics, Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology Fujian Agriculture and Forestry University, Fuzhou, 350002, P. R. China

[c] College of Chemical Engineering, Fuzhou University, 2 Xueyuan Road, Fuzhou, 350116, P. R. China.

*Authors to whom correspondence should be addressed

1

Table of Contents	2
Chemicals	3
Molecular Biology	3
Reagents	3
Cloning Procedures	3
Enzymology	3
Reagents	3
Expression and Purification of KREDs	4
Chemical Synthesis of Substrates	5
Table S1. The details of genes used in this study Figure S1-S2. SDS-PAGE analysis of His6-KREDs after IMAC purification	14 15
Table S2-S7. Screen KREDs against reduction of ketone 1a-1f. KRED-catalyzed DYRKR in semi-preparative scale using cell-free extract (CFE)	16 22
Table S8. Biological tests	36
Table S9. List of oligonucleotides used in this study ¹ H NMR and ¹³ C NMR Spectra	43 44
Chiral HPLC Spectra	166
Crystal data	206

Chemicals

Unless otherwise specified, all reagents and solvent were purchased from commercial sources and used as received. ¹H (400 MHz) and ¹³C (100 MHz) NMR were recorded on a Bruker Avance 400 spectrometer in CDCl₃ using tetramethylsilane (TMS) as internal standards. Coupling constant (*J*) values are given in Hz. Products were purified by flash column chromatography on silica gel purchased from Qingdao Haiyang Chemical Co., Ltd. Optical rotations were measured by a Rudolph AUTOPOL I Automatic Polarimeter. HRMS were recorded on a Bruker micrOTOF spectrometer. HPLC analysis were performed with Daicel Chiralpak IA column (25 cm × 4.6 mm × 5 µm), Chiralpak IC column (25 cm × 4.6 mm × 5 µm).

Molecular Biology

Reagents

Restriction enzymes (NdeI, NcoI, EcoRI and XhoI), PrimeSTAR® HS DNA Polymerase were purchased from TaKaRa (Japan). One Step Cloning Kit ClonExpress® II was purchased from Vazyme (Nanjing, China). PurePlasmid Mini Kit and Gel Extraction Kit were purchased from CWBIO (China). Chemically competent cells of *E. coli* DH5α and *E. coli* BL21 (DE3) were purchased from Transgen (China). Oligonucleotides were purchased from Genewiz (China) in standard, desalted form and used without further purification (Table S16). Synthetic genes (pET28a-KRED-F42, pET28a-CaADH, pET28a-KRED-30) were purchased from Genewiz (China) (Table S3). YDL124w was cloned into pET28a using the primers listed in Table S9. The rest of the enzymes in Table S3 were prepared as we previously described.^[1, 2] All other reagents were purchased from Sangon Biotech (China) unless otherwise specified. LB medium contained yeast extract (5 g/L), tryptone (10 g/L), NaCl (10 g/L). Antibiotics were used at the following concentration: kanamycin: 50 µg/mL.

Cloning procedures

Cloning YDL124w into pET28a vector

PCR products were amplified from genomic DNA of *Saccharomyces cerevisiae* s288c using the PrimeSTAR® HS DNA Polymerase with a T100 Thermal Cycler. The reaction began with an initial denaturing step for 180 s at 98 °C followed by 30 cycles of 10 s at 98 °C, 15 s at 55 °C and 90 s at 72 °C with a final extension step at 72 °C for 300 s. The resulting PCR products were ligated with linearized vectors, which were double digested at appropriate restriction sites (NdeI/XhoI), through homologous recombination using the One Step Cloning Kit ClonExpress® II. In general, a 10 µL reaction mixture containing 2 µL of 5×CE II Buffer, 1 µL of Exnase II, 50-200 ng linearized vector and 20-200 ng PCR amplicon was incubated at 37 °C for 30 min, and the resulting solution was used to transform chemically competent *E. coli* DH5a cells. Colony PCR and sequencing (Genewiz, China) were performed to confirm the sequence fidelity of the recombinant plasmids.

Enzymology

Reagents

Nickel(II)-nitrilotriacetic acid (Ni-NTA) agarose was purchased from Sangon. Amicon ultracentrifugal filters were purchased from EMB Millipore. PD-10 desalting columns were

3

purchased from GE Healthcare. Isopropylthio-β-D-galactoside (IPTG) was obtained from Sangon. Lysis buffer consisted of 50 mM NaPi, 300 mM NaCl, 10 mM imidazole, 10% glycerol, pH 7.5. Wash buffer consisted of 50 mM NaPi, 300 mM NaCl, 20 mM imidazole, 10% glycerol, pH 7.5. Elution buffer consisted of 50 mM NaPi, 300 mM NaCl, 250 mM imidazole, 10% glycerol, pH 7.5. Storage buffer consisted of 50 mM NaPi, 300 mM NaCl, 10% glycerol, pH 7.5.

Expression and purification of His₆-tagged recombinant proteins

An approximately 12 h culture of E. coli BL21 (DE3) cells freshly transformed with the appropriate plasmid and grown in LB medium supplemented with kanamycin (50 µg/mL) was diluted 1 : 100 into 0.5 L of the same medium in a 2 L flask. The culture was shaken at 37 °C until the optical density at 600 nm reached 0.6-0.8, then the flask was placed in an ice/water bath for ca. 30 min before the addition of isopropylthio-β-D-galactoside (IPTG) to a final concentration of 100 µM. The culture was shaken for an additional 12-14 h at 18 °C. All the following purification steps were carried out at 4 °C. The cells (1.5-2 g wet mass from 0.5 L culture) were collected by centrifugation, and then resuspended in 20 mL of lysis buffer. The cells were lysed by sonication on ice and debris was removed by centrifugation at 37,000 x gfor 30 min at 4 °C. The supernatant was loaded onto a column containing 2-3 mL of Ni-NTA resin previously equilibrated with lysis buffer. After equilibration of the resin with the lysate in an orbital shaker for ca. 30 min, the flow-through was discarded and the resin was washed with 2×20 mL of wash buffer. Resin-bound protein was eluted with elution buffer. Fractions of 1 mL were collected and the absorbance at 280 nm was measured by a NanoDrop One spectrophotometer. Fractions with strong absorbance at 280 nm were pooled and concentrated in an Amicon Ultra centrifugal filter unit with 10 kDa molecular weight cut off (MWCO) to a final volume of 2.5 mL. Imidazole and excess salt was removed by passing the protein solution through a PD-10 desalting column previously equilibrated with storage buffer. Protein was eluted with 3.5 mL of storage buffer and stored in aliquots at -80 °C. The protein concentrations were measured by a NanoDrop One spectrophotometer with calculated extinction coefficient and molecular weight.

References

[1] Li, Z.; Wang, Z.; Wang, Y.; Wu, X.; Lu, H.; Huang, Z.; Chen, F. Adv. Synth. Catal. 2019, 361, 1859-1865.

[2] Wang, Z.; Wu, X.; Li, Z.; Huang, Z.; Chen, F. Org. Biomol. Chem. 2019, 17, 3575-3580.

General procedures for the synthesis of substrates Method A



Scheme S1. General procedures for the synthesis of substrates using method A.^[1, 2]

To a stirred solution of methyl benzoate **S1** (1 equiv.) and dimethyl methylphosphonate **S2** (1.1 equiv.) in THF (9 mL) under N₂ atmosphere at -5 °C was added LDA (2.1 equiv.) dropwise. After stirring for 0.5 h, the reaction was quenched with 6 M HCl to pH 4~5. The mixture was then extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **S3**, the NMR spectra of which were in accordance with the literature. Isolated yield: 54~80%.

To a solution of phosphonate S3 (1 equiv.) in CH_2Cl_2 (20 mL) was added SO_2Cl_2 (1 equiv.) at room temperature. After stirring for 20 min at the same temperature, the resulting mixture was heated to reflux until gas evolution ceased. The mixture was then cooled to room temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products 1. Isolated yield: 60~85%.

Method B



Scheme S2. General procedures for the synthesis of substrates using method B.^[3]

To a stirred solution of **S3a** (1 equiv.) and K_2CO_3 (1.5 equiv.) in acetone (8 mL) was added CH₃I (1.5 equiv.) dropwise, and the resulting mixture was stirred at room temperature for 24 h. The reaction was quenched with saturated NH₄Cl and extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **1a.** Isolated yield: 71%.

Method C



Scheme S3. General procedures for the synthesis of substrates using method C.^[3]

To a stirred solution of **S3a** (1 equiv.) and K_2CO_3 (1.5 equiv.) in acetone (8 mL) was added C_2H_3I (1.5 equiv.) dropwise, and the resulting mixture was stirred at room temperature for 24 h. The reaction was quenched with saturated NH₄Cl and extracted with EtOAc (3×100 mL).

The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **1b**. Isolated yield: 64%.

Method D



Scheme S4. General procedures for the synthesis of substrates using method D.^[4]

A mixture of chloro(methoxy)methane S4 (1.1 equiv.) and trimethyl phosphite S5 (1 equiv.) was refluxed for 3 h. The resulting mixture was concentrated *in vacuo* to give dimethyl (methoxymethyl)phosphonate S6, which was used in the next step without further purification. To a stirred solution of methyl benzoate S1 (1 equiv.) and S6 (1.1 equiv.) in THF (9 mL) under N₂ atmosphere at -5 °C was added LDA (2.1 equiv.) dropwise. After 0.5 h, the reaction was quenched with 6 M HCl to pH 4~5. The mixture was then extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products 1c. Isolated yield: 72%.

Method E



Scheme S5. General procedures for the synthesis of substrates using method E.^[5]

A solution of phosphonate **S3a** (1 equiv.) and Selectfluor (2 equiv.) in CH₃CN (100 mL) was refluxed for 24 h. The reaction was quenched by saturated NH₄Cl (100 mL), and followed by the addition of EtOAc (100 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **1d**. Isolated yield: 30%.

Method F



Scheme S6. General procedures for the synthesis of substrates using method F.^[6]

To an ice-cooled solution of phosphonate S3a (1 equiv.) and benzenediazonium chloride S7

(1.1 equiv.), which was prepared *in situ* from aniline and sodium nitrite, in MeOH:H₂O = 5:2 (v:v) was added sodium acetate (5 equiv.). The resulting mixture was first stirred at 0 °C for 30 min, and then heated to 50 °C. The reaction was kept at 50 °C until the complete consumption of phosphonate **S3a** as monitored by TLC. The crude product **S8** was collected by filtration and used in next step without purification.

A solution of Crude **S8** in AcOH (60 mL) was treated with Zn (6 equiv.) and Ac₂O (3.2 equiv.) at room temperature. After the complete consumption of **S8** as monitored by TLC, the formed solid was filtered and washed with AcOH. The filtrate was concentrated *in vacuo* and the residue was dissolved in water (50 mL), and extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **1f**. Isolated yield: 40%.

Method G



Scheme S7. General procedures for the synthesis of substrates using method G.^[1, 2]

A mixture of CH_3I (1.1 equiv.) and triethyl phosphite **S9** (1 equiv.) was refluxed for 6 h, and was then concentrated *in vacuo* to yield diethyl methylphosphonate **S10**, which was used in the next step without further purification.

To a stirred solution of methyl benzoate **S1** (1 equiv.) and **S10** (1.1 equiv.) in THF (9 mL) under N₂ atmosphere at -5 °C was added LDA (2.1 equiv.) dropwise. After 0.5 h, the reaction was quenched with 6 M HCl to pH 4~5. The mixture was then extracted with EtOAc ($3 \times 100 \text{ mL}$). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **S11**. Isolated yields: 50~75%. To a stirred solution of phosphonate **S11** (1 equiv.) in CH₂Cl₂ (20 mL) at room temperature was added SO₂Cl₂ (1 equiv.). After 20 min, the resulting mixture was heated to reflux until gas evolution ceased. The mixture was then cooled to room temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **1**. Isolated yields: $50 \sim 75\%$.

Method H



Scheme S8. General procedures for the synthesis of substrates using method H.^[7, 8]

A mixture of ethynylbenzene **S12** (1.2 equiv.), diisopropyl phosphonate **S13** (1 equiv.), CuI (0.2 equiv.) and NEt₃ (0.3 equiv.) in DMSO (90 mL) was stirred at 55 °C for 2 days. After cooling to room temperature, brine was added. The resulting mixture was extracted with EtOAc (5×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **S14**.

A mixture of **S14** (1 equiv.), H_2O (6 equiv.), and $PdCl_2$ (0.01 equiv.) in Dioxane (15 mL) was heated at 80 °C for 2 h. The reaction mixture was concentrated *in vacuo* and extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products **S15**, the NMR spectra of which were in accordance with the literature.

To a stirred solution of phosphonate S15 (1 equiv.) in CH_2Cl_2 (20 mL) at room temperature was added SO_2Cl_2 (1 equiv.). After 20 min, the resulting mixture was heated to reflux until gas evolution ceased. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography to afford products 1s. Isolated yield: 42%.



dimethyl (1-oxo-1-phenylpropan-2-yl)phosphonate (1a) was synthesized through **Method B** as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.96-7.44 (m, Ar-H, 5H), 4.16 (dq, C₁-H, *J* = 22.9, 7.1 Hz, 1H), 3.72 (d, C₉-H, *J* = 10.9 Hz, 3H), 3.68 (d, C₁₀-H, *J* = 10.9 Hz, 3H), 1.50 (dd, C₁₁-H, *J* = 18.1, 7.1 Hz, 3H). ¹³C NMR (100 MHz,

CDCl₃) δ /ppm 196.32 (d, *J* = 5 Hz), 136.49 (d, *J* = 2 Hz), 133.51, 128.79, 128.60, 53.32 (d, *J* = 6.7 Hz), 40.86 (d, *J* = 130 Hz), 12.40 (d, *J* = 7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 27.03. HRMS Calcd. For C₁₁H₁₅NaO₄P⁺[M+Na⁺]: 265.0600. Found: 265.0604.



dimethyl (1-oxo-1-phenylbutan-2-yl)phosphonate (1b) was synthesized through **Method** C as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.98-7.48 (m, Ar-H, 5H), 4.06 (ddd, C₁-H, *J* = 22.6, 10.2, 4.1 Hz, 1H), 3.74 (d, C₉-H, *J* = 10.9 Hz, 3H), 3.68 (d, C₁₀-H, *J* = 10.9 Hz, 3H), 2.35-2.18 (m, C₁₂-H, 1H), 2.13-1.94 (m, C₁₂-H, 1H),

0.94 (t, C₁₁-H, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 196.30 (d, J = 6 Hz), 137.59 (d, J = 2 Hz), 133.50, 128.66, 128.62, 53.40 (d, J = 7 Hz), 53.24 (d, J = 7 Hz), 48.43 (d, J = 128 Hz), 21.46 (d, J = 6 Hz), 13.14 (d, J = 15 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 25.75. HRMS Calcd. For C₁₂H₁₇NaO₄P⁺[M+Na⁺]: 279.0757. Found: 279.0748.



dimethyl (1-methoxy-2-oxo-2-phenylethyl)phosphonate (1c) was synthesized through Method D as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.10-7.48 (m, Ar-H, 5H), 5.02 (d, C₁-H, *J* = 19.3 Hz, 1H), 3.82 (d, C₉-H, *J* = 10.9 Hz, 3H), 3.76 (d, C₁₀-H, *J* = 10.8 Hz, 3H), 3.49 (s, C₁₁-H, 3H). ¹³C NMR (100 MHz, CDCl₃)

 δ /ppm 194.46, 135.28, 133.90, 129.29, 128.52, 82.98 (d, J = 152 Hz), 60.36 (d, J = 13 Hz),

54.18 (d, J = 7 Hz), 53.96 (d, J = 6 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 17.12. HRMS Calcd. For C₁₁H₁₅NaO₅P⁺[M+Na⁺]: 281.0549. Found: 281.0556.



dimethyl (1-fluoro-2-oxo-2-phenylethyl)phosphonate (1d) was synthesized through **Method** E as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.03-7.50 (m, Ar-H, 5H), 6.05 (dd, C₁-H, *J* = 47.2, 13.4 Hz, 1H), 3.87 (d, C₉-H, *J* = 10.9 Hz, 3H), 3.80 (d, C₁₀-H, J = 10.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 191.08 (d, *J* = 170

Hz), 134.43, 134.04, 129.30 (d, J = 3 Hz), 128.70, 89.98 (dd, J = 196.4 Hz, 153.2Hz), 54.51 (d, J = 6 Hz), 54.42 (d, J = 6 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 27.17 (d, J = 71 Hz). HRMS Calcd. For C₁₀H₁₂FNaO₄P⁺[M+Na⁺]: 269.0349. Found: 269.0351.



dimethyl (1-chloro-2-oxo-2-phenylethyl)phosphonate (1e) was synthesized through **Method A** as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.99-7.47 (m, Ar-H, 5H), 5.48 (d, C₁-H, *J* = 15.8 Hz, 1H), 3.85 (d, C₉-H, *J* = 9 Hz, 3H). 3.82 (d, C₁₀-H, *J* = 9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 189.49 (d, *J* = 2 Hz), 134.49 (d, *J* =

2 Hz), 134.34, 129.34, 128.80, 54.92 (d, J = 7 Hz), 54.82 (d, J = 7 Hz), 51.86 (d, J = 145 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.88. HRMS Calcd. For C₁₀H₁₂ClNaO₄P⁺[M+Na⁺]: 285.0054. Found: 285.0052.



dimethyl (1-acetamido-2-oxo-2-phenylethyl)phosphonate (1f) was synthesized through Method F as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.05-7.48 (m, Ar-H, 5H), 7.20 (d, N-H, J = 8.7 Hz, 1H), 6.27 (dd, C₁-H, J = 21.2, 8.7 Hz, 1H), 3.73 (d, C₉-H, J = 11 Hz, 3H), 3.68 (d, C₁₀-H, J = 11 Hz, 3H), 2.08 (s, C₁₃-H, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 192.67, 169.66 (d, J = 4 Hz), 134.81,

134.27, 129.27, 128.66, 53.94, 53.86, 52.86 (d, J = 143 Hz), 22.82. ³¹P NMR (162 MHz, CDCl₃) δ /ppm 19.08. HRMS Calcd. For C₁₂H₁₆NNaO₅P⁺[M+Na⁺]: 308.0658. Found: 308.0664.



dimethyl (1-chloro-2-oxo-2-(p-tolyl)ethyl)phosphonate (1g) was synthesized through Method A as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.89-7.27 (m, Ar-H, 4H), 5.45 (d, C₁-H, *J* = 15.7 Hz, 1H), 3.86 (d, C₉-H, *J* = 10.5 Hz, 3H), 3.83 (d, C₁₀-H, J = 10.5 Hz, 3H), 2.40 (s, C₁₁-H, 3H). ¹³C NMR (100 MHz,

CDCl₃) δ /ppm 188.95 (d, *J* = 2 Hz), 145.57, 131.96 (d, *J* = 2 Hz), 129.52, 129.50, 54.88 (d, *J* = 7 Hz), 54.78 (d, *J* = 7 Hz), 51.76 (d, *J* = 145 Hz), 21.81. ³¹P NMR (162 MHz, CDCl₃) δ /ppm 16.10. HRMS Calcd. For C₁₁H₁₄ClNaO₄P⁺[M+Na⁺]: 299.0210. Found: 299.0207.



(1-chloro-2-(4-methoxyphenyl)-2dimethyl oxoethyl)phosphonate (1h) was synthesized through Method A as white solid. $^1\mathrm{H}\,\mathrm{NMR}$ (400 MHz, CDCl_3) δ/ppm 8.15-6.75 (m, Ar-H, 4H), 5.42 (d, C₁-H, J = 15.6 Hz, 1H), 4.02-3.70 (m, C_{9, 10, 12}-H, 9H). ¹³C NMR (100 MHz, CDCl₃)

δ/ppm 187.65 (d, J = 2 Hz), 164.52, 131.92, 127.30 (d, J = 3 Hz), 114.03, 55.62, 54.90 (d, J = 6 Hz), 54.74 (d, J = 7 Hz), 51.70 (d, J = 145 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 16.28. HRMS Calcd. For C₁₁H₁₄ClNaO₅P⁺[M+Na⁺]: 315.0160. Found: 315.0157.



O
Pdimethyl(1-chloro-2-(4-fluorophenyl)-2-
oxoethyl)phosphonate (1i) was synthesized through Method A
as white solid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 8.08-7.13 (m, Ar-H, 4H), 5.41 (d, C₁-H, J = 16.1 Hz, 1H), 3.87 (d, C₉-H, J = 6.1 Hz, 3H), 3.84 (d, C_{10} -H, J = 6.1 Hz, 3H). ¹³C NMR (100 MHz,

CDCl₃) δ/ppm 187.97, 166.38 (d, *J* = 256 Hz), 132.32 (d, *J* = 9 Hz), 130.80, 116.05 (d, *J* = 22 Hz), 55.04 (d, J = 7 Hz), 54.84 (d, J = 7 Hz), 52.06 (d, J = 144 Hz). ³¹P NMR (162 MHz, CDCl₃) δ/ppm 15.65. HRMS Calcd. For C₁₀H₁₁ClFNaO₄P⁺[M+Na⁺]: 302.9960. Found: 302.9957.



dimethyl (1-chloro-2-(4-chlorophenyl)-2-oxoethyl)phosphonate (1j) was synthesized through Method A as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.96-7.43 (m, Ar-H, 4H), 5.41 (d, C₁-H, J = 16.2 Hz, 1H), 3.86 (d, C₉-H, J =7.5 Hz, 3H), 3.83 (d, C_{10} -H, J = 7.5 Hz, 3H). ¹³C NMR (100 MHz,

CDCl₃) δ /ppm 188.41 (d, J = 2 Hz), 140.97, 132.71 (d, J = 2 Hz), 130.84, 129.13, 55.04 (d, J = 6 Hz), 54.87 (d, J = 7 Hz), 52.12 (d, J = 145 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.53. HRMS Calcd. For C₁₀H₁₁Cl₂NaO₄P⁺[M+Na⁺]: 318.9664. Found: 318.9662.



(2-(4-bromophenyl)-1-chloro-2-

oxoethyl)phosphonate (1k) was synthesized through **Method A** as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.89-7.61 (m, Ar-H, 4H), 5.40 (d, C₁-H, *J* = 16.2 Hz, 1H), 3.87 (d, C₉-H, *J* = 7.6 Hz, 3H), 3.84 (d, C_{10} -H, J = 7.6 Hz, 3H). ¹³C NMR (100 MHz,

 $CDCl_3$) δ /ppm 188.64 (d, J = 2 Hz), 133.13 (d, J = 2 Hz), 132.14, 130.88, 129.84, 55.06 (d, J = 2 Hz) 7 Hz), 54.88 (d, J = 6 Hz), 52.12 (d, J = 145 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.48. HRMS Calcd. For C₁₀H₁₁⁷⁹BrClNaO₄P⁺[M+Na⁺]: 362.9159. Found: 362.9157.



 (1-chlo
 (trifluoromethyl)phenyl)ethyl)phosphonate
 synthesized through Mathematical (1-chloro-2-oxo-2-(4-**(11)** was synthesized through **Method A** as pink solid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 8.14-7.74 (m, Ar-H, 4H), 5.44 (d, C₁-H, J = 16.5 Hz, 1H), 3.88 (d, C₉-H, J = 7.9 Hz, 3H), 3.85 (d, C₁₀-

H, J = 7.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 188.90 (d, J = 2 Hz), 137.14, 135.30 (q, J = 32 Hz), 129.79, 125.79 (q, J = 7 Hz), 123.34 (q, J = 271 Hz), 55.10 (d, J = 7 Hz), 54.90 (d, J = 7 Hz), 52.44 (d, J = 144 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.15. HRMS Calcd.



dimethyl (1-chloro-2-(3-fluorophenyl)-2oxoethyl)phosphonate (1m) was synthesized through Method A as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.80-7.29 (m, Ar-H, 4H), 5.41 (d, C₁-H, J = 16.1 Hz, 1H), 3.87 (d, C₉-H, J = 8.9 Hz, 3H), 3.84 (d, C₁₀-H, J = 8.9 Hz, 3H). ¹³C NMR (100 MHz,

CDCl₃) δ /ppm 188.49 (d, J = 2.3 Hz), 162.69 (d, J = 248.8 Hz), 136.43 (dd, J = 6.5, 2.7 Hz), 130.49 (d, J = 7.7 Hz), 125.21 (d, J = 3.1 Hz), 121.41 (d, J = 21.5 Hz), 116.09 (d, J = 23.1 Hz), 55.02 (d, J = 6.8 Hz), 54.88 (d, J = 6.9 Hz), 52.05 (d, J = 145.6 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.37. HRMS Calcd. For C₁₀H₁₁ClFNaO₄P⁺[M+Na⁺]: 302.9960. Found: 302.9955.



dimethyl (1-chloro-2-(2-fluorophenyl)-2-oxoethyl)phosphonate (1n) was synthesized through Method A as red solid. ¹H NMR (400 MHz, CDCl₃ δ /ppm 7.90-7.15 (m, Ar-H, 4H), 5.61 (dd, *J* = 17.1, 3.3 Hz, 1H), 3.86 (d, *J* = 11.0 Hz, 3H), 3.83 (d, *J* = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 187.39 (dd, *J* = 7.2, 3.1 Hz), 161.36

(d, J = 254.3 Hz), 135.90 (d, J = 9.3 Hz), 131.49 (d, J = 1.8 Hz), 124.93 (d, J = 3.2 Hz), 123.76 (dd, J = 11.8, 1.7 Hz), 116.66 (d, J = 23.8 Hz), 56.65 (d, J = 10.2 Hz), 55.22 (d, J = 10.1 Hz), 54.80 (d, J = 6.6 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.43. HRMS Calcd. For C₁₀H₁₁ClFNaO₄P⁺[M+Na⁺]: 302.9960. Found: 302.9956.



dimethyl (1-chloro-2-(naphthalen-2-yl)-2oxoethyl)phosphonate (10) was synthesized through Method A as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.56-7.54 (m, Ar-H, 7H), 5.66 (d, C₁-H, J = 15.8 Hz, 1H), 3.89 (d, C₉-H, J = 11.0 Hz, 3H), 3.86 (d, C₁₀-H, J = 11.1 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ/ppm 189.39 (d, J = 2.1 Hz), 136.01, 132.26, 131.81 (d, J = 2.8 Hz), 131.74, 129.95, 129.35, 128.78, 127.82, 127.15, 124.31, 54.97 (d, J = 3.0 Hz), 54.90 (d, J = 3.1 Hz), 51.92 (d, J = 146.0 Hz). ³¹P NMR (162 MHz, CDCl₃) δ/ppm 16.07. HRMS Calcd. For C₁₄H₁₄ClNaO₄P⁺[M+Na⁺]: 335.0210. Found: 335.0201.



dimethyl (1-chloro-2-(furan-2-yl)-2-oxoethyl)phosphonate (1p) was synthesized through Method A as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.67-6.60 (m, Ar-H, 3H), 5.34 (d, C₁-H, *J* = 16.0 Hz, 1H), 3.86 (d, C₇-H, *J* = 11.1 Hz, 3H), 3.83 (d, C₈-H, *J* = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 177.49 (d, *J* = 2.4 Hz),

150.38 (d, J = 2.9 Hz), 148.01, 120.61, 113.29, 54.94 (d, J = 3.9 Hz), 54.87 (d, J = 4.3 Hz), 51.50 (d, J = 144.3 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.40. HRMS Calcd. For C₈H₁₀ClNaO₅P⁺[M+Na⁺]: 274.9847. Found: 274.9841.



dimethyl (1-chloro-2-oxo-2-(thiophen-2-yl)ethyl)phosphonate (1q) was synthesized through Method A as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.92-7.14 (m, Ar-H, 3H), 5.26 (d, C₁-H, *J* = 15.7 Hz, 1H), 3.86 (d, C₇-H, *J* = 7.5 Hz, 3H), 3.84 (d, C₈-H, *J* = 7.7 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 181.83 (d, *J* = 1.9 Hz),

141.02 (d, J = 2.8 Hz), 136.41, 134.99, 128.65, 55.03 (d, J = 6.8 Hz), 54.90 (d, J = 6.9 Hz), 52.74 (d, J = 144.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 15.40. HRMS Calcd. For C₈H₁₀ClNaO₄PS⁺[M+Na⁺]: 290.9618. Found: 290.9594.



diethyl (1-chloro-2-oxo-2-phenylethyl)phosphonate (1r) was synthesized through Method G as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.01 -7.45 (m, Ar-H, 5H), 5.47 (d, C₁-H, *J* = 16.2 Hz, 1H), 4.29-4.15 (m, C_{9, 11}-H, 4H), 1.31 (t, C₁₀-H, *J* = 6.8 Hz, 3H), 1.24 (t, C₁₂-H, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz,

CDCl₃) δ /ppm 189.60 (d, J = 2.2 Hz), 134.83 (d, J = 2.2 Hz), 134.16, 129.35, 128.69, 64.64 (d, J = 6.9 Hz), 64.53 (d, J = 6.7 Hz), 52.71 (d, J = 143.6 Hz), 16.30 (d, J = 5.9 Hz), 16.20 (d, J = 6.2 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 13.20. HRMS Calcd. For C₁₂H₁₆ClNaO₄P⁺[M+Na⁺]: 313.0367. Found: 313.0367.



diisopropyl (1-chloro-2-oxo-2-phenylethyl)phosphonate (1s) was synthesized through Method H as white solid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 8.00 -7.44 (m, Ar-H, 5H), 5.41 (d, C₁-H, *J* = 16.7 Hz, 1H), 4.85-4.72 (m, C_{9, 11}-H, 2H), 1.33 (d, C₁₀-H, *J* = 6.2 Hz, 3H), 1.29 (d, C₁₂-H, *J* = 6.2 Hz, 3H), 1.27 (d, C₁₃-H, *J* = 6.2

Hz, 3H), 1.20 (d, C₁₄-H, J = 6.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 189.71 (d, J = 2.1 Hz), 135.11 (d, J = 1.7 Hz), 133.97, 129.39, 128.58, 73.72 (d, J = 7.2 Hz), 73.56 (d, J = 7.2 Hz), 53.46 (d, J = 144.4 Hz), 24.13 (d, J = 3.4 Hz), 23.96 (d, J = 3.4 Hz), 23.63 (d, J = 6.0 Hz), 23.55 (d, J = 5.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 11.24. HRMS Calcd. For C₁₄H₂₀ClNaO₄P⁺[M+Na⁺]: 341.0680. Found: 341.0666.



diethyl (1-chloro-2-(4-fluorophenyl)-2oxoethyl)phosphonate (1t) was synthesized through Method G as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.11-7.14 (m, Ar-H, 4H), 5.42 (d, C₁-H, J = 16.5 Hz, 1H), 4.30-4.17 (m, C_{9, 11}-H, 4H), 1.33 (t, C₁₀-H, J = 7.1 Hz, 3H), 1.28 (t, C₁₂-H, J

= 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 188.07, 166.26 (d, *J* = 259.2 Hz), 132.29 (dd, *J* = 9.6, 2.0 Hz), 131.19, 115.96, 115.94, 115.74, 115.72, 64.61 (d, *J* = 2.7 Hz), 64.56 (d, *J* = 2.7 Hz), 52.93 (d, *J* = 143.4 Hz), 16.24 (d, *J* = 6.0 Hz), 16.15 (d, *J* = 5.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 13.02. HRMS Calcd. For C₁₂H₁₅ClFNaO₄P⁺[M+Na⁺]: 331.0273. Found: 331.0276.



diethyl (1-chloro-2-(4-chlorophenyl)-2oxoethyl)phosphonate (1u) was synthesized through Method G as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 8.00-7.46 (m, Ar-H, 4H), 5.41 (d, C₁-H, J = 16.6 Hz, 1H), 4.31-4.18 (m, C_{9, 11}-H, 4H), 1.34 (t, C₁₀-H, J = 7.1 Hz, 3H),

1.28 (t, C₁₂-H, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 188.53 (d, J = 1.9 Hz), 140.78, 133.02 (d, J = 2.1 Hz), 130.87, 129.01, 64.70 (d, J = 6.9 Hz), 52.96 (d, J = 143.2 Hz), 16.31 (d, J = 5.7 Hz), 16.22 (d, J = 5.6 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 12.90. HRMS Calcd. For C₁₂H₁₅Cl₂NaO₄P⁺[M+Na⁺]: 346.9977. Found: 346.9977.

References

[1] Tao, X.; Li, W.; Ma, X.; Li, X.; Fan, W.; Zhu, L.; Xie, X.; Zhang, Z. J. Org. Chem. 2012, 77, 8401-8409.

[2] Prévost, S.; Gauthier, s.; Andrade, M. C. C.; Mordant, C.; Touati, A. R.; Lesot, P.; Savignac, P.; Ayad, T.; Phansavath, P.; Ratovelomanana-Vidal, V.; Genêt, J. *Tetrahedron: Asymmetry.* **2012**, *21*, 1436-1446.

[3] Murai, M.; Nakamura, M.; Takai, K. Org. Lett. 2014, 16, 5784-5787.

[4] Son, S.; Lee, H. J. Org. Chem. 2014, 79, 2666-2681.

[5] Radwan-Olszewska, K.; Palacios, F.; Kafarski, P. J. Org. Chem. 2011, 76, 1170-1173.

[6] Tao, X.; Li, W.; Li, X.; Xie, X.; Zhang, Z. Org. Lett. 2013, 15, 72-75.

[7] Song, W.; Zheng, N.; Li, M.; Ullah, K.; Zheng, Y. Adv. Synth. Catal. 2018, 360, 2429-2434.
[8] Li, X.; Hu, G.; Luo, P.; Tang, G.; Gao, Y.; Xu, P.; Zhao, Y. Adv. Synth. Catal. 2012, 354, 2427-2432.

Name	Accession No.	Source	aa
YHR104w	NP_011972	Saccharomyces cerevisiae	327
YDR368w	NP_010656	Saccharomyces cerevisiae	312
YGL039w	NP_011476	Saccharomyces cerevisiae	348
YNL331c	NP_014068.1	Saccharomyces cerevisiae	376
YOL151w	NP_014490.1	Saccharomyces cerevisiae	342
YDL124w	NP_010159.1	Saccharomyces cerevisiae	312
RasADH	EU485985	Ralstonia sp. DSMZ 6428	250
KmCR2	XP_022675166.1	Kluyveromyces marxianus CBS4857	341
SsCR	AF160799.1	Sporidiobolus salmonicolor	343
LkADH	WP_054768785.1	Lactobacillus kefiri	252
SyADH	EU427523.1	Sphingobium yanoikuyae	263
KRED-F42	WP_023468191.1	Exiguobacterium sp. MH3	249
CaADH	WP_010890687.1	Clostridium acetobutylicum	251
KRED-30	AHC30841.1	Chryseobacterium sp. CA49	244

Table S1. The details of genes used in this study.



Figure S1. SDS-PAGE analysis of His₆-KREDs after IMAC purification. Coomassie staining. M: RealBand 3-color Regular Range Protein Marker (Sangon Biotech, China). Lane 1: His₆-SsCR. Lane 2: His₆-YDL124w. Lane 3: His₆-LkADH. Lane 4: His₆-YHR104w. Lane 5: His₆-YNL331c. Lane 6: His₆-KRED-30. Lane 7: His₆-CaADH. Lane 8: His₆-YOL151w. Lane 9: His₆-YGL039w.



Figure S2. SDS-PAGE analysis of His₆-KREDs after IMAC purification. Coomassie staining. M: RealBand 3-color Regular Range Protein Marker (Sangon Biotech, China). Lane 1: His₆-SyADH. Lane 2: His₆-YDR368w. Lane 3: His₆-KRED-F42. Lane 4: His₆-KmCR2. Lane 5: His₆-RasADH.

Screen KREDs against reduction of ketone 1a-1f

To a solution of 10 mM ketone substrate, 20 mM glucose and 0.2 mM NADP⁺ in NaP_i buffer (50 mM, pH 7.0), were added KRED and glucose dehydrogenase (GDH) (1 mg/mL each). The 5 mL Eppendorf tube containing 1 mL of the above mixture was shaken at 200 rpm and 30 °C for 16 h. The reaction mixture was extracted with EtOAc (5 mL) and the organic layer was dried and concentrated and subjected to ¹H NMR and chiral HPLC analyses.

	KREDs GDH, glucc	se, NADP ⁺	<u>^</u>	он о		ОН	0	
	e 50 mM NaF 30 °C, 16 h	P _i , pH 7.0 , 200 rpm	→ 〔〕	* * \\`O 0M	Me e +	**	∽r∖∼OMe OMe	
1a			а	nti -2a		syn -2 a	a	
	Conv. $(\%)^b$	Stereois	Stereois	Stereois	Stereois	dr	ee	ee
		omer I	omer II	omer III	omer IV	(anti:	anti	syn
		$(\%)^c$	(%) ^c	(%) ^c	(%) ^c	syn) ^c	$(\%)^d$	$(\%)^d$
RasADH	90.6	1.82	6.03	0	92.14	11.7:1	>99	n.d. ^e
KmCR2	61.6	5.57	0.10	93.90	0.42	16.6:1	>99	n.d.
YDR368W	$N.R.^{f}$	0	0	0	0	/	/	/
KRED-F42	N.R.	0	0	0	0	/	/	/
YDL124W	N.R.	0	0	0	0	/	/	/
SyADH	12.4	2.13	4.20	3.14	90.53	14.8:1	93	n.d.
YNL331c	>99	41.34	0.13	54.41	4.11	1.4:1	86	>99
YHR104w	N.R.	0	0	0	0	/	/	/
YOL151w	N.R.	0	0	0	0	/	/	/
CaADH	10.1	2.01	97.99	0	0	<1:>9	n.d.	96
						9		
SsCR	12.2	37.40	7.06	55.54	0	1.2:1	>99	68
YGL039w	11.8	78.68	0.10	12.52	8.80	1:3.7	17	>99
LkADH	3.1	7.09	0	58.28	34.63	13.1:1	25	/
KRED-30	9.1	8.82	24.57	40.16	26.50	2:1	20	47

Table S2. Screen KREDs on reduction of α-substituted-β-keto phosphonates 1a^a

^{*a*} Reaction conditions (1 mL): **1a** (10 mM), glucose (20 mM), NADP⁺ (0.2 mM), purified KREDs and GDH (1 mg/mL each) in NaP_i buffer (50 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 16 h. ^{*b*} The reaction conversion was determined by ¹H NMR. ^{*c*} The percentage and the *dr* were determined by chiral HPLC analysis and the relative configuration of the product (syn or anti) was assigned based on the coupling constant observed in ¹H NMR. ^{*d*} The ee was determined by chiral HPLC analysis. ^{*e*} n.d.: not determined. ^{*f*}N.R.: no reaction.

	KREDs GDH, glucc	ose, NADP ⁺	→ ⌒◇		Me + I	OH	о 	e
OMe	50 mM NaF 30 °C, 16 h	P _i , pH 7.0 . 200 rpm		I OM	e ·		OMe	
1b		, _ • • • . p	а	nti -2b		syn -2	b	
	Conv. $(\%)^{b}$	Stereois	Stereois	Stereois	Stereois	dr	ee	ee
		omer I	omer II	omer III	omer IV	(anti:	anti	syn
		(%) ^c	(%) ^c	$(\%)^c$	$(\%)^c$	syn) ^c	$(\%)^d$	$(\%)^d$
RasADH	N.R. ^e	0	0	0	0	/	/	/
KmCR2	N.R.	0	0	0	0	/	/	/
YDR368W	N.R.	0	0	0	0	/	/	/
KRED-F42	N.R.	0	0	0	0	/	/	/
YDL124W	N.R.	0	0	0	0	/	/	/
SyADH	N.R.	0	0	0	0	/	/	/
YNL331c	>99	38.03	0.38	60.23	1.36	1.6:1	96	98
YHR104w	N.R.	0	0	0	0	/	/	/
YOL151w	N.R.	0	0	0	0	/	/	/
CaADH	N.R.	0	0	0	0	/	/	/
SsCR	N.R.	0	0	0	0	/	/	/
YGL039w	N.R.	0	0	0	0	/	/	/
LkADH	N.R.	0	0	0	0	/	/	/
KRED-30	N.R.	0	0	0	0	/	/	/

Table S3. Screen KREDs on reduction of α-substituted-β-keto phosphonates 1b^a

^{*a*} Reaction conditions (1 mL): **1b** (10 mM), glucose (20 mM), NADP⁺ (0.2 mM), purified KREDs and GDH (1 mg/mL each) in NaP_i buffer (50 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 16 h. ^{*b*} The reaction conversion was determined by ¹H NMR. ^{*c*} The percentage and the *dr* were determined by chiral HPLC analysis and the relative configuration of the product (syn or anti) was assigned based on the coupling constant observed in ¹H NMR. ^{*d*} The ee was determined by chiral HPLC analysis. ^{*e*} N.R.: no reaction.

	KREDs GDH, gluco 50 mM NaF 30 °C, 16 h	ose, NADP ⁺ P _i , pH 7.0 , 200 rpm	•		e +	OH	O II P_OMe OMe	
1c	·	•	а	nti -2c		syn -2	с	
	Conv. $(\%)^{b}$	Stereois	Stereois	Stereois	Stereois	dr	ee	ee
		omer I	omer II	omer III	omer IV	(anti:	anti	syn
		(%) ^c	(%) ^c	(%) ^c	$(\%)^c$	syn) ^c	$(\%)^d$	$(\%)^d$
RasADH	31.5	0	71.87	0	28.13	2.6:1	>99	>99
KmCR2	7.4	47.77	49.03	3.20	0	30.2:1	racemi	n.d. ^{<i>e</i>}
							С	
YDR368W	13.8	0	0	0	>99	<1:>9	n.d.	>99
						9		
KRED-F42	32.0	0	60.32	0	39.68	1.5:1	>99	>99
YDL124W	9.9	0	0	36.20	63.80	<1:>9	n.d.	28
						9		
SyADH	7.4	0	46.57	0	53.43	1:1.1	>99	>99
YNL331c	>99	31.24	0.20	68.10	0.47	1:2.2	99	99
YHR104w	7.4	0	0	0	>99	<1:>9	n.d.	>99
						9		
YOL151w	13.0	3.11	87.33	0	9.57	9.5:1	93	>99
CaADH	9.9	0	58.31	0	41.69	1.4:1	>99	>99
SsCR	15.2	2.81	56.90	0.56	39.74	1.5:1	90.6	97
YGL039w	9.9	0	>99	0	0	>99:<	>99	n.d.
						1		
LkADH	7.4	10.43	6.96	30.37	52.25	1:4.8	17	26
KRED-30	33.3	5.19	9.80	6.73	78.28	1:5.7	31	84

Table S4. Screen KREDs on reduction of α-substituted-β-keto phosphonates 1c^a

^{*a*} Reaction conditions (1 mL): **1c** (10 mM), glucose (20 mM), NADP⁺ (0.2 mM), purified KREDs and GDH (1 mg/mL each) in NaP_i buffer (50 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 16 h. ^{*b*} The reaction conversion was determined by ¹H NMR. ^{*c*} The percentage and the *dr* were determined by chiral HPLC analysis and the relative configuration of the product (syn or anti) was assigned based on the coupling constant observed in ¹H NMR. ^{*d*} The ee was determined by chiral HPLC analysis. ^{*e*} n.d.: not determined.

	KREDs GDH, gluco 50 mM NaF	ose, NADP ⁺ P _i , pH 7.0	→ ()		Me e +	OH + +	O II - P_ OMe OMe	9
1d	30 °C, 16 h	, 200 rpm	a	nti-2d		syn- 2	d	
	Conv. $(\%)^b$	Stereois	Stereois	Stereois	Stereois	dr	ee	ee
		omer I	omer II	omer III	omer IV	(anti:	anti	syn
		$(\%)^c$	$(\%)^c$	$(\%)^c$	$(\%)^c$	syn) ^c	$(\%)^d$	$(\%)^d$
RasADH	>99	8.10	79.50	5.06	7.35	7.1:1	82	18
KmCR2	>99	65.33	0.51	8.22	25.95	1.9:1	98	52
YDR368W	69.4	3.52	0	0	96.48	1:27.4	n.d. ^e	>99
KRED-F42	>99	0.66	17.62	81.72	0	1:4.5	93	>99
YDL124W	>99	0	98.85	1.15	0	86.3:1	>99	n.d.
SyADH	>99	12.30	73.97	11.62	2.11	6.3:1	72	69
YNL331c	>99	32.01	9.61	7.37	51.01	1:1.4	54	75
YHR104w	30.6	2.78	93.62	3.60	0	26.7:1	94	n.d.
YOL151w	>99	48.04	13.87	9.60	28.49	1.6:1	55	50
CaADH	>99	19.54	13.99	60.18	6.29	1:2	16	81
SsCR	>99	16.42	10.35	5.06	68.17	1:2.7	23	86
YGL039w	>99	35.75	2.15	0	62.10	1:1.6	89	>99
LkADH	75.2	86.43	0.08	0.22	13.27	6.4:1	>99	97
KRED-30	>99	19.00	0.21	21.55	59.24	1:4.2	98	47

Table S5. Screen KREDs on reduction of α-substituted-β-keto phosphonates 1d^{*a*}

^{*a*} Reaction conditions (1 mL): **1d** (10 mM), glucose (20 mM), NADP⁺ (0.2 mM), purified KREDs and GDH (1 mg/mL each) in NaP_i buffer (50 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 16 h. ^{*b*} The reaction conversion was determined by ¹H NMR. ^{*c*} The percentage and the *dr* were determined by chiral HPLC analysis and the relative configuration of the product (syn or anti) was assigned based on the coupling constant observed in ¹H NMR. ^{*d*} The ee was determined by chiral HPLC analysis. ^{*e*} n.d.: not determined.

	KREDs GDH, glucc 50 mM NaF 30 °C, 16 h	ose, NADP ⁺ P _i , pH 7.0 , 200 rpm	•		Me +	OH * * C	O P OMe	
1e			а	nti -2e		syn- 2 0	е	
	Conv. $(\%)^b$	Stereois	Stereois	Stereois	Stereois	dr	ee	ee
		omer I	omer II	omer III	omer IV	(anti:	anti	syn
		(%) ^c	(%) ^c	(%) ^c	(%) ^c	syn) ^c	$(\%)^d$	$(\%)^d$
RasADH	>99	0.50	8.45	2.99	88.06	7.7:1	99	65
KmCR2	>99	96.31	3.56	0.14	0	26.1:1	>99	n.d. ^e
YDR368W	25.5	1.09	98.91	0	0	1:90.6	n.d.	>99
KRED-F42	71.9	11.30	4.07	61.22	23.41	1:1.9	35	88
YDL124W	47.4	0.52	32.56	7.50	59.42	1.5:1	98	62
SyADH	91.7	10.31	1.16	3.30	85.23	21.4:1	78	n.d.
YNL331c	>99	15.62	57.85	0.20	26.33	1:1.4	26	>99
YHR104w	$N.R.^{f}$	0	0	0	0	/	/	/
YOL151w	24.2	83.21	11.89	4.90	0	5:1	>99	42
CaADH	25.5	22.07	6.38	57.85	13.71	1:1.8	23	80
SsCR	>99	34.03	65.96	0	0	1:1.9	>99	>99
YGL039w	>99	25.57	70.14	0.09	4.20	1:2.4	72	>99
LkADH	39.4	99.47	0.53	0	0	>99:<	>99	n.d.
						1		
KRED-30	>99	29.14	7.10	7.93	55.83	5.6:1	31	6

Table S6. Screen KREDs on reduction of α-substituted-β-keto phosphonates 1e^a

^{*a*} Reaction conditions (1 mL): **1e** (10 mM), glucose (20 mM), NADP⁺ (0.2 mM), purified KREDs and GDH (1 mg/mL each) in NaP_i buffer (50 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 16 h. ^{*b*} The reaction conversion was determined by ¹H NMR. ^{*c*} The percentage and the *dr* were determined by chiral HPLC analysis and the relative configuration of the product (syn or anti) was assigned based on the coupling constant observed in ¹H NMR. ^{*d*} The ee was determined by chiral HPLC analysis. ^{*e*} n.d.: not determined. ^{*f*} N.R.: no reaction.

	KREDs GDH, glucc 50 mM NaF 30 °C, 16 h	ose, NADP ⁺ P _i , pH 7.0 , 200 rpm	•		e +	OH + HN	O II P_OMe OMe)
1f			а	nti- 2f		syn -2	f	
	Conv. $(\%)^b$	Stereois	Stereois	Stereois	Stereois	dr	ee	ee
		omer I	omer II	omer III	omer IV	(anti:	anti	syn
		$(\%)^c$	(%) ^c	(%) ^c	$(\%)^c$	syn) ^c	$(\%)^d$	$(\%)^d$
RasADH	N.R. ^e	0	0	0	0	/	/	/
KmCR2	N.R.	0	0	0	0	/	/	/
YDR368W	N.R.	0	0	0	0	/	/	/
KRED-F42	N.R.	0	0	0	0	/	/	/
YDL124W	N.R.	0	0	0	0	/	/	/
SyADH	N.R.	0	0	0	0	/	/	/
YNL331c	>99	0	99.68	0.17	0.14	>99:<	>99	n.d.
						1		
YHR104w	N.R.	0	0	0	0	/	/	/
YOL151w	N.R.	0	0	0	0	/	/	/
CaADH	N.R.	0	0	0	0	/	/	/
SsCR	N.R.	0	0	0	0	/	/	/
YGL039w	N.R.	0	0	0	0	/	/	/
LkADH	N.R.	0	0	0	0	/	/	/
KRED-30	N.R.	0	0	0	0	/	/	/

Table S7. Screen KREDs on reduction of α-substituted-β-keto phosphonates 1f^a

^{*a*} Reaction conditions (1 mL): **1f** (10 mM), glucose (20 mM), NADP⁺ (0.2 mM), purified KREDs and GDH (1 mg/mL each) in NaP_i buffer (50 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 16 h. ^{*b*} The reaction conversion was determined by ¹H NMR. ^{*c*} The percentage and the *dr* were determined by chiral HPLC analysis and the relative configuration of the product (syn or anti) was assigned based on the coupling constant observed in ¹H NMR. ^{*d*} The ee was determined by chiral HPLC analysis. ^{*e*} N.R.: no reaction.

KRED-catalyzed DYRKR in semi-preparative scale using cell-free extract (CFE)

The semi-preparative scale reaction was carried out with ketone (125 mg), glucose (1 g), NADP⁺ (50 mg), 35 mL 30% (w/v) CFE of KREDs in NaP_i buffer (50 mM, pH 7.0), 10 mL 15% (w/v) CFE of GDH in NaP_i buffer (50 mM, pH 7.0) and 5 mL DMSO at 30 °C and 900 rpm for 24 h. Silica gel was added to the reaction mixture and subjected to centrifugation. The supernatant was extracted with EtOAc. The organic layer was then washed with brine and dried with anhydrous Na₂SO₄, then filtered, and the filtrate was evaporated to dryness. The product was purified by flash chromatography. The *dr* was determined by chiral HPLC analysis and the relative configuration of the product (*syn* or *anti*) was assigned based on the coupling constant observed in ¹H NMR. The ee was determined by chiral HPLC analysis, and the absolute configuration was assigned by X-ray crystallography.



anti-(1*S*, 2*S*)-dimethyl (1-hydroxy-1-phenylpropan-2yl)phosphonate (*anti*-(1*S*, 2*S*)-2a) which catalyzed by KmCR2 was purified by chromatography on silica gel with 70.6% yield as white solid.¹H NMR (400 MHz, CDCl₃) δ /ppm 7.37-7.28 (m, Ar-H, 5H), 4.74 (dd, C₂-H, *J* = 10.3, 10.3 Hz, 1H), 4.49 (br, O-H,1H), 3.84 (d,

 C_9 -H, J = 10.6 Hz, 3H), 3.76 (d, C_{11} -H, J = 10.8 Hz, 3H), 2.27 (ddq, C_1 -H, J = 16.8, 9.4, 7.4 Hz, 1H), 0.90 (dd, C_{10} -H, J = 17.5, 7.3 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 141.42 (d, J = 15.5 Hz), 128.41, 128.05, 126.88, 74.76 (d, J = 3.6 Hz), 52.77 (d, J = 6.7 Hz), 38.81 (d, J = 135.6 Hz), 11.79 (d, J = 5.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 35.96. [α]₂₅^D = 27.18 (c = 0.5, CHCl₃). HPLC Chiracel[®] IF, 250 × 4.6 mm column, hexane/2-propanol 93:7, 0.8 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=14.2 min, t₂=15.7 min, t₃=21.0 min (major), t₄=22.2 min. ee = 99.0%, dr = 12.7:1. HRMS Calcd. For C₁₁H₁₇NaO₄P⁺[M+Na⁺]: 267.0757. Found: 267.0748.



anti-(1*R*, 2*R*)-dimethyl (1-hydroxy-1-phenylpropan-2yl)phosphonate (*anti*-(1*R*, 2*R*)-2a) which catalyzed by RasADH was purified by chromatography on silica gel with 55.5% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.43-7.22 (m, Ar-H, 5H), 4.75 (dd, C₂-H, *J* = 11.1, 9.4 Hz, 1H), 4.47 (s, O-H, 1H), 3.85

(d, C₉-H, J = 10.5 Hz, 3H), 3.77 (d, C₁₀-H, J = 10.7 Hz, 3H), 2.28 (ddq, C₁-H, J = 16.9, 9.4, 7.4 Hz, 1H), 0.90 (dd, C₁₀-H, J = 17.5, 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 141.39 (d, J = 15.6 Hz), 128.43, 128.07, 126.88, 74.79 (d, J = 3.7 Hz), 52.78 (d, J = 7.4 Hz), 38.82 (d, J = 135.6 Hz), 11.81 (d, J = 5.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 35.97. [α]₂₅^D= -23.18 (c = 0.5, CHCl₃). HPLC Chiracel[®] IF, 250 × 4.6 mm column, hexane/2-propanol 93:7, 0.8 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=14.2 min, t₂=15.7 min, t₃=21.0 min, t₄=22.2 min (major). ee = >99%, dr = 26.3:1. HRMS Calcd. For C₁₁H₁₇NaO₄P⁺[M+Na⁺]: 267.0757. Found: 267.0751.



anti-(1*R*, 2*R*)-dimethyl (1-fluoro-2-hydroxy-2phenylethyl)phosphonate (*anti*-(1*R*, 2*R*)-2d) which catalyzed by YDL124w was purified by chromatography on silica gel with 71.4% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.53-7.30 (m, Ar-H, 5H), 5.18-5.08 (m, C₂-H, 1H), 4.79 (ddd, C₁-H, *J* = 45.4,

7.8, 2.1 Hz, 1H), 4.18-3.96 (br, O-H, 1H), 3.85 (d, C₉-H, J = 10.6 Hz, 3H), 3.74 (d, C₁₀-H, J = 10.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.70 (dd, J = 9.8, 2.1 Hz), 128.52, 128.46, 126.88, 89.74 (dd, J = 189.4, 162.9 Hz), 71.92 (dd, J = 22.3, 1.6 Hz), 54.23 (dd, J = 6.9, 2.0 Hz), 53.30 (d, J = 6.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 19.93 (d, J = 73.4 Hz). [α]₂₅D = -59.96 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 0.3 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=43.3 min, t₂=85.6 min (major), t₃=95.4 min, t₄=104.4 min. ee = 73.4%, dr = 6.7:1. HRMS Calcd. For C₁₀H₁₄FNaO₄P⁺[M+Na⁺]: 271.0506. Found: 271.0491.



syn-dimethyl (1-fluoro-2-hydroxy-2-phenylethyl)phosphonate (*syn*-2d) which catalyzed by YDR368w was purified by chromatography on silica gel with 35.7% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.51-7.32 (m, Ar-H, 5H), 5.19 (ddd, C₂-H, *J* = 25.3, 4.1, 4.1 Hz, 1H), 4.89 (ddd, C₁-H, *J* = 45.2, 5.6, 3.7

catalyzd by YDR368w

catalyzd by YDR368w Hz, 1H), 3.85 (d, C₉-H, J = 10.7 Hz, 3H), 3.71 (d, C₁₀-H, J = 10.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.30 (dd, J = 9.7, 3.4 Hz), 128.50, 128.47, 126.91, 90.93 (dd, J = 190.5, 165.9 Hz), 72.26 (dd, J = 18.6, 2.6 Hz), 54.35 (dd, J = 6.4, 1.5 Hz), 52.92 (d, J = 7.0 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 18.85 (d, J = 78.3 Hz). [α]₂₅^D = 56.10 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 0.3 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=43.3 min, t₂=85.6 min, t₃=95.4 min, t₄=104.4 min (major). ee = 98.6%, dr = 18.6:1. HRMS Calcd. For C₁₀H₁₄FNaO₄P⁺[M+Na⁺]: 271.0506. Found: 271.0499.



syn-dimethyl (1-fluoro-2-hydroxy-2-phenylethyl)phosphonate (*syn*-2d) which catalyzed by KRED-F42 was purified by chromatography on silica gel with 78.6% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm7.60-7.17 (m, Ar-H, 5H), 5.19 (ddd, C₂-H, *J* = 25.5, 4.2, 4.2 Hz, 1H), 4.89 (ddd, C₁-H, *J* = 45.3, 5.7, 3.6 Hz, 1H), 3.85 (d, C₉-H, *J* = 10.7 Hz, 3H), 3.71 (d, C₁₀-H, *J* = 10.8

Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.30 (dd, J = 9.6, 3.5 Hz), 128.50, 128.46, 126.91, 126.87, 90.94 (dd, J = 190.4, 165.9 Hz), 72.25 (dd, J = 18.6, 2.6 Hz), 54.35 (dd, J = 6.3, 1.4 Hz), 52.93 (d, J = 6.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 18.86 (d, J = 78.3 Hz). [α]₂₅^D = -249.41 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 0.3 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=43.3 min, t₂=85.6 min, t₃=95.4 min (major), t₄=104.4 min. ee > 99%, dr = 5.6:1. HRMS Calcd. For C₁₀H₁₄FNaO₄P⁺[M+Na⁺]: 271.0506. Found: 271.0489.



anti-(1*S*, 2*S*)- dimethyl (1-chloro-2-hydroxy-2phenylethyl)phosphonate (*anti*-(1*S*, 2*S*)-2e) which catalyzed by KmCR2 was purified by chromatography on silica gel with 79.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.49-7.30 (m, Ar-H, 5H), 5.01 (dd, C₂-H, *J* = 10.1, 10.1 Hz, 1H), 4.25 (br, O-

H, 1H), 4.07 (dd, C₁-H, J = 8.8, 8.8Hz, 1H), 3.91 (d, C₉-H, J = 10.7 Hz, 3H), 3.77 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 139.44 (d, J = 10.7 Hz), 128.59, 128.33, 127.16, 74.59, 54.90 (d, J = 7.3 Hz), 54.11 (d, J = 152.1 Hz), 53.83 (d, J = 6.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.26. [α]₂₅^D = 35.17 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=11.6 min (major), t₂=14.9 min, t₃=16.6 min, t₄=44.5 min. ee > 99%, dr = 21.5:1. HRMS Calcd. For C₁₀H₁₄ClNaO₄P⁺[M+Na⁺]: 287.0210. Found: 287.0207.



anti-(1*R*, 2*R*)- dimethyl (1-chloro-2-hydroxy-2phenylethyl)phosphonate (*anti*-(1*R*, 2*R*)-2e) which catalyzed by RasADH was purified by chromatography on silica gel with 66.7% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.53-7.30 (m, Ar-H, 5H), 5.04 (dd, C₂-H, *J* = 11.7, 8.4 Hz, 1H), 4.37 (br, O-H,

1H), 4.10 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.92 (d, C₉-H, J = 10.6 Hz, 1H), 3.79 (d, C₁₀-H, J = 11.0 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 139.42 (d, J = 10.8 Hz), 128.60, 128.33, 127.16, 74.60, 54.91 (d, J = 7.0 Hz), 54.09 (d, J = 151.3 Hz), 53.84 (d, J = 6.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.25. [α]₂₅^D = -34.97 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=11.6 min, t₂=14.9 min, t₃=16.6 min, t₄=44.5 min (major). ee = 99.6%, dr = 9.8:1. HRMS Calcd. For C₁₀H₁₄ClNaO₄P⁺[M+Na⁺]: 287.0210. Found: 287.0208.



anti-dimethyl (1-acetamido-2-hydroxy-2phenylethyl)phosphonate (*anti*-2f) which catalyzed by YNL331c was purified by chromatography on silica gel with 82.6% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.94-7.65 (m, Ar-H, 5H), 7.08 (d, N-H, J = 9.8 Hz, 1H), 5.46 (dd, C₂-H, J = 21.9, 5.3 Hz, 1H), 5.32 (ddd, C₁-H, J = 15.5, 9.9, 5.3 Hz, 1H), 4.14 (d, C₉-H, J = 10.8 Hz, 3H), 3.87 (d, C₁₀-H, J = 11.0 Hz, 3H), 2.41 (s, C₁₂-H,

3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 169.71 (d, *J* = 5.1 Hz), 139.46 (d, *J* = 5.1 Hz), 128.26, 127.98, 126.26, 74.05 (d, *J* = 1.9 Hz), 53.43 (d, *J* = 7.0 Hz), 52.99 (d, *J* = 6.8 Hz), 51.19 (d, *J* = 151.6 Hz)., 22.94. ³¹P NMR (162 MHz, CDCl₃) δ /ppm 25.84. [α]₂₅^D = -1.0 (*c* = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=28.7 min, t₂=31.0 min (major), t₃=34.6 min, t₄=41.3 min. *ee* > 99.6%, *dr* = 31.2:1. HRMS Calcd. For C₁₂H₁₈NNaO₅P⁺[M+Na⁺]: 310.0815. Found: 310.0817.



anti-(1*S*, 2*S*)-2gimethyl (1-chloro-2-hydroxy-2-(p-tolyl)ethyl)phosphonate *anti*-(1*S*, 2*S*)-2g which catalyzed by KmCR2 was purified by chromatography on silica gel with 56.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.35-7.13 (m, Ar-H, 4H), 4.97 (ddd, C₂-H, *J* = 11.6, 8.5, 3.3 Hz, 1H),

4.20 (d, O-H, J = 3.6 Hz, 1H), 4.06 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.90 (d, C₉-H, J = 10.7 Hz, 3H), 3.78 (d, C₁₀-H, J = 11.0 Hz, 3H), 2.35 (s, C₁₁-H, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.41, 136.44 (d, J = 11.2 Hz), 129.04, 127.05, 74.45, 54.88 (d, J = 7.1 Hz), 54.07 (d, J = 150.9 Hz), 53.85 (d, J = 6.8 Hz), 21.23. ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.41. [α]₂₅^D = 25.18 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=14.9 min (major), t₂=18.9 min, t₃=24.7 min, t₄=45.8 min. ee = 99.0%, dr = 47.2:1. HRMS Calcd. For C₁₁H₁₆ClNaO₄P⁺[M+Na⁺]: 301.0367. Found: 301.0374.



anti-(1*R*, 2*R*)-2gimethyl (1-chloro-2-hydroxy-2-(p-tolyl)ethyl)phosphonate *anti*-(1*R*, 2*R*)-2g which catalyzed by RasADH was purified by chromatography on silica gel with 77.8% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.44-7.05 (m, Ar-H, 4H), 5.05-4.95 (m, C₂-H, 1H), 4.32 (d, O-H, *J* =

3.7 Hz, 1H), 4.08 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.91 (d, C₉-H, J = 10.7 Hz, 3H), 3.80 (d, C₁₀-H, J = 10.9 Hz, 3H), 2.37 (s, C₁₁-H, 1H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.35, 136.60 (d, J = 10.9 Hz), 129.01, 127.09, 74.36, 54.83 (d, J = 7.0 Hz), 54.23 (d, J = 151.6 Hz), 53.86 (d, J = 6.8 Hz), 21.24. ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.41. [α]₂₅^D = -23.58 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=14.9 min, t₂=18.9 min, t₃=24.7 min, t₄=45.8 min (major). *ee* = 99.7%, *dr* = 11.4:1. HRMS Calcd. For C₁₁H₁₆ClNaO₄P⁺[M+Na⁺]: 301.0367. Found: 301.0373.



anti-(1*S*, 2*S*)-dimethyl (1-chloro-2-hydroxy-2-(4methoxyphenyl)ethyl)phosphonate (*anti*-(1*S*, 2*S*)-2h) which catalyzed by KmCR2 was purified by chromatography on silica gel with 42.9% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.42-6.74 (m, 4H), 4.96 (dd, C₂-H, *J* =

10.9, 8.6 Hz, 1H), 4.18 (br, O-H, 1H), 4.04 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.91 (d, C₉-H, J = 10.7 Hz, 3H), 3.81 (s, C₁₁-H, 3H), 3.79 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 159.77, 131.59 (d, J = 11.3 Hz), 128.38, 113.71, 74.15, 55.28, 54.86 (d, J = 7.0 Hz), 54.22 (d, J = 150.5 Hz), 53.87 (d, J = 6.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.41. [α]₂₅^D = 21.78 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 70:30, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=12.2 min (major), t₂=15.3 min, t₃=19.2 min, t₄=40.2 min. ee = 88.0%, dr = 9.8:1. HRMS Calcd. For C₁₁H₁₆ClNaO₅P⁺[M+Na⁺]: 317.0316. Found: 317.0317.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-hydroxy-2-(4methoxyphenyl)ethyl)phosphonate (*anti*-(1*R*, 2*R*)-2h) which catalyzed by RasADH was purified by chromatography on silica gel with 79.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.45-6.63 (m, Ar-H, 4H), 4.96 (ddd, C₂-

H, J = 10.9, 8.5, 2.5 Hz, 1H), 4.24 (d, O-H, J = 3.5 Hz, 1H), 4.04 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.89 (d, C₉-H, J = 10.7 Hz, 3H), 3.80 (s, C₁₁-H), 3.79 (d, C₁₀-H, J = 11.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 159.76, 131.61 (d, J = 11.1 Hz), 128.38, 113.70, 74.14, 55.28, 54.85 (d, J = 7.0 Hz), 54.25 (d, J = 150.8 Hz), 53.87 (d, J = 6.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.39. [α]₂₅^D = -23.98 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 70:30, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=12.2 min, t₂=15.3 min, t₃=19.2 min, t₄=40.2 min (major). ee = 99.8%, dr = 20.6:1. HRMS Calcd. For C₁₁H₁₆ClNaO₅P⁺[M+Na⁺]: 317.0316. Found: 317.0316.



anti- (1*S*, 2*S*)-dimethyl (1-chloro-2-(4-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*- (1*S*, 2*S*)-2i) which catalyzed by KmCR2 was purified by chromatography on silica gel with 75.5% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.51-6.93 (m, Ar-H, 4H), 5.02 (dd, C₂-H, *J* = 9.7, 9.7 Hz, 1H), 4.49

(br, O-H, 1H), 4.03 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.92 (d, C₉-H, J = 10.7 Hz, 3H), 3.81 (d, C₁₀-H, J = 10.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 162.76 (d, J = 247.0 Hz), 35.28 (dd, J = 11.2, 3.2 Hz), 128.94 (d, J = 8.2 Hz), 115.21 (d, J = 21.6 Hz), 73.87, 54.95 (d, J = 7.0 Hz), 54.10 (d, J = 151.3 Hz), 53.91 (d, J = 6.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.04. [α]₂₅^D = 30.18 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=8.1 min (major), t₂=9.9 min, t₃=10.6 min, t₄=36.0 min. ee = 98.9%, dr = 13.2:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0132.



anti- (1*R*, 2*R*)-dimethyl (1-chloro-2-(4-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*- (1*R*, 2*R*)-2i) which catalyzed by RasADH was purified by chromatography on silica gel with 89.0% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.50-7.02 (m, Ar-H, 4H), 5.02 (dd, C₂-H, *J* = 10.9, 8.6 Hz, 1H),

4.47 (br, O-H, 1H), 4.03 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.93 (d, C₉-H, J = 10.7 Hz, 1H), 3.82 (d, C₁₀-H, J = 10.9 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 1 162.77 (d, J = 246.9 Hz), 135.26 (dd, J = 11.1, 3.1 Hz), 128.94 (d, J = 8.4 Hz), 115.21 (d, J = 21.6 Hz), 73.89, 54.96 (d, J = 7.2 Hz), 54.08 (d, J = 151.2 Hz), 53.91 (d, J = 6.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.04. [α]₂₅^D = -33.38 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=8.1 min, t₂=9.9 min, t₃=10.6 min, t₄=36.0 min (major). ee = 99.6%, dr = 6.7:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0114.



anti-(1*S*, 2*S*)-dimethyl (1-chloro-2-(4-chlorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*S*, 2*S*)-2j) which catalyzed by KmCR2 was purified by chromatography on silica gel with 64.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.43-7.30 (m, Ar-H, 4H), 4.98 (dd, C₂-H, *J* = 11.2, 8.5 Hz, 1H),

4.44 (br, O-H, 1H), 3.99 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.91 (d, C₉-H, J = 10.7 Hz, 3H), 3.79 (d, C₁₀-H, J = 10.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 137.90 (d, J = 11.3 Hz), 134.39, 128.59, 128.49, 73.94, 55.01 (d, J = 7.2 Hz), 53.93 (d, J = 6.8 Hz), 53.87 (d, J = 151.1 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.94. [α]₂₅^D = 24.58 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.5 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=19.6 min (major), t₂=24.6 min, t₃=25.8 min, t₄=82.3 min. *ee* = 88.8%, *dr* = 11.2:1. HRMS Calcd. For C₁₀H₁₃Cl₂NaO₄P⁺[M+Na⁺]: 320.9821. Found: 320.9822.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-(4-chlorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2j) which catalyzed by RasADH was purified by chromatography on silica gel with 66.7% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.49-7.30 (m, Ar-H, 4H), 5.01 (dd, C₂-H, *J* = 11.0, 8.5 Hz, 1H),

anti-(1*R*, 2*R*)-**2**j 7.49-7.30 (m, Ar-H, 4H), 5.01 (dd, C₂-H, *J* = 11.0, 8.5 Hz, 1H), 4.57 (br, O-H, 1H), 4.03 (dd, C₁-H, *J* = 9.5, 8.4 Hz, 1H), 3.92 (d, C₉-H, *J* = 10.7 Hz, 3H), 3.81 (d, C₁₀-H, *J* = 10.9 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.00 (d, *J* = 11.2 Hz), 128.61, 128.46, 73.89, 54.97 (d, *J* = 7.0 Hz), 53.98 (d, *J* = 151.6 Hz), 53.92 (d, *J* = 6.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.92. [α]₂₅^D = -16.39 (*c* = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.5 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=19.6 min, t₂=24.6 min, t₃=25.8 min, t₄=82.3 min (major). *ee* = 99.7%, *dr* = 7.4:1. HRMS Calcd. For C₁₀H₁₃Cl₂NaO₄P⁺[M+Na⁺]: 320.9821. Found: 320.9822.



anti-(1*S*, 2*S*)-dimethyl (2-(4-bromophenyl)-1-chloro-2hydroxyethyl)phosphonate (*anti*-(1*S*, 2*S*)-2k) which catalyzed by KmCR2 was purified by chromatography on silica gel with 50.9% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.58-7.28 (m, Ar-H, 4H), 4.97 (ddd, C₂-H, *J* = 11.4, 8.6, 2.7 Hz,

1H), 4.41 (d, O-H, J = 3.5 Hz, 1H), 3.99 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.92 (d, C₉-H, J = 10.7 Hz, 3H), 3.79 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.47 (d, J = 11.3 Hz), 131.43, 128.92, 122.55, 73.98, 55.00 (d, J = 7.0 Hz), 53.93 (d, J = 6.9 Hz), 53.85 (d, J = 151.3 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.92. [α]₂₅^D = 14.39 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.7 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=14.2 min (major), t₂=17.8 min, t₃=18.7 min, t₄=56.1 min. ee = 90.0%, dr = 15.1:1. HRMS Calcd. For C₁₀H₁₃⁷⁹BrClNaO₄P⁺[M+Na⁺]: 364.9316. Found: 364.9318.



anti-(1*R*, 2*R*)-dimethyl (2-(4-bromophenyl)-1-chloro-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2k) which catalyzed by RasADH was purified by chromatography on silica gel with 68.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.55-7.30 (m, Ar-H, 4H), 4.99 (dd, C₂-H, *J* = 9.8, 9.8 Hz, 1H),

4.55 (br, O-H, 1H), 4.02 (dd, C₁-H, J = 9.5, 8.4 Hz, 1H), 3.92 (d, C₉-H, J = 10.7 Hz, 3H), 3.81 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.51 (d, J = 11.1 Hz), 131.42, 128.93, 122.54, 73.96, 54.98 (d, J = 7.2 Hz), 53.93 (d, J = 6.8 Hz), 53.89 (d, J = 151.5 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.89. [α]₂₅^D = -13.59 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.7 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=14.2 min, t₂=17.8 min, t₃=18.7 min, t₄=56.1 min (major). *ee* = 99.8%, *dr* = 10.9:1. HRMS Calcd. For C₁₀H₁₃⁷⁹BrClNaO₄P⁺[M+Na⁺]: 364.9316. Found: 364.9319.



anti-(1*S*, 2*S*)-dimethyl (1-chloro-2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)phosphonate (*anti*-(1*S*, 2*S*)-2l) which catalyzed by KmCR2 was purified by chromatography on silica gel with 51.7% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 7.75-7.45 (m, Ar-H, 4H),

5.06 (dd, C₂-H, J = 9.8, 9.8 Hz, 1H), 4.56 (d, O-H, J = 3.6 Hz, 1H), 4.02 (dd, C₁-H, J = 9.5, 8.5 Hz, 1H), 3.93 (d, C₉-H, J = 10.7 Hz, 3H), 3.79 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 143.58 (d, J = 10.9 Hz), 130.60 (q, J = 32.4 Hz), 127.69, 125.18 (q, J = 3.7 Hz), 124.03 (q, J = 272.0 Hz), 73.90, 54.98 (d, J = 7.0 Hz), 53.98 (d, J = 153.0 Hz), 53.93 (d, J = 6.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.76. [α]₂₅^D = 12.79 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 90:10, 0.5 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=16.5 min (major), t₂=19.0 min, t₃=20.0 min, t₄=61.8 min. ee = 86.8%, dr = 12.3:1. HRMS Calcd. For C₁₁H₁₃ClF₃NaO₄P⁺[M+Na⁺]: 355.0084. Found: 355.0085.



anti-(1R,2R)-dimethyl(1-chloro-2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)phosphonate(anti-(1R,2R)-2l)which catalyzed by RasADH was purified bychromatography on silica gel with 77.1% yield as white solid.¹H NMR (400 MHz, CDCl₃) δ/ppm 7.72-7.47 (m, Ar-H, 4H),

5.09 (dd, C₂-H, J = 11.5, 8.4 Hz, 1H), 4.65 (d, O-H, J = 3.6 Hz, 1H), 4.05 (dd, C₁-H, J = 9.5, 8.4 Hz, 1H), 3.94 (d, C₉-H, J = 10.7 Hz, 3H), 3.81 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 143.61 (d, J = 10.8 Hz), 130.58 (q, J = 32.4 Hz), 127.70, 124.02 (q, J = 272.1 Hz), 125.16 (q, J = 3.8 Hz), 73.87, 54.95 (d, J = 7.2 Hz), 54.01 (d, J = 152.9 Hz), 53.92 (d, J = 6.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.73. [α]₂₅^D = -19.79 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 90:10, 0.5 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=16.5 min, t₂=19.0 min, t₃=20.0 min, t₄=61.8 min (major). ee = 99.3%, dr = 9.8:1. HRMS Calcd. For C₁₁H₁₃ClF₃NaO₄P⁺[M+Na⁺]: 355.0084. Found: 355.0085.



anti-(1*S*, 2*S*)-dimethyl (1-chloro-2-(3-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*S*, 2*S*)-2m) which catalyzed by KmCR2 was purified by chromatography on silica gel with 69.1% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.40-6.94 (m, Ar-H, 4H), 5.02 (dd, C₂-H, *J* = 9.9, 9.9 Hz, 1H), 4.64

(br, O-H, 1H), 4.05 (dd, C₁-H, J = 9.5, 8.3 Hz, 1H), 3.92 (dd, C₂-H, J = 10.6 Hz, 3H), 3.80 (d, C₁₀-H, J = 11.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm ¹³C NMR (100 MHz, CDCl₃) δ /ppm 162.72 (d, J = 246.2 Hz), 142.09 (dd, J = 11.0, 7.0 Hz), 129.75 (d, J = 8.1 Hz), 123.00 (d, J = 3.0 Hz), 115.45 (d, J = 21.1 Hz), 114.14 (d, J = 22.3 Hz), 74.00 (d, J = 2.0 Hz), 54.96 (d, J = 7.0 Hz), 53.93 (d, J = 152.1 Hz), 53.90 (d, J = 6.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.89. [α]₂₅^D = 31.38 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 95:5, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=24.8 min (major), t₂=33.4 min, t₃=36.6 min, t₄=118.9 min. ee > 99%, dr = 15.3:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0106.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-(3-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2m) which catalyzed by RasADH was purified by chromatography on silica gel with 73.9% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.47-6.93 (m, Ar-H, 4H), 5.03 (dd, C₂-H, *J* = 10.1, 10.1 Hz, 1H), 4.57 (br, O-H, 1H), 4.04 (dd, C₁-H, *J* = 9.0, 9.0 Hz, 1H), 3.93 (d,

C₉-H, J = 10.7 Hz, 3H), 3.81 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ/ppm 162.99 (d, J = 245.9 Hz), 142.63 (dd, J = 10.8, 6.9 Hz), 130.05 (d, J = 8.0 Hz), 123.38 (d, J = 2.9 Hz), 115.72 (d, J = 21.1 Hz), 114.48 (d, J = 22.3 Hz), 74.19 (d, J = 1.9 Hz), 55.22 (d, J = 7.2 Hz), 54.45 (d, J = 152.8 Hz), 54.24 (d, J = 6.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ/ppm 21.90. [α]₂₅^D = -21.38 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 95:5, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=24.8 min, t₂=33.4 min, t₃=36.6 min, t₄=118.9 min (major). ee = 99.7%, dr = 9.4:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0115.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-(3-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2m) which catalyzed by SyADH was purified by chromatography on silica gel with 96.1% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.41-6.94 (m, Ar-H, 4H), 5.02 (dd, C₂-H, *J* = 11.5, 8.4 Hz, 1H), 4.72-4.47 (m, O-H, 1H), 4.04 (dd, C₁-H, *J* = 9.5, 8.4 Hz, 1H), 3.93 (d,

C₉-H, J = 10.7 Hz, 1H), 3.81 (d, C₁₀-H, J = 11.0 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 162.72 (d, J = 246.2 Hz), 142.04 (dd, J = 11.0, 7.1 Hz), 129.77 (d, J = 8.2 Hz), 122.99 (d, J = 3.0 Hz), 115.48 (d, J = 21.2 Hz), 114.14 (d, J = 22.2 Hz), 74.02 (d, J = 2.0 Hz), 54.99 (d, J = 7.1 Hz), 53.91 (d, J = 6.9 Hz), 53.86 (d, J = 151.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.91. [α]₂₅^D = -29.38 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 95:5, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=24.8 min, t₂=33.4 min, t₃=36.6 min, t₄=118.9 min (major). ee = 93.7%, dr = 14.7:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0117.



anti-(1*S*, 2*S*)-dimethyl (1-chloro-2-(2-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*S*, 2*S*)-2n) which catalyzed by KmCR2 was purified by chromatography on silica gel with 70.7% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.65-6.91 (m, Ar-H, 4H), 5.34 (dd, C₂-H, *J* = 16.8, 7.6 Hz, 1H), 4.37 (br, O-H,

1H), 4.25 (dd, C₁-H, J = 9.7, 7.6 Hz, 1H), 3.94 (d, C₉-H, J = 10.7 Hz, 3H), 3.68 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 160.28 (d, J = 246.8 Hz), 130.02 (d, J = 8.4 Hz), 128.43 (d, J = 3.9 Hz), 126.86 (dd, J = 12.3, 9.7 Hz), 124.29 (d, J = 3.9 Hz), 115.30 (d, J = 21.9 Hz), 69.23, 55.07 (d, J = 7.0 Hz), 53.66 (d, J = 6.9 Hz), 53.16 (d, J = 152.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.95. [α]₂₅^D = 24.78 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.8 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=13.2 min, t₂=14.6 min, t₃=16.1 min (major), t₄=26.4 min. ee = 99.8%, dr > 99:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0116.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-(2-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2n) which catalyzed by RasADH was purified by chromatography on silica gel with 66.7% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.61-7.04 (m, Ar-H, 4H), 5.37 (dd, C₂-H, *J* = 16.3, 12.3 Hz, 1H), 4.49 (d, O-H,

J = 5.1 Hz, 1H), 4.27 (dd, C₁-H, J = 9.7, 7.6 Hz, 1H), 3.95 (d, C₉-H,

anti-(1*R*, 2*R*)-**2n** catalyzed by RasADH

J = 10.7 Hz, 3H), 3.70 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 160.27 (d, J = 246.6 Hz), 130.02 (d, J = 8.4 Hz), 128.42 (d, J = 3.9 Hz), 126.84 (dd, J = 12.8, 9.5 Hz), 124.29 (d, J = 3.5 Hz), 115.30 (d, J = 21.9 Hz), 69.25, 55.07 (d, J = 7.0 Hz), 53.65 (d, J = 6.9 Hz), 53.15 (dd, J = 152.7, 2.3 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.94. [α]₂₅^D = -18.59 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.8 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=13.2 min, t₂=14.6 min, t₃=16.1 min, t₄=26.4 min (major). ee = 99.7%, dr = 5.1:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0117.



anti-(1R, 2R)-**2n** catalyzed by SyADH *anti*-(1*R*, 2*R*)-dimethyl (1-chloro-2-(2-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2n) which catalyzed by SyADH was purified by chromatography on silica gel with 93.7% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.64-6.81 (m, Ar-H, 4H), 5.37 (dd, C₂-H, *J* = 16.5, 7.6 Hz, 1H), 4.51 (br, O-H, 1H), 4.27 (dd, C₁-H, *J* = 9.7, 7.6 Hz, 1H), 3.95 (d, C₉-H, *J* = 10.7 Hz,

3H), 3.70 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 160.30 (d, J = 246.7 Hz), 130.05 (d, J = 8.5 Hz), 128.37 (d, J = 3.9 Hz), 126.71 (dd, J = 13.0, 9.4 Hz), 124.31 (d, J = 3.5 Hz), 115.32 (d, J = 21.7 Hz), 69.38, 55.13 (d, J = 7.0 Hz), 53.75 (d, J = 2.4 Hz), 53.62 (d, J = 6.9 Hz), 52.24 (d, J = 2.5 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.96. [α]₂₅^D = -28.98 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 85:15, 0.8 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=13.2 min, t₂=14.6 min, t₃=16.1 min, t₄=26.4 min (major). ee = 97.4%, dr = 23.4:1. HRMS Calcd. For C₁₀H₁₃ClFNaO₄P⁺[M+Na⁺]: 305.0116. Found: 305.0116.



anti-(1*S*, 2*S*)-dimethyl (1-chloro-2-hydroxy-2-(naphthalen-2-yl)ethyl)phosphonate (*anti*-(1*S*, 2*S*)-20) which catalyzed by KmCR2 was purified by chromatography on silica gel with 58.0% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 7.96-7.45 (m, Ar-H, 7H), 5.21 (dd, C₂-

H, J = 11.7, 8.5 Hz, 1H), 4.52 (br, O-H, 1H), 4.21 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.94 (d, C₉-H, J = 10.7 Hz, 3H), 3.78 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 136.74 (d, J = 10.9 Hz), 133.43, 132.99, 128.21 (d, J = 6.0 Hz), 127.73, 126.90, 126.32 (d, J = 3.1 Hz), 124.27, 74.82, 54.96 (d, J = 7.1 Hz), 53.97 (d, J = 151.2 Hz), 53.87 (d, J = 6.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.29. [α]₂₅^D = 17.79 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=13.2 min (major), t₂=17.4 min, t₃=19.7 min, t₄=38.6 min. ee > 99%, dr = 29.2:1. HRMS Calcd. For C₁₄H₁₆ClNaO₄P⁺[M+Na⁺]: 337.0367. Found: 337.0366.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-hydroxy-2-(naphthalen-2-yl)ethyl)phosphonate (*anti*-(1*R*, 2*R*)-2o) which catalyzed by RasADH was purified by chromatography on silica gel with 68.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.99-7.43 (m, Ar-H, 4H), 5.21 (dd, C₂-

H, J = 11.8, 8.5 Hz, 1H), 4.48 (br, O-H, 1H), 4.21 (dd, C₁-H, J = 8.9, 8.9 Hz, 1H), 3.95 (d, C₉-H, J = 10.6 Hz, 3H), 3.78 (d, C₁₀-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 136.71 (d, J = 11.0 Hz), 133.44, 132.99, 128.21 (d, J = 7.0 Hz), 127.73, 126.90, 126.32 (d, J = 3.2 Hz), 124.25, 74.84, 54.98 (d, J = 7.0 Hz), 53.93 (d, J = 151.1 Hz), 53.87 (d, J = 6.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.29. [α]₂₅^D = -29.38 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=13.2 min, t₂=17.4 min, t₃=19.7 min, t₄=38.6 min (major). ee = 99.3%, dr = 10.7:1. HRMS Calcd. For C₁₄H₁₆ClNaO₄P⁺[M+Na⁺]: 337.0367. Found: 337.0371.



anti-(1S, 2S)-dimethyl (1-chloro-2-(furan-2-yl)-2 hydroxyethyl)phosphonate (anti-(1S, 2S)-2p) which catalyzed by KmCR2 was purified by chromatography on silica gel with 72.2% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 7.54-6.15 (m, Ar-H, 3H), 5.08 (dd, C₂-H, J = 9.8, 9.8 Hz, 1H), 4.51 (br, O-H,

1H), 4.35 (dd, C₁-H, J = 9.0, 9.0 Hz, 1H), 3.87 (d, C₇-H, J = 10.7 Hz, 3H), 3.77 (d, C₈-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 151.79 (d, J = 11.0 Hz), 142.67, 110.38, 109.03, 68.88, 54.97 (d, J = 6.9 Hz), 53.89 (d, J = 6.8 Hz), 51.86 (d, J = 153.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 22.72. [α]₂₅^D = 7.59 (c = 0.5, CHCl₃). HPLC Chiracel[®] IA, 250 × 4.6 mm column, hexane/2-propanol 95:5, 0.4 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=40.9 min (major), t₂=47.2 min, t₃=49.8 min, t₄=52.5 min. ee > 99%, dr > 99:1. HRMS Calcd. For C₈H₁₂ClNaO₅P⁺[M+Na⁺]: 277.0003. Found: 276.9999.



anti-(1R, 2*R*)-dimethyl (1-chloro-2-(furan-2-yl)-2hydroxyethyl)phosphonate (anti-(1R, 2R)-2p) which catalyzed by RasADH was purified by chromatography on silica gel with 69.0% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 7.50-6.26 (m, Ar-H, 3H), 5.09 (dd, C_2 -H, J = 8.5, 8.5 Hz, 1H), 4.44-4.39 (br, O-H, 1H), 4.36 (dd, C₁-H, *J* = 9.7, 8.0 Hz, 1H), 3.91 (d, C₇-H, *J* = 10.7

Hz, 3H), 3.79 (d, C₈-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 151.83 (d, J =10.8 Hz), 142.66, 110.38, 109.01, 68.84, 54.97 (d, J = 7.1 Hz), 53.89 (d, J = 6.7 Hz), 51.88 (d, J = 153.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.72. [α]₂₅^D = 2.4 (c = 0.5, CHCl₃). HPLC Chiracel® IA, 250 × 4.6 mm column, hexane/2-propanol 95:5, 0.4 mL/min flow rate, 215 nm UV lamp, 30 °C, t_1 =40.9 min, t_2 =47.2 min (major), t_3 =49.8 min, t_4 =52.5 min. ee = 65.4%, dr =4.4:1. HRMS Calcd. For C₈H₁₂ClNaO₅P⁺[M+Na⁺]: 277.0003. Found: 276.9997.



2*R*)-dimethyl (1-chloro-2-(furan-2-vl)-2anti-(1R, hydroxyethyl)phosphonate (anti-(1R, 2R)-2p) which catalyzed by SyADH was purified by chromatography on silica gel with 62.7% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 7.56-6.24 (m, Ar-H, 3H), 5.09 (dd, C₂-H, J = 12.9, 12.9 Hz, 1H), 4.40 (br, O-H, 1H), 4.35 (dd, C₁-H, *J* = 9.7, 8.0 Hz, 1H), 3.91 (d, C₇-H, *J* = 10.7 Hz,

3H), 3.79 (d, C₈-H, J = 11.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 151.82 (d, J = 11.1 Hz), 142.66, 110.38, 109.02, 68.85, 54.96 (d, J = 7.0 Hz), 53.89 (d, J = 6.8 Hz), 51.88 (d, J = 153.8Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.71. $[\alpha]_{25}^{D} = -7.39$ (c = 0.5, CHCl₃). HPLC Chiracel® IA, 250 × 4.6 mm column, hexane/2-propanol 95:5, 0.4 mL/min flow rate, 215 nm UV lamp, 30 °C, t_1 =40.9 min, t_2 =47.2 min (major), t_3 =49.8 min, t_4 =52.5 min. ee = 99.4%, $dr > 10^{-1}$ 99:1. HRMS Calcd. For C₈H₁₂ClNaO₅P⁺[M+Na⁺]: 277.0003. Found: 276.9994.



2*S*)-dimethyl (1-chloro-2-hydroxy-2-(thiophen-2anti-(1S, ⁸ yl)ethyl)phosphonate (*anti*-(1*S*, 2*S*)-2q) which catalyzed by KmCR2 was purified by chromatography on silica gel with 72.2% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ/ppm 7.40-6.70 (m, Ar-H, 3H), 5.30 (dd, C_2 -H, J = 9.3, 9.3 Hz, 1H), 4.37 (br, O-H, 1H), 4.08 (dd, C₁-H, J = 9.5, 8.2 Hz, 1H), 3.89 (d, C₇-H, J = 10.7 Hz, 3H), 3.80 (d, C₈-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 143.02 (d, J = 12.1 Hz), 126.50, 126.47, 125.82, 70.93, 54.90 (d, J = 6.9 Hz), 54.77 (d, J = 151.8 Hz), 54.01 (d, J = 6.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.54. [α]₂₅^D = 12.79 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, $t_1=12.9 \text{ min (major)}, t_2=17.7 \text{ min, } t_3=19.1 \text{ min, } t_4=61.7 \text{ min. } ee > 99\%, dr = 18.4:1. HRMS$ Calcd. For C₈H₁₂ClNaO₄PS⁺[M+Na⁺]: 292.9775. Found: 292.9773.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-hydroxy-2-(thiophen-2-yl)ethyl)phosphonate (*anti*-(1*R*, 2*R*)-2q) which catalyzed by RasADH was purified by chromatography on silica gel with 77.8% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.47-6.78 (m, Ar-H, 3H), 5.32 (ddd, C₂-H, *J* = 9.5, 7.6, 3.2 Hz, 1H), 4.65 (d, O-H, *J* = 4.0 Hz, 1H), 4.11 (dd, C₁-H, *J* = 9.6, 8.1 Hz, 1H), 3.88 (d, C₇-

H, J = 10.7 Hz, 3H), 3.81 (d, C₈-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 143.03 (d, J = 11.9 Hz), 126.47, 126.45, 125.79, 70.93, 54.88 (d, J = 7.0 Hz), 54.78 (d, J = 152.0 Hz), 53.98 (d, J = 8.2 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.51. [α]₂₅^D = 2.4 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=12.9 min, t₂=17.7 min, t₃=19.1 min, t₄=61.7 min (major). *ee* = 93.5%, *dr* = 1.9:1. HRMS Calcd. For C₈H₁₂ClNaO₄PS⁺[M+Na⁺]: 292.9775. Found: 292.9771.



anti-(1*R*, 2*R*)-dimethyl (1-chloro-2-hydroxy-2-(thiophen-2-yl)ethyl)phosphonate (*anti*-(1*R*, 2*R*)-2q) which catalyzed by SyADH was purified by chromatography on silica gel with 51.6% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.54-6.64 (m, Ar-H, 3H), 5.37-5.29 (m, C₂-H, 1H), 4.60 (br, O-H, 1H), 4.11 (dd, C₁-H, *J* = 8.9, 8.9 Hz, 1H), 3.88 (d, C₇-H, *J* = 10.7 Hz, 3H), 3.81 (d,

C₈-H, J = 11.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 143.00 (d, J = 12.1 Hz), 126.48, 126.46, 125.80, 70.96, 54.88 (d, J = 7.0 Hz), 54.74 (d, J = 151.6 Hz), 53.98 (d, J = 6.7 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.50. [α]₂₅^D = -7.79 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=12.9 min, t₂=17.7 min, t₃=19.1 min, t₄=61.7 min (major). ee = 96.1%, dr = 21.7:1. HRMS Calcd. For C₈H₁₂ClNaO₄PS⁺[M+Na⁺]: 292.9775. Found: 292.9775.

anti-(1S,2S)-diethyl



anti-(1S, 2S)-2r

phenylethyl)phosphonate (*anti*-(1*S*, 2*S*)-2r) which catalyzed by KmCR2 was purified by chromatography on silica gel with 79.4% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.56-7.30 (m, Ar-H, 5H), 5.02 (dd, C₂-H, *J* = 11.8, 8.4 Hz, 1H), 4.57 (br, O-H, 1H), 4.37-4.10 (m, C_{9,11}-H, 4H), 4.06 (dd, C₁-H, *J* = 8.8,

(1-chloro-2-hydroxy-2-

8.8 Hz, 1H), 1.41 (t, C₁₀-H, J = 7.1 Hz, 3H), 1.31 (t, C₁₂-H, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 139.51 (d, J = 10.9 Hz), 128.50, 128.26, 127.21, 74.61, 64.63 (d, J = 7.1 Hz), 63.67 (d, J = 6.8 Hz), 54.28 (d, J = 150.3 Hz), 16.45 (d, J = 5.8 Hz), 16.24 (d, J = 6.0 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 21.50. [α]₂₅^D = 29.58 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=10.7 min (major), t₂=13.8 min, t₃=17.1 min, t₄=61.1 min. ee > 99%, dr = 24.5:1. HRMS Calcd. For C₁₂H₁₈ClNaO₄P⁺[M+Na⁺]: 315.0523. Found: 315.0516.



anti-(1R,2R)-diethyl(1-chloro-2-hydroxy-2-
phenylethyl)phosphonate (anti-(1R, 2R)-2r) which catalyzed by
RasADH was purified by chromatography on silica gel with 92.2%
yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.53-
7.31 (m, Ar-H, 5H), 5.02 (dd, C₂-H, J = 11.9, 8.4 Hz, 1H), 4.55
(br, O-H, 1H), 4.38-4.09 (m, C_{9,11}-H, 4H), 4.05 (dd, C₁-H, J = 8.8,

8.8 Hz, 1H), 1.41 (t, C₁₀-H, J = 7.1 Hz, 3H), 1.32 (t, C₁₂-H, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 139.49 (d, J = 10.9 Hz), 128.50, 128.26, 127.21, 74.61, 64.63 (d, J = 7.2 Hz), 63.67 (d, J = 6.8 Hz), 54.25 (d, J = 150.1 Hz), 16.45 (d, J = 5.8 Hz), 16.24 (d, J = 5.8 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 19.85. [α]₂₅^D = -23.98 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=10.7 min, t₂=13.8 min, t₃=17.1 min, t₄=61.1 min (major). ee = 99.8%, dr = 22.3:1. HRMS Calcd. For C₁₂H₁₈ClNaO₄P⁺[M+Na⁺]: 315.0523. Found: 315.0534.



anti-(1*S*, 2*S*)-diisopropyl (1-chloro-2-hydroxy-2-phenylethyl)phosphonate (*anti*-(1*S*, 2*S*)-2s) which catalyzed by KmCR2 was purified by chromatography on silica gel with 92.2% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.53-7.31 (m, Ar-H, 5H), 4.97 (dd, C₂-H, *J* = 10.7, 9.1 Hz, 1H), 4.90 (dq, C₉-H, *J* = 12.6, 6.3 Hz, 1H), 4.78 (dq, C₁₀-H, *J* = 12.2, 6.3 Hz,

anti-(1S, 2S)-**2s**

1H), 3.96 (dd, C₁-H, J = 8.7, 8.7 Hz, 1H), 1.44 (d, C₁₁-H, J = 3.1 Hz, 3H), 1.43 (d, C₁₂-H, J = 3.2 Hz, 3H), 1.37 (d, C₁₃-H, J = 6.2 Hz, 3H), 1.31 (d, C₁₄-H, J = 6.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 139.59 (d, J = 11.6 Hz), 128.46, 128.22, 127.34, 74.58, 73.66 (d, J = 7.3 Hz), 72.96 (d, J = 7.0 Hz), 54.50 (d, J = 150.4 Hz), 24.14 (d, J = 3.5 Hz), 23.99 (d, J = 3.9 Hz), 23.87 (d, J = 5.3 Hz), 23.76 (d, J = 4.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 19.85. [α]₂₅^D = 55.76 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=7.7 min, t₂=9.3 min (major), t₃=10.6 min, t₄=15.3 min. ee = 99.8%, dr = 68.4:1. HRMS Calcd. For C₁₄H₂₂ClNaO₄P⁺[M+Na⁺]: 343.0836. Found: 343.0836.



anti-(1*R*, 2*R*)-diisopropyl (1-chloro-2-hydroxy-2phenylethyl)phosphonate (*anti*-(1*R*, 2*R*)-2s) which catalyzed by RasADH was purified by chromatography on silica gel with 87.4% yield as white solid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.50-7.31 (m, Ar-H, 5H), 4.97 (dd, C₂-H, *J* = 11.0, 8.8 Hz, 1H), 4.90 (dq, C₉-H, *J* = 12.6, 6.3 Hz, 1H), 4.77 (dp, C₁₀-H, *J* = 12.5, 6.4 Hz,

1H), 3.96 (dd, C₁-H, J = 8.7, 8.7 Hz, 1H), 1.44 (d, C₁₁-H, J = 3.2 Hz, 3H), 1.43 (d, C₁₂-H, J = 3.4 Hz, 3H), 1.37 (d, C₁₃-H, J = 6.2 Hz, 3H), 1.31 (d, C₁₄-H, J = 6.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 139.58 (d, J = 11.5 Hz), 128.46, 128.22, 127.34, 74.58, 73.66 (d, J = 7.3 Hz), 72.95 (d, J = 7.0 Hz), 54.48 (d, J = 150.3 Hz), 24.14 (d, J = 3.6 Hz), 23.99 (d, J = 3.9 Hz), 23.88 (d, J = 5.3 Hz), 23.76 (d, J = 4.9 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 18.16. [α]₂₅^D = -70.95 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=7.7 min, t₂=9.3 min, t₃=10.6 min, t₄=15.3

min (major). ee = 94.6%, dr = 48.1:1. HRMS Calcd. For C₁₄H₂₂ClNaO₄P⁺[M+Na⁺]: 343.0836. Found: 343.0835.



anti-(1R, 2R)-2t

anti-(1*R*, 2*R*)-diethyl (1-chloro-2-(4-fluorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2t) which catalyzed by RasADH was purified by chromatography on silica gel with 79.5% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.54-6.88 (m, Ar-H, 4H), 4.99 (dd, C₂-H, *J* = 10.9, 8.5 Hz, 1H), 4.68 (br, O-H, 1H), 4.37-4.10 (m, C_{9,10}-H,

4H), 3.98 (dd, C₁-H, J = 8.8, 8.8 Hz, 1H), 1.40 (t, C₁₁-H, J = 7.0 Hz, 3H), 1.33 (t, C₁₂-H, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 162.73 (d, J = 246.7 Hz), 135.37 (dd, J = 11.2, 3.2 Hz), 129.00 (d, J = 8.2 Hz), 115.11 (d, J = 21.6 Hz), 73.85, 64.66 (d, J = 7.0 Hz), 63.77 (d, J = 6.8 Hz), 54.32 (d, J = 150.0 Hz), 16.44 (d, J = 5.8 Hz), 16.26 (d, J = 6.0 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 19.62. [α]₂₅^D = -58.36 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=7.6 min, t₂=9.2 min, t₃=11.6 min, t₄=51.4 min (major). *ee* = 94.7%, *dr* = 11.9:1. HRMS Calcd. For C₁₂H₁₇ClFNaO₄P⁺[M+Na⁺]: 333.0429. Found: 333.0422.



anti-(1R, 2R)-**2u**

anti-(1*R*, 2*R*)-diethyl (1-chloro-2-(4-chlorophenyl)-2hydroxyethyl)phosphonate (*anti*-(1*R*, 2*R*)-2u) which catalyzed by RasADH was purified by chromatography on silica gel with 68.4% yield as yellow liquid. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.49-7.34 (m, Ar-H, 4H), 4.99 (dd, C₂-H, J = 11.3, 8.5 Hz, 1H), 4.68 (br, O-H, 1H), 4.39-4.10 (m, C₉, 10-

H, 4H), 3.98 (dd, C₁-H, J = 8.8, 8.8 Hz, 1H), 1.41 (t, C₁₁-H, J = 7.1 Hz, 3H), 1.33 (t, C₁₂-H, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ /ppm 138.04 (d, J = 11.3 Hz), 128.65, 128.41, 127.81, 73.92, 64.75 (d, J = 7.1 Hz), 63.81 (d, J = 6.8 Hz), 54.10 (d, J = 150.3 Hz), 16.45 (d, J = 5.8 Hz), 16.25 (d, J = 6.0 Hz). ³¹P NMR (162 MHz, CDCl₃) δ /ppm 19.56. [α]₂₅^D = -32.78 (c = 0.5, CHCl₃). HPLC Chiracel[®] IC, 250 × 4.6 mm column, hexane/2-propanol 80:20, 1.0 mL/min flow rate, 215 nm UV lamp, 30 °C, t₁=7.6 min, t₂=8.8 min, t₃=11.5 min, t₄=46.0 min (major). ee = 98.8%, dr = 13.8:1. HRMS Calcd. For C₁₂H₁₇Cl₂NaO₄P⁺[M+Na⁺]: 349.0134. Found: 349.0120.

Table S8. Biological tests.

Test Strain	Compounds with weak inhibitory activity
Bacillus subtilis 168	#17: <i>anti</i> -(1 <i>S</i> , 2 <i>S</i>)-2 r , #28: <i>anti</i> -(1 <i>S</i> , 2 <i>S</i>)-2 s , #35:
(labeled as 090)	<i>anti-</i> (1 <i>R</i> , 2 <i>R</i>)- 2u
Staphylococcus aureus CMCC(B) 26003	#30: anti-(1S, 2R)-2d, #31: syn-2d, #20: anti-
(labeled as 031)	(1S, 2S)-2i, #19: anti-(1R, 2R)-2l, #32: anti-(1R,
	2R)-2p, #33: anti-(1R, 2R)-2q, #28: anti-(1S,
	2S)-2s, #38: anti-(1R, 2R)-2s, #35: anti-(1R, 2R)-
	2u
E. coli BL21	#35: anti-(1R, 2R)- 2u
(labeled as 033)	

(labeled as 029)

Pseudomonas aeruginosa CICC10351 #11: *anti-*(1*S*, 2*S*)-**2p**, #35: *anti-*(1*R*, 2*R*)-**2u**



Antibacterial activities of the synthesized compounds were evaluated by using a paper disc diffusion assay (Mearns-Spragg et al., 1998). A sterile paper disc (6 mm in diameter) saturated with 5 μ L of DMSO-dissolved tested compounds (20 μ g/ μ L) was placed on LB agar plates inoculated with test organisms, including *Pseudomonas aeruginosa* CICC10351, *Staphylococcus aureus* CMCC(B) 26003, *Bacillus subtilis* 168 and *E. coli* BL21. Chloramphenicol (25 μ g) was used as the positive control and 5 μ L of DMSO was used as the negative control. Plates were incubated at 30 °C (or 37 °C for *E. coli*) overnight.






















Name	Sequence (5'→3') ^a
pET28a-NdeI-YDL124w_fw	cggcctggtgccgcggcggcagccatATGTCATTTCACCAAC AGTTCT
pET28a-XhoI-YDL124w_rc	agtggtggtggtggtggtggtgctcgagTTATACTTTTTGAGCAG CGTAGT

Table S9. List of oligonucleotides used in this study

^aNucleotides colored in red indicate cleavage sites







fl (ppm)

























fl (ppm)



fl (ppm)




























f1 (ppm)

















Compound 1t, ¹H NMR













f1 (ppm)
















































































































Compound *anti-*(1*S*, 2*S*)-**2p**, ³¹P NMR

anti-(1S, 2S)-**2p**

approxibits with the automation and the and the any meter is a second strain of the barrier of the	ter a part de la participa de la desta de participa de la desta
աներակությունի հատերեներությունը կանությունը որդությունը կությունը կաներությունը հատերեներին հատերերին հատերեր Հայ	ngolf dir der full der referent bestandelte gereinigt bereinigt in der bestander gertannen g

-21.7170

50 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 f1 (ppm)












anti-(1S, 2S)-**2q**

Compound *anti-*(1*S*, 2*S*)-**2q**, ³¹P NMR

21.5406

50 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 f1 (ppm)





































spectrum is the chiral HPLC analysis of RasADH-catalyzed biotransformation.







spectrum is the chiral HPLC analysis of YDR368w-catalyzed biotransformation.



spectrum is the chiral HPLC analysis of KRED-F42-catalyzed biotransformation.







spectrum is the chiral HPLC analysis of YNL331c-catalyzed biotransformation.





anti-(1R, 2R)-dimethyl (1-chloro-2-hydroxy-2-(p-tolyl)ethyl)phosphonate (*anti-(1R, 2R)*-2g)



anti-(1S, 2S)-(dimethyl (1-chloro-2-hydroxy-2-(4-methoxyphenyl)ethyl)phosphonate (*anti-(1S, 2S)-*2h)







anti-(1S, 2S)-dimethyl (1-chloro-2-(4-fluorophenyl)-2-hydroxyethyl)phosphonate (*anti-(1S, 2S)*-2i)





anti-(1S, 2S)-dimethyl (1-chloro-2-(4-chlorophenyl)-2-hydroxyethyl)phosphonate (*anti-(1S, 2S)*-2j)






anti-(1S, 2S)-dimethyl (2-(4-bromophenyl)-1-chloro-2-hydroxyethyl)phosphonate (*anti-(1S, 2S)*-2k)



anti-(1R, 2R)-dimethyl (2-(4-bromophenyl)-1-chloro-2-hydroxyethyl)phosphonate (*anti-(1R, 2R)*-2k)







anti-(1S, 2S)-dimethyl (1-chloro-2-(3-fluorophenyl)-2-hydroxyethyl)phosphonate (*anti-(1S, 2S)*-2m)



anti-(1R, 2R)-dimethyl (1-chloro-2-(3-fluorophenyl)-2-hydroxyethyl)phosphonate (*anti-(1R, 2R)*-2m)



anti-(1R, 2R)-dimethyl (1-chloro-2-(3-fluorophenyl)-2-hydroxyethyl)phosphonate (*anti-(1R, 2R)*-2m)



anti-(1S, 2S)-dimethyl (1-chloro-2-(2-fluorophenyl)-2-hydroxyethyl)phosphonate (anti-

The top spectrum is the chiral HPLC analysis of the racemic synthetic standard. The bottom spectrum is the chiral HPLC analysis of KmCR2-catalyzed biotransformation.



2R)-dimethyl anti-(1R, (1-chloro-2-(2-fluorophenyl)-2-hydroxyethyl)phosphonate







anti-(1S, 2S)-dimethyl (1-chloro-2-hydroxy-2-(naphthalen-2-yl)ethyl)phosphonate (*anti-(1S, 2S)*-20)



anti-(1R, 2R)-dimethyl (1-chloro-2-hydroxy-2-(naphthalen-2-yl)ethyl)phosphonate (*anti-(1R, 2R)*-20)







anti-(1R, 2R)-dimethyl (1-chloro-2-(furan-2-yl)-2-hydroxyethyl)phosphonate (anti-(1R,



anti-(1R, 2R)-dimethyl (1-chloro-2-(furan-2-yl)-2-hydroxyethyl)phosphonate (anti-(1R,



anti-(1S, 2S)-dimethyl (1-chloro-2-hydroxy-2-(thiophen-2-yl)ethyl)phosphonate (anti-(1S, 2S)-2a)



anti-(1R, 2R)-dimethyl (1-chloro-2-hydroxy-2-(thiophen-2-yl)ethyl)phosphonate (anti-



spectrum is the chiral HPLC analysis of SyADH-catalyzed biotransformation.













anti-(1R, 2R)-diethyl (1-chloro-2-(4-fluorophenyl)-2-hydroxyethyl)phosphonate (anti-



anti-(1R, 2R)-diethyl (1-chloro-2-(4-chlorophenyl)-2-hydroxyethyl)phosphonate (anti-

Crystal data

Crystallographic data for compound *anti*-(1*S*, 2*S*)-**2k** (CCDC-1975103) has been deposited with the Cambridge Crystallographic Data Centre, Copies of the data can be obtained, free of charge, on application to CCDC (Email:deposit@ccdc.cam.ac.uk)



Table 1. Crystal data and structure refinement for ga_91205aa_a.

Identification code	ga_91205aa_a	
Empirical formula	C10 H13 Br Cl O4 P	
Formula weight	343.53	
Temperature	173(2) K	
Wavelength	1.34138 Å	
Crystal system	Monoclinic	
Space group	P21/n	
Unit cell dimensions	a = 8.7086(8) Å	$\alpha = 90$ °.
	b = 9.0359(7) Å	β = 102.801(3) °.
	c = 17.1804(16) Å	$\gamma = 90$ °.
Volume	1318.3(2) Å ³	
Z	4	
Density (calculated)	1.731 Mg/m ³	
Absorption coefficient	4.829 mm ⁻¹	
F(000)	688	
Crystal size	$0.040 \ge 0.031 \ge 0.027 \text{ mm}^3$	
Theta range for data collection	4.592 to 58.483 °.	

Index ranges	$-11 <\!\!=\!\!h <\!\!=\!\!11, -\!\!11 <\!\!=\!\!k <\!\!=\!\!11, -\!\!21 <\!\!=\!\!l <\!\!=\!\!21$
Reflections collected	17962
Independent reflections	2825 [R(int) = 0.0489]
Completeness to theta = 53.594 $^{\circ}$	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.752 and 0.618
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2825 / 1 / 160
Goodness-of-fit on F ²	1.150
Final R indices [I>2sigma(I)]	R1 = 0.0440, wR2 = 0.1134
R indices (all data)	R1 = 0.0450, wR2 = 0.1140
Extinction coefficient	n/a
Largest diff. peak and hole	1.047 and -0.831 e.Å ⁻³

Table 2. Atomic coordinates ($x \ 10^4$) and equivalent isotropic displacement parameters (Å²x 10^3)

	х	у	Z	U(eq)
Br(1)	10228(1)	7592(1)	4189(1)	59(1)
P(1)	3255(1)	443(1)	3622(1)	27(1)
Cl(1)	6692(1)	597(1)	3898(1)	39(1)
O(1)	3464(3)	3734(3)	3558(1)	32(1)
O(2)	2784(3)	356(3)	2752(1)	36(1)
O(3)	3703(3)	-1070(2)	4058(1)	35(1)
O(4)	1994(3)	1029(3)	4060(1)	38(1)
C(1)	8662(4)	6080(4)	3985(2)	39(1)
C(2)	8330(5)	5409(4)	3259(2)	45(1)
C(3)	7110(4)	4388(4)	3087(2)	39(1)
C(4)	6260(3)	4030(3)	3652(2)	28(1)
C(5)	6679(4)	4693(4)	4395(2)	35(1)
C(6)	7874(4)	5725(4)	4569(2)	41(1)
C(7)	4850(3)	3018(3)	3439(2)	27(1)
C(8)	4969(3)	1626(3)	3957(2)	26(1)
C(9)	4092(6)	-2344(4)	3639(3)	51(1)
C(10)	539(4)	1716(5)	3639(3)	50(1)

for ga_91205aa_a. U(eq) is defined as one third of $\ \ the trace of the orthogonalized <math display="inline">U^{ij}$ tensor.

Br(1)-C(1)	1.908(3)
P(1)-O(2)	1.462(2)
P(1)-O(4)	1.556(2)
P(1)-O(3)	1.567(2)
P(1)-C(8)	1.822(3)
Cl(1)-C(8)	1.787(3)
O(1)-C(7)	1.424(4)
O(1)-H(1)	0.819(19)
O(3)-C(9)	1.438(4)
O(4)-C(10)	1.452(5)
C(1)-C(2)	1.358(5)
C(1)-C(6)	1.374(5)
C(2)-C(3)	1.388(5)
C(2)-H(2)	0.9500
C(3)-C(4)	1.383(4)
C(3)-H(3)	0.9500
C(4)-C(5)	1.384(5)
C(4)-C(7)	1.510(4)
C(5)-C(6)	1.380(5)
C(5)-H(5)	0.9500
C(6)-H(6)	0.9500
C(7)-C(8)	1.531(4)
C(7)-H(7)	1.0000
C(8)-H(8)	1.0000
C(9)-H(9A)	0.9800
C(9)-H(9B)	0.9800
C(9)-H(9C)	0.9800
C(10)-H(10A)	0.9800
C(10)-H(10B)	0.9800
C(10)-H(10C)	0.9800
O(2)-P(1)-O(4)	116.44(14)
O(2)-P(1)-O(3)	115.21(13)
O(4)-P(1)-O(3)	101.08(13)
O(2)-P(1)-C(8)	112.35(13)
O(4)-P(1)-C(8)	105.31(14)

Table 3. Bond lengths [Å] and angles [] for ga_91205aa_a.

O(3)-P(1)-C(8)	105.10(13)
C(7)-O(1)-H(1)	101(4)
C(9)-O(3)-P(1)	121.4(2)
C(10)-O(4)-P(1)	122.4(2)
C(2)-C(1)-C(6)	121.8(3)
C(2)-C(1)-Br(1)	119.2(3)
C(6)-C(1)-Br(1)	119.0(3)
C(1)-C(2)-C(3)	119.3(3)
C(1)-C(2)-H(2)	120.4
C(3)-C(2)-H(2)	120.4
C(4)-C(3)-C(2)	120.6(3)
C(4)-C(3)-H(3)	119.7
C(2)-C(3)-H(3)	119.7
C(3)-C(4)-C(5)	118.3(3)
C(3)-C(4)-C(7)	120.3(3)
C(5)-C(4)-C(7)	121.3(3)
C(6)-C(5)-C(4)	121.6(3)
C(6)-C(5)-H(5)	119.2
C(4)-C(5)-H(5)	119.2
C(1)-C(6)-C(5)	118.3(3)
C(1)-C(6)-H(6)	120.9
C(5)-C(6)-H(6)	120.9
O(1)-C(7)-C(4)	111.1(2)
O(1)-C(7)-C(8)	103.9(2)
C(4)-C(7)-C(8)	114.2(2)
O(1)-C(7)-H(7)	109.2
C(4)-C(7)-H(7)	109.2
C(8)-C(7)-H(7)	109.2
C(7)-C(8)-Cl(1)	110.1(2)
C(7)-C(8)-P(1)	110.62(19)
Cl(1)-C(8)-P(1)	108.44(16)
C(7)-C(8)-H(8)	109.2
Cl(1)-C(8)-H(8)	109.2
P(1)-C(8)-H(8)	109.2
O(3)-C(9)-H(9A)	109.5
O(3)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
O(3)-C(9)-H(9C)	109.5

H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
O(4)-C(10)-H(10A)	109.5
O(4)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
O(4)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5

Symmetry transformations used to generate equivalent atoms:

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
Br(1)	51(1)	57(1)	63(1)	10(1)	-1(1)	-32(1)
P(1)	29(1)	24(1)	26(1)	1(1)	2(1)	-6(1)
Cl(1)	29(1)	36(1)	50(1)	4(1)	5(1)	3(1)
O(1)	27(1)	32(1)	35(1)	8(1)	2(1)	1(1)
O(2)	40(1)	32(1)	31(1)	-2(1)	-1(1)	-8(1)
O(3)	47(1)	24(1)	33(1)	3(1)	4(1)	-6(1)
O(4)	33(1)	42(1)	40(1)	2(1)	11(1)	-4(1)
C(1)	30(2)	37(2)	45(2)	9(1)	0(1)	-14(1)
C(2)	49(2)	47(2)	43(2)	7(2)	15(2)	-17(2)
C(3)	45(2)	41(2)	31(2)	0(1)	8(1)	-12(2)
C(4)	27(1)	25(1)	30(1)	4(1)	3(1)	-3(1)
C(5)	36(2)	38(2)	32(2)	0(1)	8(1)	-11(1)
C(6)	42(2)	42(2)	36(2)	-3(1)	1(1)	-15(2)
C(7)	27(1)	26(1)	25(1)	3(1)	1(1)	-3(1)
C(8)	25(1)	25(1)	25(1)	2(1)	2(1)	-2(1)
C(9)	71(3)	28(2)	55(2)	-1(2)	12(2)	4(2)
C(10)	27(2)	58(2)	62(2)	-9(2)	6(2)	-3(2)

Table 4.Anisotropic displacement parameters $(Å^2x \ 10^3)$ for ga_91205aa_a.The anisotropicdisplacement factor exponent takes the form: $-2\pi^2$ [$h^2 \ a^{*2}U^{11} + ... + 2 \ h \ k \ a^* \ b^* \ U^{12}$]

	x	У	Z	U(eq)
H(1)	3090(60)	4040(60)	3108(17)	67(16)
H(2)	8926	5635	2874	55
H(3)	6857	3932	2577	47
H(5)	6130	4430	4795	42
H(6)	8145	6179	5080	49
H(7)	4693	2720	2866	32
H(8)	5032	1921	4524	31
H(9A)	3578	-2266	3072	77
H(9B)	3727	-3242	3862	77
H(9C)	5236	-2394	3696	77
H(10A)	758	2724	3483	75
H(10B)	-208	1752	3990	75
H(10C)	86	1136	3162	75

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å²x 10^3) for ga_91205aa_a.

O(2)-P(1)-O(3)-C(9)	18.4(3)
O(4)-P(1)-O(3)-C(9)	144.8(3)
C(8)-P(1)-O(3)-C(9)	-105.8(3)
O(2)-P(1)-O(4)-C(10)	-11.4(3)
O(3)-P(1)-O(4)-C(10)	-137.0(3)
C(8)-P(1)-O(4)-C(10)	113.8(3)
C(6)-C(1)-C(2)-C(3)	3.2(6)
Br(1)-C(1)-C(2)-C(3)	-175.1(3)
C(1)-C(2)-C(3)-C(4)	-1.4(6)
C(2)-C(3)-C(4)-C(5)	-1.3(5)
C(2)-C(3)-C(4)-C(7)	174.9(3)
C(3)-C(4)-C(5)-C(6)	2.2(5)
C(7)-C(4)-C(5)-C(6)	-173.9(3)
C(2)-C(1)-C(6)-C(5)	-2.2(6)
Br(1)-C(1)-C(6)-C(5)	176.1(3)
C(4)-C(5)-C(6)-C(1)	-0.5(6)
C(3)-C(4)-C(7)-O(1)	-121.1(3)
C(5)-C(4)-C(7)-O(1)	55.0(4)
C(3)-C(4)-C(7)-C(8)	121.8(3)
C(5)-C(4)-C(7)-C(8)	-62.1(4)
O(1)-C(7)-C(8)-Cl(1)	-178.63(18)
C(4)-C(7)-C(8)-Cl(1)	-57.5(3)
O(1)-C(7)-C(8)-P(1)	61.5(3)
C(4)-C(7)-C(8)-P(1)	-177.4(2)
O(2)-P(1)-C(8)-C(7)	41.2(2)
O(4)-P(1)-C(8)-C(7)	-86.5(2)
O(3)-P(1)-C(8)-C(7)	167.2(2)
O(2)-P(1)-C(8)-Cl(1)	-79.69(18)
O(4)-P(1)-C(8)-Cl(1)	152.60(15)
O(3)-P(1)-C(8)-Cl(1)	46.32(18)

Table 6. Torsion angles [°] for ga_91205aa_a.

Symmetry transformations used to generate equivalent atoms:

D-HA	d(D-H)	d(HA)	d(DA)	<(DHA)
O(1)-H(1)O(2)#1	0.819(19)	1.92(3)	2.703(3)	161(5)

Table 7. Hydrogen bonds for ga_91205aa_a [Å and °].

Symmetry transformations used to generate equivalent atoms:

#1 -x+1/2,y+1/2,-z+1/2

Crystallographic data for compound *anti*-(1R, 2R)-**2k** (CCDC-1975107) has been deposited with the Cambridge Crystallographic Data Centre, Copies of the data can be obtained, free of charge, on application to CCDC (Email:deposit@ccdc.cam.ac.uk)



Table 1. Crystal data and structure refinement for ga_91204ea_a.

ga_91204ea_a	
C10 H13 Br Cl O4 P	
343.53	
293(2) K	
1.34138 Å	
Orthorhombic	
P212121	
a = 8.7884(3) Å	α= 90 °.
b = 9.1062(3) Å	β= 90 °.
c = 34.6640(10) Å	$\gamma=90~^\circ\!\!.$
2774.12(15) Å ³	
8	
1.645 Mg/m ³	
4.590 mm ⁻¹	
1376	
$0.370 \ge 0.170 \ge 0.080 \text{ mm}^3$	
4.367 to 56.467 °.	
-10<=h<=10, -11<=k<=10, -43<=l<=42	
	ga_91204ea_a C10 H13 Br Cl O4 P 343.53 293(2) K 1.34138 Å Orthorhombic P212121 a = 8.7884(3) Å b = 9.1062(3) Å c = 34.6640(10) Å 2774.12(15) Å ³ 8 1.645 Mg/m ³ 4.590 mm ⁻¹ 1376 0.370 x 0.170 x 0.080 mm ³ 4.367 to 56.467 °. -10<=h<=10, -11<=k<=10, -43
Reflections collected	32076
---	---
Independent reflections	5576 [R(int) = 0.0478]
Completeness to theta = 53.594 $^{\circ}$	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.702 and 0.412
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	5576 / 2 / 319
Goodness-of-fit on F ²	1.057
Final R indices [I>2sigma(I)]	R1 = 0.0424, wR2 = 0.1136
R indices (all data)	R1 = 0.0441, wR2 = 0.1148
Absolute structure parameter	0.079(8)
Extinction coefficient	n/a
Largest diff. peak and hole	0.532 and -0.601 e.Å ⁻³

Table 2. Atomic coordinates ($x \ 10^4$) and equivalent isotropic displacement parameters (Å²x 10^3)

	X	У	Z	U(eq)
Br(1)	-332(1)	9348(1)	4183(1)	98(1)
Cl(1)	6738(2)	5857(2)	4312(1)	78(1)
P(1)	6796(2)	2685(2)	4432(1)	61(1)
O(1)	3421(5)	3049(5)	4496(1)	67(1)
O(2)	7047(6)	2693(6)	4846(1)	81(1)
O(3)	8227(5)	2804(6)	4172(1)	80(1)
O(4)	6072(6)	1268(5)	4263(1)	79(1)
C(1)	1142(7)	7895(7)	4283(2)	65(1)
C(2)	1526(8)	6910(7)	3999(2)	69(2)
C(3)	2540(7)	5800(7)	4085(2)	64(1)
C(4)	3212(6)	5677(6)	4444(1)	54(1)
C(5)	2831(8)	6709(8)	4714(2)	75(2)
C(6)	1808(9)	7847(9)	4636(2)	84(2)
C(7)	4215(6)	4396(6)	4546(1)	54(1)
C(8)	5611(6)	4219(6)	4285(1)	57(1)
C(9)	9716(9)	3226(11)	4307(3)	102(3)
C(10)	5250(13)	238(8)	4491(3)	110(3)
Br(2)	2963(1)	10417(1)	3315(1)	124(1)
Cl(2)	6485(1)	3676(2)	3195(1)	65(1)
P(2)	9768(2)	3499(2)	3049(1)	53(1)
O(5)	9511(4)	6771(4)	3000(1)	60(1)
O(6)	9786(5)	3354(5)	2632(1)	70(1)
O(7)	11232(4)	4138(5)	3239(1)	69(1)
O(8)	9589(5)	2045(5)	3282(1)	73(1)
C(11)	4488(7)	9008(7)	3203(2)	74(2)
C(12)	4469(8)	8279(8)	2863(2)	79(2)
C(13)	5642(7)	7306(7)	2784(2)	68(2)
C(14)	6779(6)	7040(5)	3040(1)	49(1)
C(15)	6762(8)	7782(7)	3390(2)	68(2)
C(16)	5616(9)	8766(9)	3475(2)	84(2)
C(17)	8112(6)	6046(5)	2944(1)	50(1)
C(18)	8227(5)	4687(5)	3206(1)	47(1)

for ga_91204ea_a. U(eq) is defined as one third of % U(eq) the trace of the orthogonalized U^{ij} tensor.

C(19)	12415(8)	4807(12)	3028(3)	103(3)
C(20)	8943(12)	771(9)	3128(3)	116(3)

Br(1)-C(1)	1.884(6)
Cl(1)-C(8)	1.793(6)
P(1)-O(2)	1.452(4)
P(1)-O(3)	1.551(5)
P(1)-O(4)	1.553(5)
P(1)-C(8)	1.815(5)
O(1)-C(7)	1.421(7)
O(1)-H(1)	0.83(5)
O(3)-C(9)	1.442(9)
O(4)-C(10)	1.425(9)
C(1)-C(6)	1.358(9)
C(1)-C(2)	1.374(9)
C(2)-C(3)	1.380(8)
C(2)-H(2)	0.9300
C(3)-C(4)	1.383(8)
C(3)-H(3)	0.9300
C(4)-C(5)	1.370(8)
C(4)-C(7)	1.504(8)
C(5)-C(6)	1.398(9)
C(5)-H(5A)	0.9300
C(6)-H(6)	0.9300
C(7)-C(8)	1.532(7)
C(7)-H(7)	0.9800
C(8)-H(8)	0.9800
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
Br(2)-C(11)	1.896(6)
Cl(2)-C(18)	1.787(5)
P(2)-O(6)	1.453(4)
P(2)-O(7)	1.558(4)
P(2)-O(8)	1.559(4)
P(2)-C(18)	1.817(5)

Table 3. Bond lengths [Å] and angles [] for ga_91204ea_a.

O(5)-C(17)	1.409(7)
O(5)-H(5)	0.79(2)
O(7)-C(19)	1.409(9)
O(8)-C(20)	1.398(10)
C(11)-C(12)	1.352(10)
C(11)-C(16)	1.386(10)
C(12)-C(13)	1.387(9)
C(12)-H(12)	0.9300
C(13)-C(14)	1.359(8)
C(13)-H(13)	0.9300
C(14)-C(15)	1.388(8)
C(14)-C(17)	1.518(7)
C(15)-C(16)	1.380(9)
C(15)-H(15)	0.9300
C(16)-H(16)	0.9300
C(17)-C(18)	1.538(6)
C(17)-H(17)	0.9800
C(18)-H(18)	0.9800
C(19)-H(19A)	0.9600
C(19)-H(19B)	0.9600
C(19)-H(19C)	0.9600
C(20)-H(20A)	0.9600
C(20)-H(20B)	0.9600
C(20)-H(20C)	0.9600
O(2)-P(1)-O(3)	116.7(3)
O(2)-P(1)-O(4)	116.1(3)
O(3)-P(1)-O(4)	99.8(3)
O(2)-P(1)-C(8)	111.1(3)
O(3)-P(1)-C(8)	104.4(3)
O(4)-P(1)-C(8)	107.4(3)
C(7)-O(1)-H(1)	103(6)
C(9)-O(3)-P(1)	124.4(5)
C(10)-O(4)-P(1)	123.0(5)
C(6)-C(1)-C(2)	121.3(6)
C(6)-C(1)-Br(1)	119.1(5)
C(2)-C(1)-Br(1)	119.6(5)
C(1)-C(2)-C(3)	118.8(5)

C(1)-C(2)-H(2)	120.6
C(3)-C(2)-H(2)	120.6
C(2)-C(3)-C(4)	121.9(5)
C(2)-C(3)-H(3)	119.0
C(4)-C(3)-H(3)	119.0
C(5)-C(4)-C(3)	117.2(5)
C(5)-C(4)-C(7)	121.0(5)
C(3)-C(4)-C(7)	121.6(5)
C(4)-C(5)-C(6)	122.2(6)
C(4)-C(5)-H(5A)	118.9
C(6)-C(5)-H(5A)	118.9
C(1)-C(6)-C(5)	118.5(6)
C(1)-C(6)-H(6)	120.8
C(5)-C(6)-H(6)	120.8
O(1)-C(7)-C(4)	110.7(4)
O(1)-C(7)-C(8)	103.4(4)
C(4)-C(7)-C(8)	114.4(4)
O(1)-C(7)-H(7)	109.4
C(4)-C(7)-H(7)	109.4
C(8)-C(7)-H(7)	109.4
C(7)-C(8)-Cl(1)	109.0(4)
C(7)-C(8)-P(1)	112.1(4)
Cl(1)-C(8)-P(1)	108.0(3)
C(7)-C(8)-H(8)	109.3
Cl(1)-C(8)-H(8)	109.3
P(1)-C(8)-H(8)	109.3
O(3)-C(9)-H(9A)	109.5
O(3)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
O(3)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
O(4)-C(10)-H(10A)	109.5
O(4)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
O(4)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5

O(6)-P(2)-O(7)	116.4(3)
O(6)-P(2)-O(8)	116.1(3)
O(7)-P(2)-O(8)	100.4(3)
O(6)-P(2)-C(18)	111.2(2)
O(7)-P(2)-C(18)	105.5(2)
O(8)-P(2)-C(18)	106.0(2)
C(17)-O(5)-H(5)	108(4)
C(19)-O(7)-P(2)	123.5(5)
C(20)-O(8)-P(2)	123.2(5)
C(12)-C(11)-C(16)	121.5(5)
C(12)-C(11)-Br(2)	120.2(5)
C(16)-C(11)-Br(2)	118.3(5)
C(11)-C(12)-C(13)	118.5(6)
C(11)-C(12)-H(12)	120.8
C(13)-C(12)-H(12)	120.8
C(14)-C(13)-C(12)	122.1(6)
C(14)-C(13)-H(13)	119.0
C(12)-C(13)-H(13)	119.0
C(13)-C(14)-C(15)	118.5(5)
C(13)-C(14)-C(17)	122.0(5)
C(15)-C(14)-C(17)	119.4(5)
C(16)-C(15)-C(14)	120.7(6)
C(16)-C(15)-H(15)	119.7
C(14)-C(15)-H(15)	119.7
C(15)-C(16)-C(11)	118.7(6)
C(15)-C(16)-H(16)	120.6
C(11)-C(16)-H(16)	120.6
O(5)-C(17)-C(14)	111.4(4)
O(5)-C(17)-C(18)	103.8(4)
C(14)-C(17)-C(18)	113.6(4)
O(5)-C(17)-H(17)	109.3
C(14)-C(17)-H(17)	109.3
C(18)-C(17)-H(17)	109.3
C(17)-C(18)-Cl(2)	110.2(3)
C(17)-C(18)-P(2)	110.5(3)
Cl(2)-C(18)-P(2)	109.0(3)
C(17)-C(18)-H(18)	109.0
Cl(2)-C(18)-H(18)	109.0

P(2)-C(18)-H(18)	109.0
O(7)-C(19)-H(19A)	109.5
O(7)-C(19)-H(19B)	109.5
H(19A)-C(19)-H(19B)	109.5
O(7)-C(19)-H(19C)	109.5
H(19A)-C(19)-H(19C)	109.5
H(19B)-C(19)-H(19C)	109.5
O(8)-C(20)-H(20A)	109.5
O(8)-C(20)-H(20B)	109.5
H(20A)-C(20)-H(20B)	109.5
O(8)-C(20)-H(20C)	109.5
H(20A)-C(20)-H(20C)	109.5
H(20B)-C(20)-H(20C)	109.5

Symmetry transformations used to generate equivalent atoms:

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
Br(1)	96(1)	98(1)	98(1)	2(1)	-6(1)	47(1)
Cl(1)	68(1)	72(1)	95(1)	16(1)	13(1)	-6(1)
P(1)	58(1)	70(1)	54(1)	4(1)	-2(1)	16(1)
O(1)	68(2)	71(2)	62(2)	11(2)	16(2)	-6(2)
O(2)	87(3)	97(3)	59(2)	9(2)	-11(2)	28(3)
O(3)	49(2)	116(4)	75(3)	0(3)	1(2)	22(2)
O(4)	87(3)	71(3)	79(3)	-4(2)	12(2)	5(2)
C(1)	58(3)	69(3)	68(3)	3(3)	1(3)	14(3)
C(2)	71(4)	78(4)	59(3)	2(3)	-10(3)	13(3)
C(3)	74(3)	68(3)	52(3)	-4(2)	-3(2)	13(3)
C(4)	47(2)	65(3)	50(2)	5(2)	4(2)	4(2)
C(5)	74(4)	99(5)	51(3)	-11(3)	-8(3)	28(4)
C(6)	84(4)	96(5)	72(4)	-16(3)	3(3)	37(4)
C(7)	50(2)	67(3)	46(2)	7(2)	4(2)	4(2)
C(8)	53(3)	72(3)	45(2)	10(2)	2(2)	10(3)
C(9)	61(4)	112(6)	132(7)	-4(5)	1(4)	-2(4)
C(10)	144(8)	67(4)	117(6)	9(4)	15(6)	-13(5)
Br(2)	109(1)	128(1)	136(1)	14(1)	31(1)	74(1)
Cl(2)	46(1)	68(1)	80(1)	8(1)	6(1)	-2(1)
P(2)	49(1)	59(1)	52(1)	-1(1)	5(1)	15(1)
O(5)	47(2)	68(2)	66(2)	18(2)	9(2)	-2(2)
O(6)	74(3)	77(3)	59(2)	-10(2)	4(2)	23(2)
O(7)	47(2)	89(3)	73(2)	0(2)	1(2)	10(2)
O(8)	76(3)	63(2)	80(3)	8(2)	7(2)	20(2)
C(11)	61(3)	71(4)	89(4)	13(3)	15(3)	29(3)
C(12)	63(3)	94(5)	80(4)	9(4)	-12(3)	29(4)
C(13)	66(3)	75(4)	62(3)	-3(3)	-10(3)	23(3)
C(14)	45(2)	48(2)	55(3)	7(2)	6(2)	5(2)
C(15)	70(3)	78(4)	57(3)	-4(3)	-5(3)	26(3)
C(16)	86(4)	98(5)	69(4)	-11(3)	6(3)	32(4)
C(17)	49(2)	56(3)	44(2)	5(2)	7(2)	9(2)
C(18)	40(2)	55(2)	47(2)	0(2)	6(2)	4(2)
C(19)	44(3)	147(8)	120(6)	-13(6)	14(3)	-10(4)

Table 4.Anisotropic displacement parameters $(Å^2x \ 10^3)$ for ga_91204ea_a.The anisotropicdisplacement factor exponent takes the form: $-2\pi^2$ [$h^2 \ a^{*2}U^{11} + ... + 2 \ h \ k \ a^* \ b^* \ U^{12}$]

	Х	у	Z	U(eq)
H(1)	2990(100)	2930(90)	4708(18)	90(30)
H(2)	1109	6990	3753	83
H(3)	2778	5115	3895	77
H(5A)	3267	6652	4958	90
H(6)	1589	8554	4822	100
H(7)	4546	4486	4814	65
H(8)	5276	4070	4018	68
H(9A)	9788	4277	4314	152
H(9B)	10477	2841	4136	152
H(9C)	9875	2840	4562	152
H(10A)	5414	445	4760	164
H(10B)	5598	-738	4434	164
H(10C)	4184	312	4434	164
H(5)	9620(60)	7340(50)	2830(11)	37(12)
H(12)	3686	8425	2686	95
H(13)	5652	6820	2548	81
H(15)	7531	7614	3569	82
H(16)	5602	9258	3710	101
H(17)	8034	5729	2675	60
H(18)	8424	5007	3471	57
H(19A)	12619	4244	2800	155
H(19B)	13316	4850	3184	155
H(19C)	12117	5784	2957	155
H(20A)	7979	1004	3013	174
H(20B)	8796	63	3330	174
H(20C)	9609	369	2936	174

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å²x 10^3) for ga_91204ea_a.

O(2)-P(1)-O(3)-C(9)	-13.6(7)
O(4)-P(1)-O(3)-C(9)	-139.6(6)
C(8)-P(1)-O(3)-C(9)	109.5(6)
O(2)-P(1)-O(4)-C(10)	19.8(7)
O(3)-P(1)-O(4)-C(10)	146.3(7)
C(8)-P(1)-O(4)-C(10)	-105.1(7)
C(6)-C(1)-C(2)-C(3)	4.0(11)
Br(1)-C(1)-C(2)-C(3)	-176.2(5)
C(1)-C(2)-C(3)-C(4)	-1.9(10)
C(2)-C(3)-C(4)-C(5)	-0.1(9)
C(2)-C(3)-C(4)-C(7)	174.9(6)
C(3)-C(4)-C(5)-C(6)	0.0(10)
C(7)-C(4)-C(5)-C(6)	-174.9(7)
C(2)-C(1)-C(6)-C(5)	-4.0(12)
Br(1)-C(1)-C(6)-C(5)	176.2(6)
C(4)-C(5)-C(6)-C(1)	2.0(12)
C(5)-C(4)-C(7)-O(1)	117.6(6)
C(3)-C(4)-C(7)-O(1)	-57.1(6)
C(5)-C(4)-C(7)-C(8)	-126.0(6)
C(3)-C(4)-C(7)-C(8)	59.3(7)
O(1)-C(7)-C(8)-Cl(1)	179.5(3)
C(4)-C(7)-C(8)-Cl(1)	59.0(5)
O(1)-C(7)-C(8)-P(1)	-61.1(5)
C(4)-C(7)-C(8)-P(1)	178.4(4)
O(2)-P(1)-C(8)-C(7)	-46.4(5)
O(3)-P(1)-C(8)-C(7)	-173.1(4)
O(4)-P(1)-C(8)-C(7)	81.5(4)
O(2)-P(1)-C(8)-Cl(1)	73.6(4)
O(3)-P(1)-C(8)-Cl(1)	-53.1(3)
O(4)-P(1)-C(8)-Cl(1)	-158.5(3)
O(6)-P(2)-O(7)-C(19)	11.8(7)
O(8)-P(2)-O(7)-C(19)	138.1(6)
C(18)-P(2)-O(7)-C(19)	-112.0(6)
O(6)-P(2)-O(8)-C(20)	-22.9(7)
O(7)-P(2)-O(8)-C(20)	-149.3(6)
C(18)-P(2)-O(8)-C(20)	101.1(7)

Table 6. Torsion angles [°] for ga_91204ea_a.

C(16)-C(11)-C(12)-C(13)	-2.2(11)
Br(2)-C(11)-C(12)-C(13)	177.1(5)
C(11)-C(12)-C(13)-C(14)	1.8(11)
C(12)-C(13)-C(14)-C(15)	-0.7(10)
C(12)-C(13)-C(14)-C(17)	-176.3(6)
C(13)-C(14)-C(15)-C(16)	0.0(10)
C(17)-C(14)-C(15)-C(16)	175.7(6)
C(14)-C(15)-C(16)-C(11)	-0.5(11)
C(12)-C(11)-C(16)-C(15)	1.6(12)
Br(2)-C(11)-C(16)-C(15)	-177.7(6)
C(13)-C(14)-C(17)-O(5)	125.2(6)
C(15)-C(14)-C(17)-O(5)	-50.4(6)
C(13)-C(14)-C(17)-C(18)	-118.0(6)
C(15)-C(14)-C(17)-C(18)	66.4(6)
O(5)-C(17)-C(18)-Cl(2)	176.6(3)
C(14)-C(17)-C(18)-Cl(2)	55.4(5)
O(5)-C(17)-C(18)-P(2)	-62.9(4)
C(14)-C(17)-C(18)-P(2)	175.9(4)
O(6)-P(2)-C(18)-C(17)	-42.8(4)
O(7)-P(2)-C(18)-C(17)	84.2(4)
O(8)-P(2)-C(18)-C(17)	-169.8(3)
O(6)-P(2)-C(18)-Cl(2)	78.4(3)
O(7)-P(2)-C(18)-Cl(2)	-154.5(3)
O(8)-P(2)-C(18)-Cl(2)	-48.6(3)

Symmetry transformations used to generate equivalent atoms:

D-HA	d(D-H)	d(HA)	d(DA)	<(DHA)
O(5)-H(5)O(6)#1 O(1)-H(1)O(2)#2	0.79(2) 0.83(5)	1.92(3) 1.85(6)	2.693(5) 2.667(6)	166(5) 169(8)

Table 7. Hydrogen bonds for ga_91204ea_a [Å and].

Symmetry transformations used to generate equivalent atoms:

#1 -x+2,y+1/2,-z+1/2 #2 x-1/2,-y+1/2,-z+1