Supporting Information

A biocatalytic redox cascade approach for one-pot deracemization of carboxyl-substituted tetrahydroisoquinolines by stereoinversion

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1. Microorganism strains and plasmids

Pseudomonas putida KT2440, *Pseudomonas aeruginosa* PAO1, *Pseudomonas fluorescens* Pf0-1, and *Pseudomonas entomophila str.* L48 were stored in our laboratory. *Escherichia coli* strain DH5α and BL21 (DE3) were used as cloning host and expression host, respectively. The pGEM-T was used as cloning vector. The pET-28a (+) was used as expression vector. The genes encoding D-amino acid oxidase from *Fusarium solani* (*Fs*DAAO, GenBank accession number: BAA00692.1) and alcohol dehydrogenase from *Thermoanaerobacter brockii* (*Tb*ADH, GenBank accession number: ADV78851.1) were codon optimized and previously cloned into pET-28a (+), respectively. The recombinant plasmid pET-28a-*Fs*DAAO or pET-28a-*Tb*ADH was transformed into *E. coli* strain BL21 (DE3) and stored in our laboratory.

2. Cloning of DpkAs

The Pip2C/Pyr2C reductase genes from *Pseudomonas putida* KT2440, *Pseudomonas aeruginosa* PAO1, putative dehydrogenase gene from *Pseudomonas fluorescens* Pf0-1, and putative malate dehydrogenase gene from *Pseudomonas entomophila str.* L48 were amplified from the corresponding genome DNA of these strains via polymerase chain reaction (PCR) with a series of primers (listed in Table S2) and ligated into the vector pET-28a (+). The recombinant plasmids pET-28a-DpkAs were subsequently transformed into *E. coli* BL21 (DE3) for expression. Multiple alignment was performed using the MUSCLE server (https://www.ebi.ac.uk/Tools/msa/muscle/) and displayed using Esprit (http://espript.ibcp.fr).

3. Expression of recombinant *Fs*DAAO, DpkAs, and *Tb*ADH

The *E. coli* BL21 (DE3) cells harboring recombinant plasmid pET-28a-*Fs*DAAO, pET-28a-DpkAs, or pET-28a-*Tb*ADH were cultivated in 50 mL of liquid LB medium supplemented with kanamycin (50 μg/mL) at 37 ℃ with shaking at 200 rpm until OD_{600} reached 0.6–0.8, and then induced by addition of IPTG (final concentration of 0.1 mM) at 18 ℃ for further 15 h. After medium removal by centrifugation (4000 rpm, 10 min, 4 ℃), the cells pellet were washed with phosphate buffer (50 mM, pH 8.0) for twice and resuspended in the same buffer. The cells were disrupted by sonication. After cell debris removal by centrifugation (12000 rpm, 20 min, 4 ℃), the crude lysates were obtained and analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

4. Purification of recombinant *Fs*DAAO, *Pp*DpkA and *Tb*ADH

Recombinant *E.coli* cells were harvested by centrifugation and resuspended in buffer A (20 mM sodium phosphate buffer, pH 7.5, 500 mM NaCl, 20 mM imidazole). The cells were disrupted by sonication and the cell lysate were removed by centrifugation (12000 rpm at 4 ℃ for 20 min). Then the supernatant was loaded onto a Ni-NTA-Sefinose column (5 mL, Sangon Biotech), and the proteins were eluted with an increasing gradient of imidazole from 20 to 250 mM in buffer A at a flow rate of 1 mL/min. The pure protein was collected, concentrated by ultrafiltration and stored at -80 ℃ with 20% glycerol for further study. The expression and purity of the protein were identified by SDS-PAGE. The protein concentration was determined using the Bradford Protein Assay Kit (Quick Start™, Bio-Rad, USA).

5. Enzyme activity assay

The DpkA enzyme activity was determined using a HPLC method or by monitoring the decrease in the absorbance of NADPH at 340 nm. In the HPLC method, the standard reaction mixture (400 μL) contained 10 mM substrate 3,4-dihydroisoquinoline-1-carboxylic acid (**1b**), 10 mM NADPH and appropriate amount of enzyme in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0). The reaction was performed at 30 °C for 5 min with shaking at 600 rpm and then quenched by addition of 400 μL methanol (containing 50 mM formic acid and 25 mM diethylamine). The amount of product was then determined by HPLC. One unit (U) of DpkA activity in the imine reduction was defined as the amount of protein that catalyzed the formation of 1 μmol (*S*)-**1a** per minute. On the other hand, the standard reaction mixture (1 mL) contained 10 mM substrate,

0.1 mM NADPH and appropriate amount of enzyme in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0) at 30 °C, and the absorbance was continuously measured at 340 nm for one minute. A molar extinction coefficient for NADPH of 6220 M^{-1} cm^{-1} was used for calculating the enzyme activity. One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol NADPH per minute.

The *Fs*DAAO activity was determined using a HPLC method or a coupled *o*-dianisidine/peroxidase method. In the HPLC method, the reaction mixture contained FAD (50 μM), *rac*-**1a** (10 mM) and an appropriate amount of crude extract or purified enzyme in NaH_2PO_4-Na_2HPO_4 buffer (50 mM, pH 8.0). After incubation at 30 °C with 600 rpm shaking for 5 min, the reaction was terminated by adding an equal volume of methanol (containing 50 mM formic acid and 25 mM diethylamine). The sample was then analyzed by chiral HPLC. One unit (U) of DAAO was defined as the amount of protein that catalyzed the oxidation of 1 μmol (*R*)-**1a** per minute. In the coupled *o*-dianisidine/peroxidase method, unless otherwise stated, the reaction mixture contained substrate, *o*-dianisidine (0.86 mM), horseradish peroxidase (0.05 mg/ml), appropriate amount of purified *Fs*DAAO and FAD (20 μM) in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0) at 30 °C.

The *Tb*ADH activity was determined using a by monitoring the increase in the absorbance of NADPH at 340 nm. The standard reaction mixture (1 mL) contained 10 mM substrate isopropanol, 0.25 mM $NADP^+$ and appropriate amount of enzyme in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0) at 30 °C, and the absorbance was continuously measured at 340 nm for one minute. A molar extinction coefficient for NADPH of 6220 M^{-1} cm^{-1} was used for calculating the enzyme activity. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol NADPH per minute.

6. Effects of pH and temperature on *Pp*DpkA

The effect of pH on enzyme activity was determined at different pH levels (5.0–10.0) in the following buffers (50 mM): NaOAc-HOAc (pH 5.0, 5.5, 6.0), Na_2HPO_4-NaH_2PO_4 (pH 6.0, 6.5, 7.0, 7.5, 8.0), Tris-HCl (pH 8.0, 8.5, 9.0), Gly-NaOH (pH 9.0, 10.0). The pH stability of *Pp*DpkA was determined by preincubating the enzyme protein (0.1 mg/mL) in different pH (5.0–11.0) buffers for 24 h at 4 ℃, followed by analysis of the residual activity at 30 ℃ and pH 8.0. The highest enzyme activity was taken as 100%. The effect of temperature on enzyme activity was determined at different temperatures ranging from 20 to 55 °C. The thermostability of *Pp*DpkA was determined by preincubating the pure protein (0.1 mg/mL) at different temperatures (30, 40 and 50 °C) for proper time followed by measuring the residual activity. The half-life of the enzyme was calculated according to the equation $t_{1/2} = \ln 2/k$.

7. Determination of kinetic parameters

The kinetic parameters of purified *Pp*DpkA toward **1b** were determined by measuring the activities at varied **1b** concentrations (0.1–20 mM) at fixed NADPH (10 mM) using the standard HPLC method. The Michaelis-Menten constant (*K*m) and the catalytic number (*k*cat) of the enzyme were calculated according to the Lineweaver-Burk plots.

8. Effects of NADPH regeneration system on the activity of *Fs*DAAO and *Pp*DpkA

The effect of the NADPH regeneration system on the specific activity of purified *Fs*DAAO was determined using HPLC method at pH 8.0 and 30 °C. Different concentrations of NADPH (0–10.0 mM), $NADP^+$ (0–10.0 mM), isopropanol (0–100 mM), and acetone (0–100 mM) were added to the standard reaction mixture, respectively.

The effects of the isopropanol (0–100 mM) and acetone (0–100 mM) on the specific activity of purified *Pp*DpkA were determined by monitoring the decrease in the absorbance of NADPH at 340 nm.

9. Preparation and isolation of 1b

Reaction was performed as previously described.[1] (*R*)-**1a** (200 mg) was dissolved in 100 ml deionized water and the pH was carefully adjusted to 8.0 with ammonium hydroxide. The purified *Fs*DAAO and excessive catalase were added to the substrate solution. The reactor was kept at 30 ℃ through a thermostatic water bath. The pH was kept at 8.0 by bumping ammonium

hydroxide into the reactor. The reaction was monitored by chiral HPLC. After 24 h, the product was obtained through ultrafiltration and lyophilization, and analyzed using NMR and HR-MS, as described in the "Analytical methods" section.

10. General procedure for multi-enzymatic synthesis of (*S*)-2a–4a

General procedure for deracemization of *rac*-**2a**–**4a** was carried out as follows:

The substrate *rac*-**2a** hydrochloride salt (200 mg, 0.81 mmol), *rac*-**3a** (200 mg, 1.04 mmol) or *rac*-**4a** hydrochloride salt (200 mg, 0.73 mmol) was dissolved in water (8 mL), respectively. The pH was carefully adjusted to a value of pH 8.0 with ammonium hydroxide. Isopropanol (0.49 or 0.62 or 0.44 mmol) and $NADP^+$ (0.4 or 0.52 or 0.37 μmol) were added to the substrate solution. The lyophilized *E.coli* cells containing *Fs*DAAO or *Pp*DpkA or *Tb*ADH from 200 or 50 or 200 mL ferments was resuspended in 12.5 or 5 or 10 mL water, and the crude lysate was prepared by sonication and centrifugation. 5, 1 and 6 mL crude lysate and 50 mg catalase were added to the substrate solution. The pH was kept at 8.0. The reactor was kept at 30 ℃ through a thermostatic water bath. The reaction was monitored by chiral HPLC. Upon the completion of reaction, the proteins were removed through ultrafiltration. The solution were then evaporated off under vacuum, and the residue was washed with warm water. Compounds (*S*)-**1a**–**4a** were obtained.

11. Analytical methods

HPLC of **1b** and **1a** were performed with Pntulips® QS-C18 column (5 um, 250 × 4.6 mm) at 40 ℃. Chiral HPLC of *rac*-**1a**–**4a** were performed with CHIRALPAK ZWIX® (-) (150 × 4 mm, DAICEL Chiral Technologies) at 25 ℃. Absolute configuration was determined by comparison with literature data or by analogue.[2] Melting points were determined using a WRS-1B melting point apparatus. NMR spectra were recorded on a Bruker Avance DMX 500 spectrometer with D_2O as solvent (500 MHz for ^1H, 125 MHz for ^{13}C). Optical rotations were measured with a Perkin-Elmer 341 polarimeter at 20 ℃. HRMS were measured on a time-of-flight mass (TOF) mass spectrometer equipped with an electrospray ionization (ESI).

12. References

1 S. Ju, M. Qian, G. Xu, L. Yang and J. Wu, *Advanced Synthesis & Catalysis*, 2019, **361**, 3191-3199.

2 I. Ilisz, Z. Gecse, Z. Pataj, F. Fulop, G. Toth, W. Lindner and A. Peter, *J Chromatogr A*, 2014, **1363**, 169-177.

Supplementary data

1. Cloning, expression, purification and characterization

1.1 Cloning of candidate imine-reducing enzymes

Table S1. List of protein information of imine-reducing enzymes used in this study.

Source	GenBank Accession No.	Protein sequence identity
Pseudomonas putida KT2440	SKC02707.1 (*Pp*DpkA)	100%
Pseudomonas entomophila str. L48	CAK15457.1 (putative *Pe*DpkA)	87%
Pseudomonas fluorescens Pf0-1	ABA74308.1 (putative *Pf*DpkA)	73%
Pseudomonas aeruginosa PAO1	NP_249943.1 (*Pa*DpkA)	43%

Table S2. Primers used in this study.

Prime name	Primers (5' to 3')
*Pp*DpkA-F	CGGGATCCATGTCCGCACCTTCCACCAGCAC (*Bam*H I)
*Pp*DpkA-R	CCCAAGCTTTCAGCCAAGCAGCTCTTTCAGG (*Hin*d III)
*Pe*DpkA-F	CGGGATCCGTGCGCGTAGCCTTCAAC (*Bam*H I)
*Pe*DpkA-R	CCCAAGCTTTCACCTCGCCAGCGCCTTC (*Hin*d III)
*Pf*DpkA-F	CGGGATCCATGTCTGCGCCACACGATC (*Bam*H I)
*Pf*DpkA-R	CCGCTCGAGTTACTCGCCGGCCAGTTCAC (*Xho* I)
*Pa*DpkA-F	CGGGATCCGTGATCCGAATGACGCTGGAC (*Bam*H I)
*Pa*DpkA-R	CCCAAGCTTTCACTCCAGCAACGCCAGC (*Hin*d III)

1.2 Multiple sequence alignment

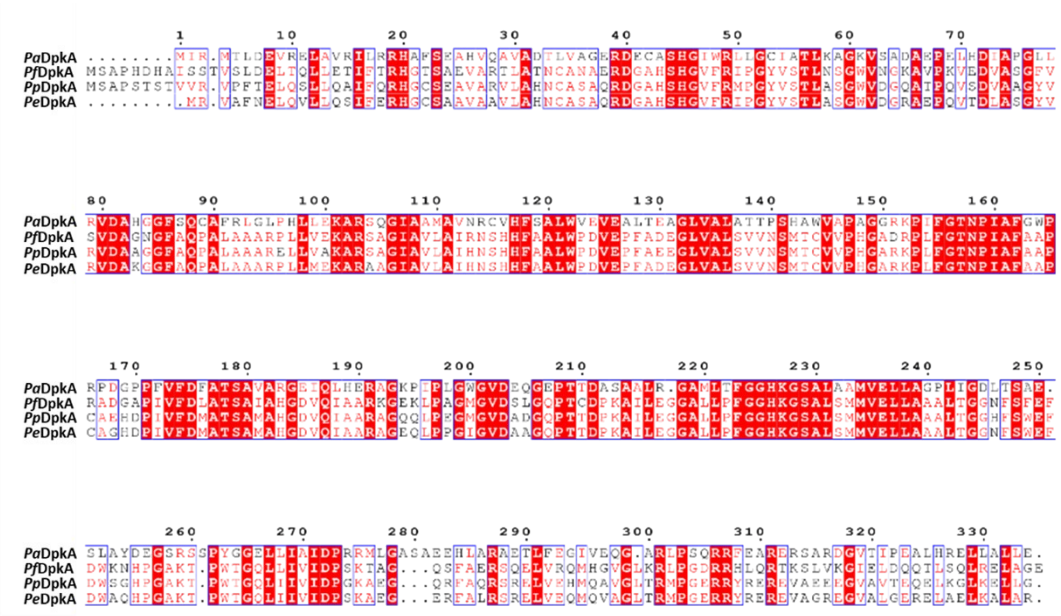


Figure S1. Multiple sequence alignment of four DpkAs. Multiple alignment was performed using the MUSCLE server (https://www.ebi.ac.uk/Tools/msa/muscle/) and displayed using Espript (http://espript.ibcp.fr).

1.3 Expression of four DpkAs

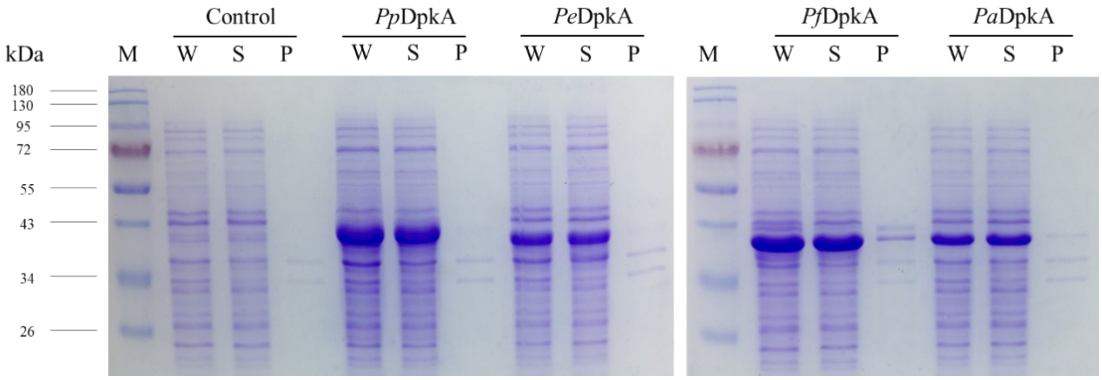


Figure S2. SDS-PAGE analysis of four DpkAs. Control, *E.coli* BL21 (DE3) expressing empty plasmid pET-28a (+); *Pp*DpkA, *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Pp*DpkA; *Pe*DpkA, *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Pe*DpkA; *Pf*DpkA, *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Pf*DpkA; *Pa*DpkA, *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Pa*DpkA. Lane M, protein ruler; lane W, crude extract of whole cell; lane S, soluble fraction; lane P, precipitate (insoluble fraction).

1.4 Purification and characterization of *Pp*DpkA

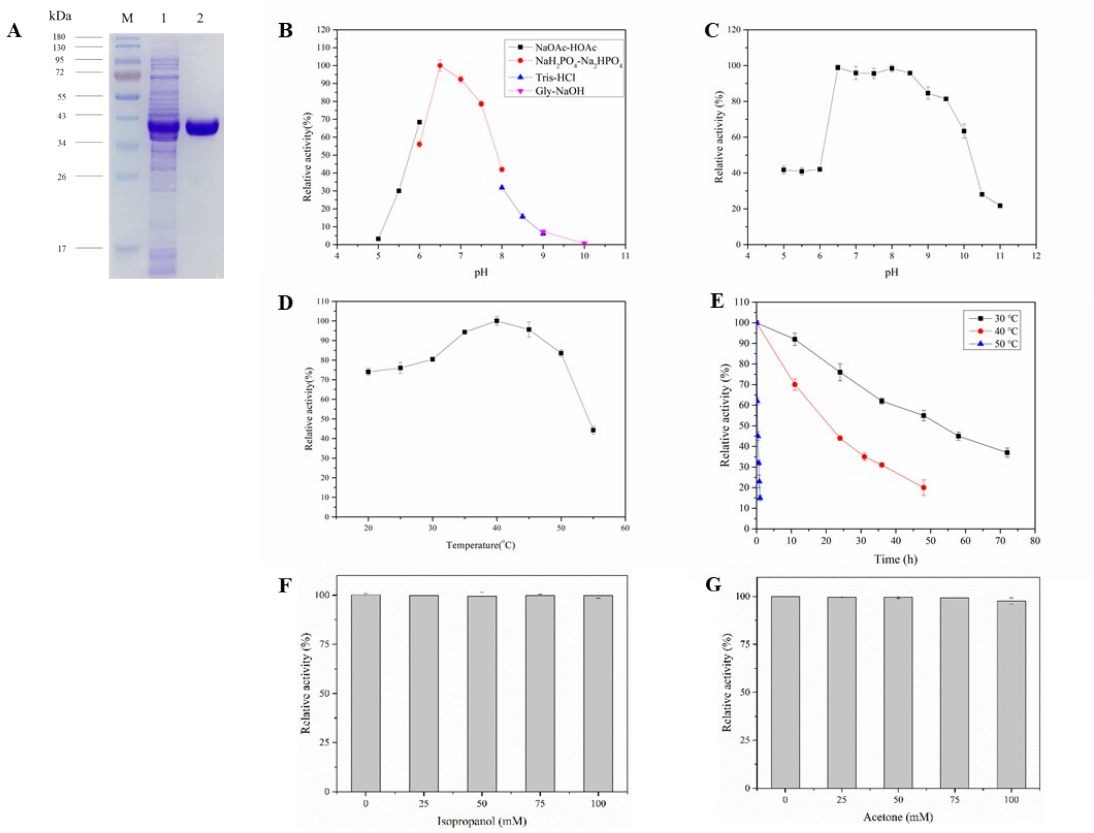


Figure S3. Purification and characterization of *Pp*DpkA. **A**) SDS-PAGE analysis of recombinant *Pp*dpkA before and after purification. Lane M, protein ruler; lane 1, crude extract of *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Pp*dpkA; lane 2, purified recombinant *Pp*dpkA. **B**) Effect of pH on the enzyme activity was determined by measuring the activity at various buffers (pH 5.0–10.0) at 30 °C. The value at pH 6.5 was set as 100%. **C**) Effect of pH on the enzyme stability was determined by measuring the remaining activity after incubation of the enzyme (0.1 mg/mL) in different buffers (pH 5.0–11.0) for 24 h at 4 °C. **D**) Effect of temperature on the enzyme activity was determined by measuring the activity at various temperatures (20–55 °C) at pH 8.0. **E**) Effect of temperature on the enzyme stability was determined by measuring the remaining activity after incubation of the enzyme (0.1 mg/mL) at 30, 40 and 50 °C. **F**) Effect of isopropanol on the enzyme activity was determined with different concentrations of isopropanol (0–100 mM) at 30 °C and pH 8.0. The value without isopropanol was set as 100%. **G**) Effect of acetone on the enzyme activity was determined with different concentrations of acetone (0–100 mM) at 30 °C and pH 8.0. The value without acetone was set as 100%. All experiments were performed in triplicate. Each data represents the mean ± the standard deviation of three measurements.

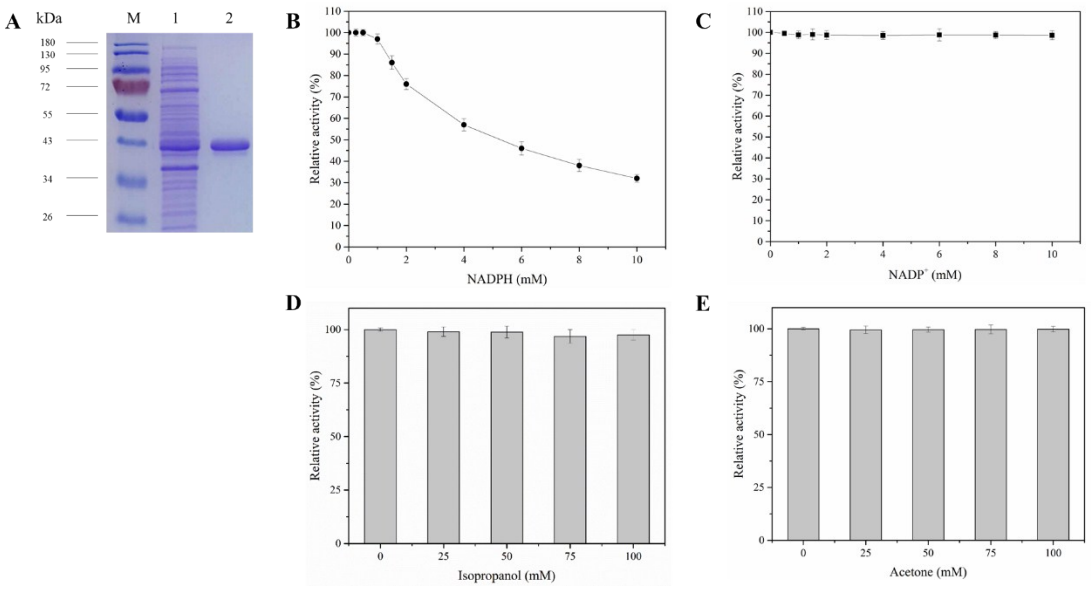
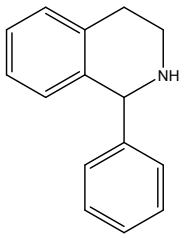


Figure S4. Effects of cofactor NADPH regeneration system on the specific activities of purified *Fs*DAAO. **A**) SDS-PAGE analysis of recombinant *Fs*DAAO before and after purification. Lane M, protein ruler; lane 1, crude extract of *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Fs*DAAO; lane 2, purified recombinant *Fs*DAAO. **B**) Effect of NADPH on the enzyme activity was determined with different concentrations of NADPH (0–10.0 mM) at 30 °C and pH 8.0. The value without NADPH was set as 100%. **C**) Effect of $NADP^+$ on the enzyme activity was determined with different concentrations of $NADP^+$ (0–10.0 mM) at 30 °C and pH 8.0. The value without $NADP^+$ was set as 100%. **D**) Effect of isopropanol on the enzyme activity was determined with different concentrations of isopropanol (0–100 mM) at 30 °C and pH 8.0. The value without isopropanol was set as 100%. **E**) Effect of acetone on the enzyme activity was determined with different concentrations of acetone (0–100 mM) at 30 °C and pH 8.0. The value without acetone was set as 100%. All experiments were performed in triplicate. Each data represents the mean±the standard deviation of three measurements.

Table S3. The activity of purified *Fs*DAAO toward 1-phenyl-1,2,3,4-tetrahydroisoquinoline[a]

Substrate	Specific activity
	0

[a] The assay was performed using a coupled *o*-dianisidine/peroxidase method.

Condon-optimized gene sequence of *Tb*ADH

ATGAAGGGTTTTGCAATGCTGAGTATTGGCAAAGTTGGTTGGATTGAAAAAGAAAAACCGGCACCGGGT
CCGTTTGATGCAATTGTGCGCCCGCTGGCCGTGGCACCGTGTACAAGCGATATTCATACCGTGTTTGAAG
GTGCCATTGGCGAACGTCATAATATGATTCTGGGTCATGAAGCAGTGGGTGAAGTGGTTGAAGTGGGTA
GCGAAGTTAAAGATTTTAAACCGGGTGACCGTGTTGTTGTGCCGGCCATTACCCCGGATTGGCGCACCAG
TGAAGTTCAGCGTGGCTATCATCAGCATAGCGGCGGTATGCTGGCAGGTTGGAAATTTTCAAATGTTAAA
GATGGTGTGTTCGGTGAATTTTTCCATGTTAATGATGCCGATATGAATCTGGCACATCTGCCGAAAGAAAT
TCCGCTGGAAGCAGCCGTGATGATTCCGGATATGATGACCACCGGCTTTCATGGTGCCGAACTGGCCGAT
ATTGAACTGGGTGCAACCGTTGCCGTTCTGGGTATTGGCCCGGTTGGTCTGATGGCCGTTGCAGGTGCCA
AACTGCGTGGCGCCGGCCGTATTATTGCCGTTGGCAGCCGCCCGGTTTGCGTGGATGCCGCTAAATATTA
TGGTGCAACCGATATTGTTAACTATAAAGATGGTCCGATTGAAAGCCAGATTATGAATCTGACCGAAGGT
AAAGGTGTTGATGCAGCAATTATTGCAGGTGGCAATGCAGATATTATGGCAACCGCCGTGAAAATTGTGA
AACCGGGCGGTACAATTGCCAATGTGAATTATTTTGGTGAAGGTGAAGTTCTGCCGGTTCCGCGTCTGGA
ATGGGGCTGCGGCATGGCCCATAAAACCATTAAGGGTGGCCTGTGCCCGGGCGGTCGTCTGAGAATGGA
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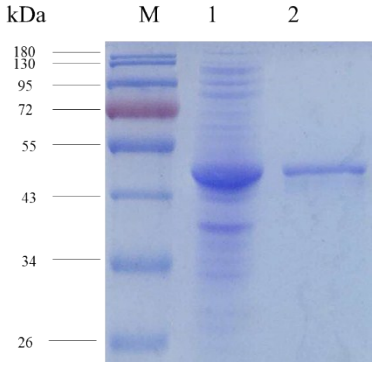


Figure S5. SDS-PAGE analysis of recombinant *Tb*ADH before and after purification. Lane M, protein ruler; lane 1, crude extract of *E.coli* BL21 (DE3) expressing recombinant plasmid pET-28a-*Tb*ADH; lane 2, purified recombinant *Tb*ADH.

Preparation and characterization of compound 1b

3, 4-dihydroisoquinoline-1-carboxylic acid (1b):

Yellow solid; 94% yield (186 mg); m.p. 206-208 °C; ^{1}H NMR (500 MHz, D_2O): δ 7.69-7.42 (m, 2H), 7.33-7.17 (m, 2H), 3.70 (t, *J* = 7.8 Hz, 2H), 2.93 (t, 2H); ^{13}C NMR (125 MHz, D_2O): δ 170.5, 164.9, 138.6, 137.1, 131.5, 128.6, 128.0, 122.4, 41.4, 24.2; HRMS (ESI-TOF) m/z: calcd. for $C_{10}H_{10}NO_2$ $[M+H]^+$: 176.0706; found: 176.0732.

Multi-enzymatic deracemization of *rac*-1a–4a

(*S*)-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ((*S*)-1a)

White solid; 89% yield (158 mg), *ee* >99%; m.p. 263-266 °C; $[\alpha]_D^{20}$ = +69 [*c* 0.5, 1M HCl]; ^{1}H NMR (500 MHz, D_2O): δ 7.49-7.37 (m, 1H), 7.33-7.15 (m, 3H), 4.87 (s, 1H), 3.56-3.46 (m, 1H), 3.42-3.32 (m, 1H), 3.03-2.94 (m, 2H); ^{13}C NMR (125 MHz, D_2O): δ171.9, 131.7, 128.7, 128.3, 128.1, 127.9, 126.9, 58.6, 39.8, 24.5; HRMS (ESI-TOF) m/z: calcd. for $C_{10}H_{12}NO_2$ $[M+H]^+$: 178.0863; found: 178.0869.

(*S*)-6-chloro-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ((*S*)-2a)

White solid; 91% yield (156 mg), *ee* >99%; m.p. 269-271 °C; $[\alpha]_D^{20}$ = +67 [*c* 0.5, 1M HCl]; ^{1}H NMR (500 MHz, D_2O) δ 7.43 (d, *J* = 8.3 Hz, 1H), 7.30-7.12 (m, 2H), 5.25 (s, 1H), 3.59-3.37 (m, 2H), 3.06-2.87 (m, 2H); ^{13}C NMR (125 MHz, D_2O): δ169.4, 134.2, 133.7, 129.8, 128.6, 127.1, 124.3, 55.9, 39.4, 24.2; HRMS (ESI-TOF) m/z: calcd. for $C_{10}H_{11}ClNO_2$ $[M+H]^+$: 212.0473; found: 212.0466.

(*S*)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ((*S*)-3a)

White solid; 86% yield (173 mg), *ee* >99%; m.p. 253-255 °C; $[\alpha]_D^{20}$ = +65 [*c* 0.5, 1M HCl]; ^{1}H NMR (500 MHz, D_2O) δ 7.24 (d, *J* = 8.6 Hz, 1H), 6.69 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 4.84 (s, 1H), 3.42 (dt, *J* = 13.2, 6.7 Hz, 1H), 3.30 (dt, *J* = 12.6, 6.1 Hz, 1H), 2.86 (t, *J* = 6.4 Hz, 2H). ^{13}C NMR (125 MHz, D_2O): δ171.6, 155.5, 133.5, 129.5, 119.3, 114.8, 114.4, 57.5, 39.6, 24.5; HRMS (ESI-TOF) m/z: calcd. for $C_{10}H_{12}NO_3$ $[M+H]^+$: 194.0812; found: 194.0802.

(*S*)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ((*S*)-4a)

White solid; 88% yield (152 mg), *ee* >99%; m.p. 256-258 °C; $[\alpha]_D^{20}$ = +66 [*c* 0.5, 1M HCl]; ^{1}H NMR (500 MHz, D_2O) δ 7.01 (s, 1H), 6.74 (s, 1H), 5.07 (s, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.52-3.37 (m, 2H), 2.97-2.82 (m, 2H); ^{13}C NMR (125 MHz, D_2O): δ170.2, 148.5, 147.0, 124.7, 118.1, 111.5, 110.8, 56.2, 55.7, 55.6, 39.7, 23.9; HRMS (ESI-TOF) m/z: calcd. for $C_{12}H_{16}NO_4$ $[M+H]^+$: 238.1074; found: 238.1061.

NMR and HR-MS spectra of 1b

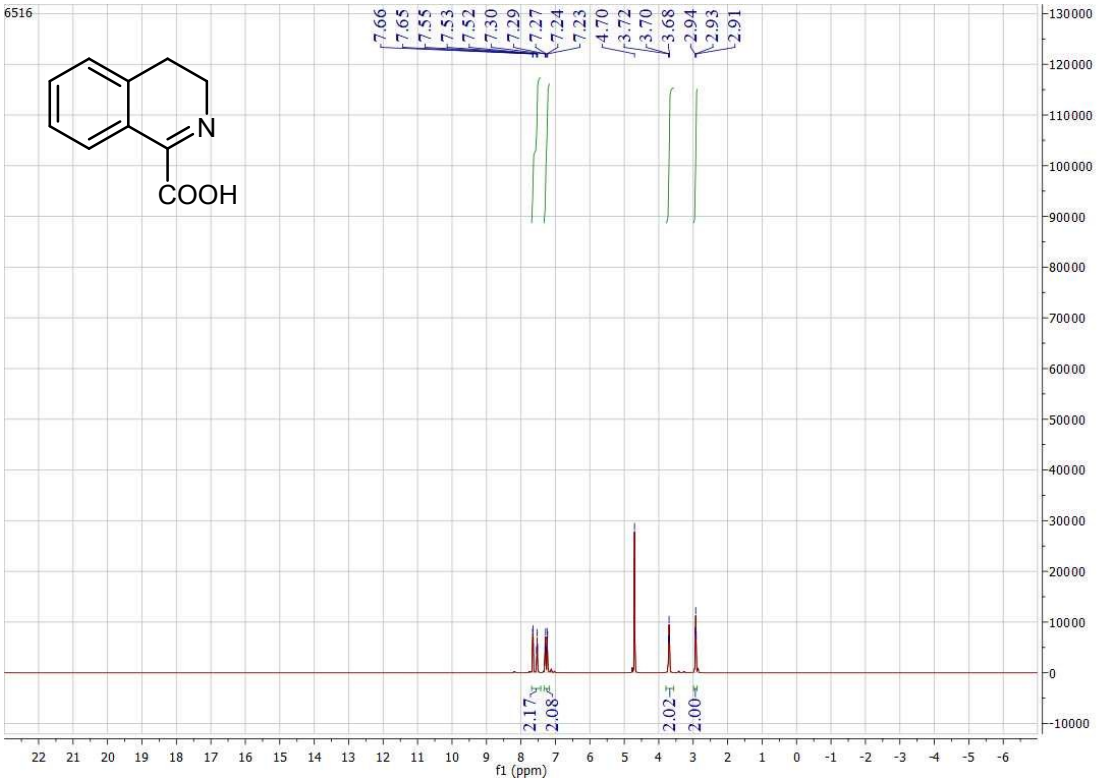


Figure S6. [1]H-NMR spectrum of compound **1b**.

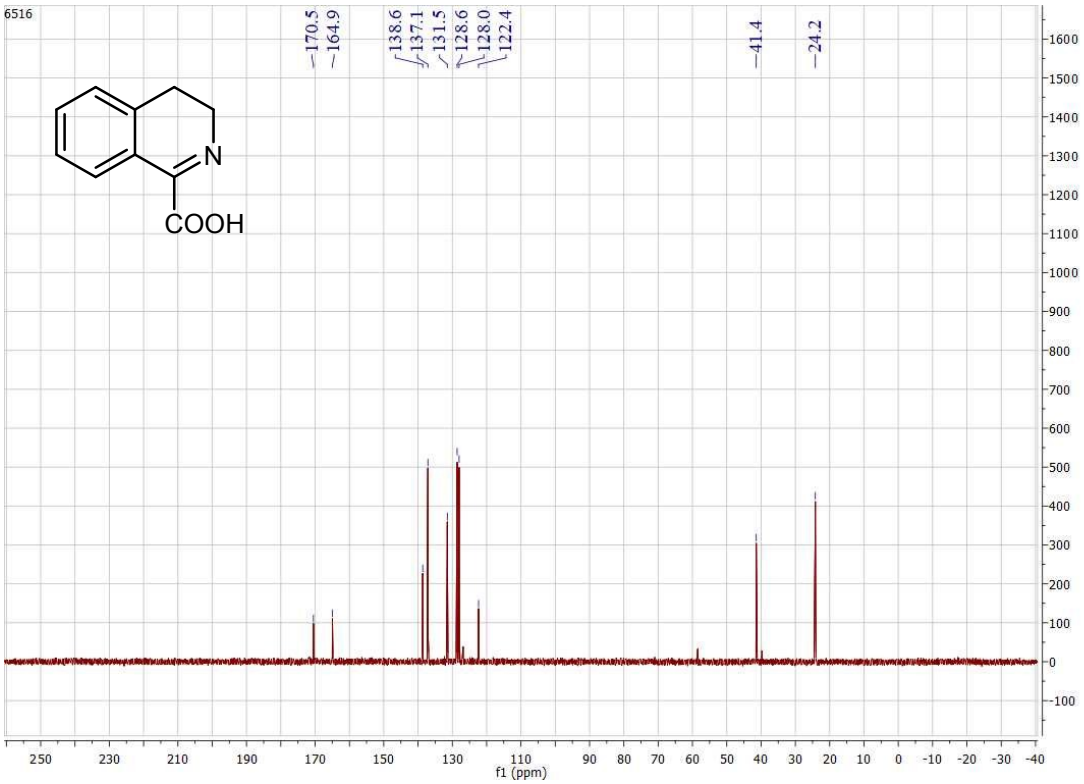


Figure S7. ^{13}C-NMR spectrum of compound **1b**.

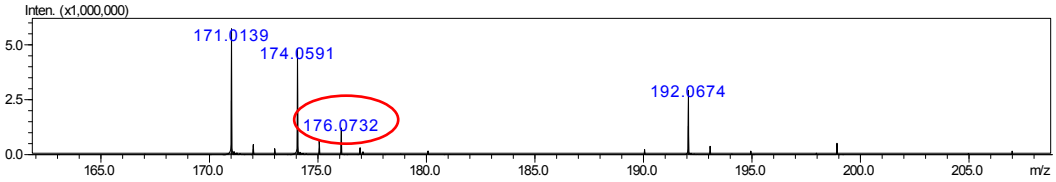


Figure S8. HRMS spectrum of compound **1b**.

NMR and HR-MS spectra of products (*S*)-1a–4a

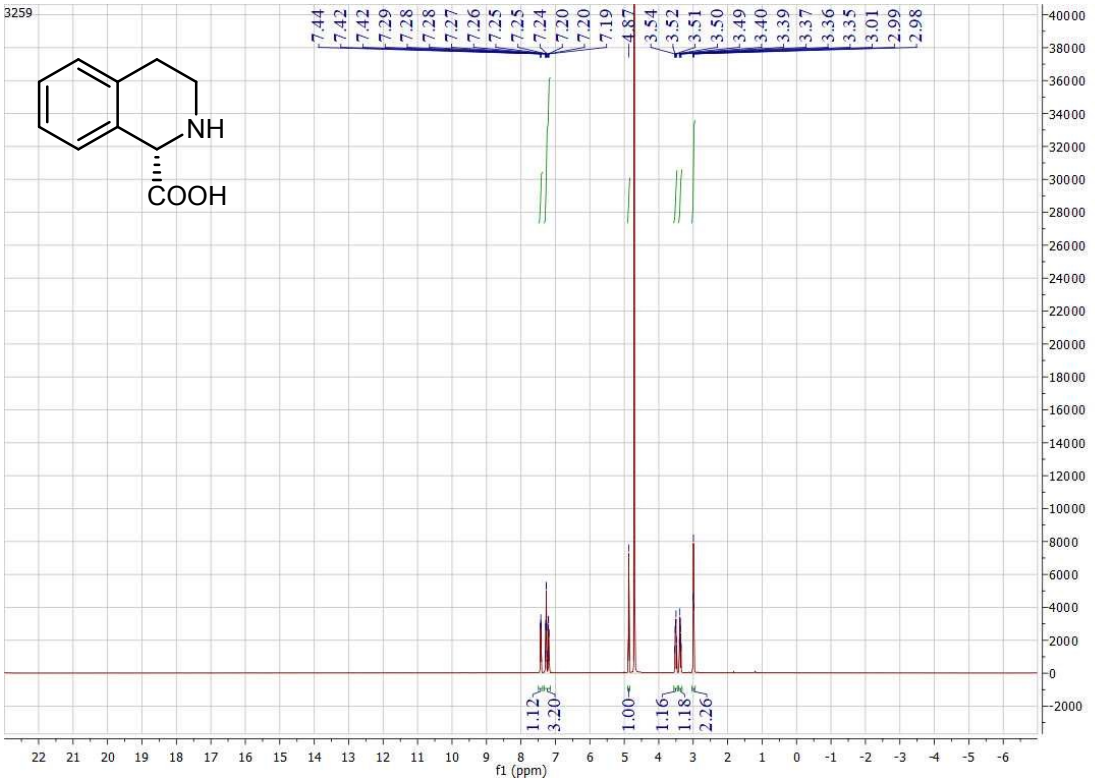


Figure S9. [1]H-NMR spectrum of product (*S*)-**1a**.

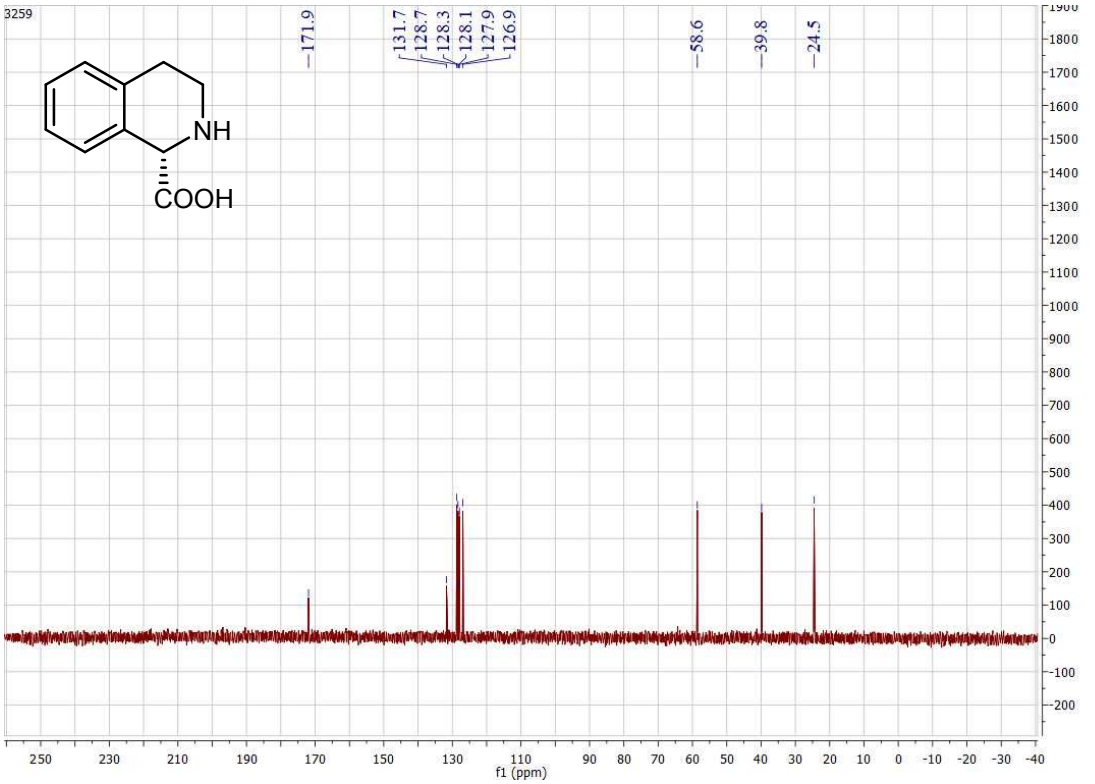


Figure S10. ^{13}C-NMR spectrum of product (*S*)-**1a**.

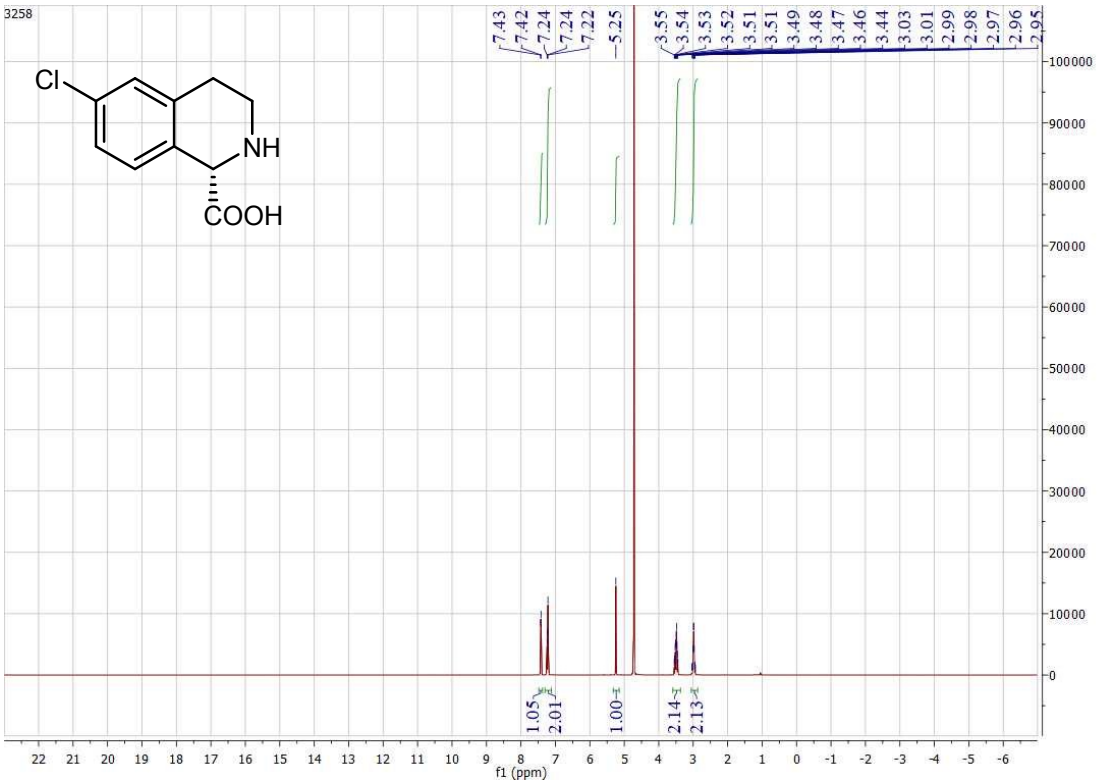


Figure S11. ^{1}H-NMR spectrum of product (*S*)-**2a**.

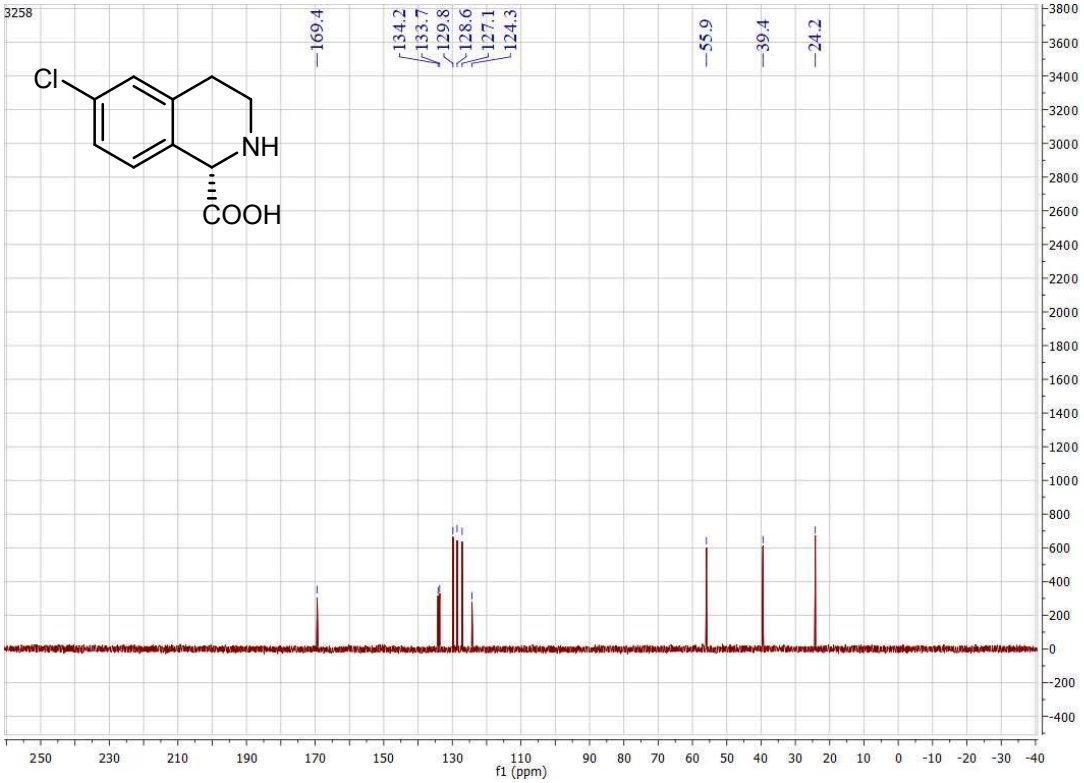


Figure S12. ^{13}C-NMR spectrum of product (*S*)-**2a**.

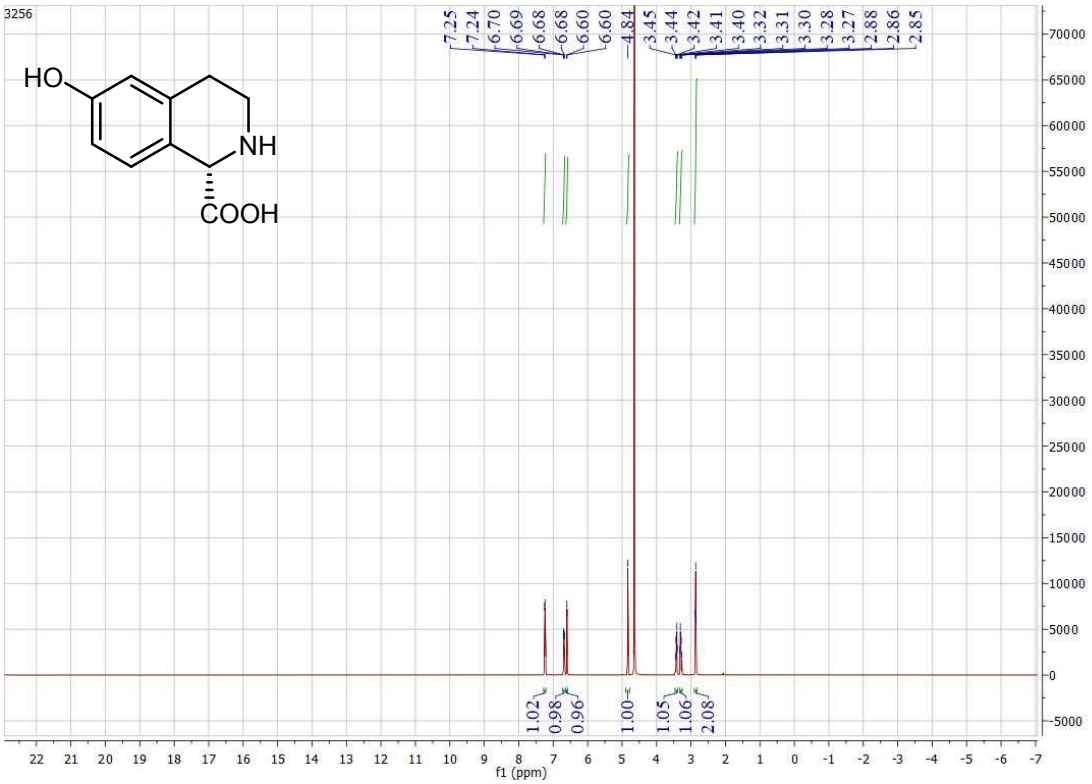


Figure S13. ^{1}H-NMR spectrum of product (*S*)-**3a**.

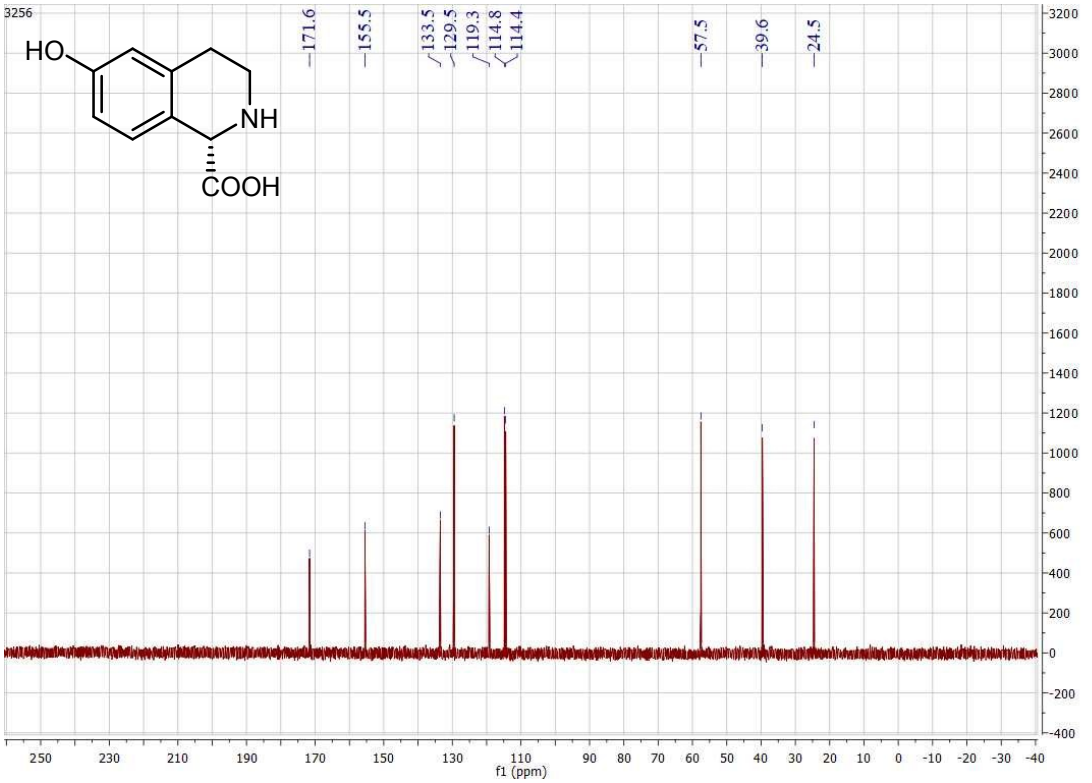


Figure S14. ^{13}C-NMR spectrum of product (*S*)-**3a**.

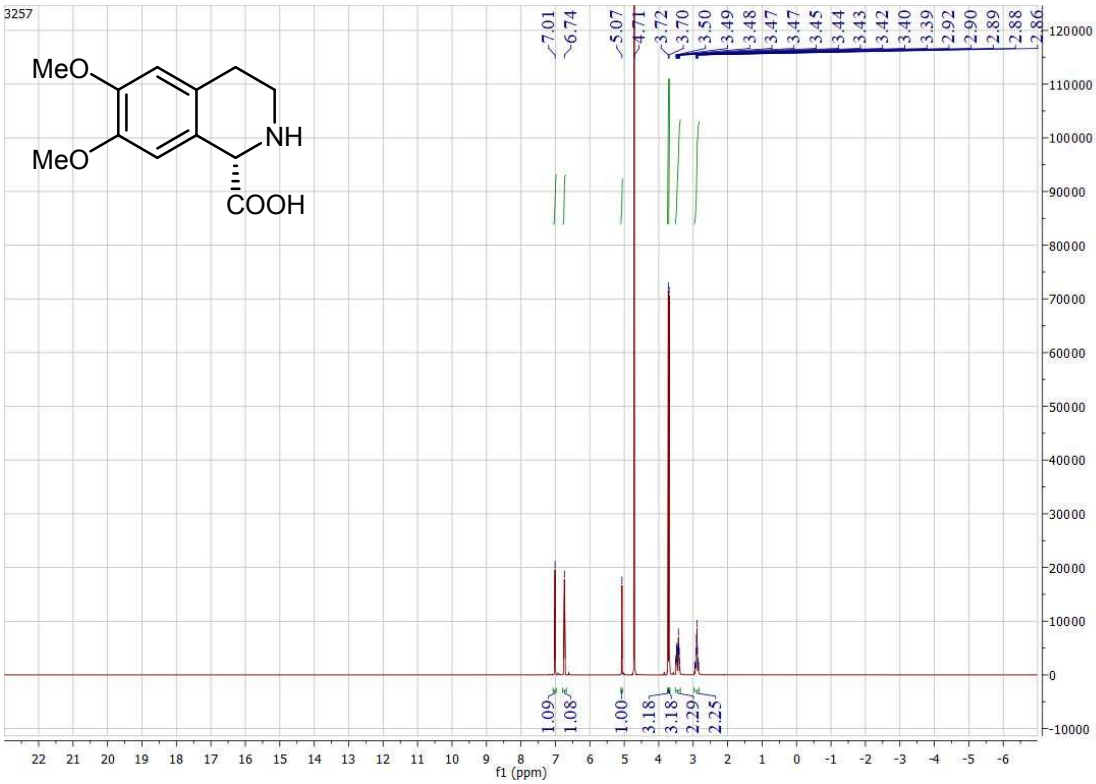


Figure S15. ^{1}H-NMR spectrum of product (*S*)-**4a**.

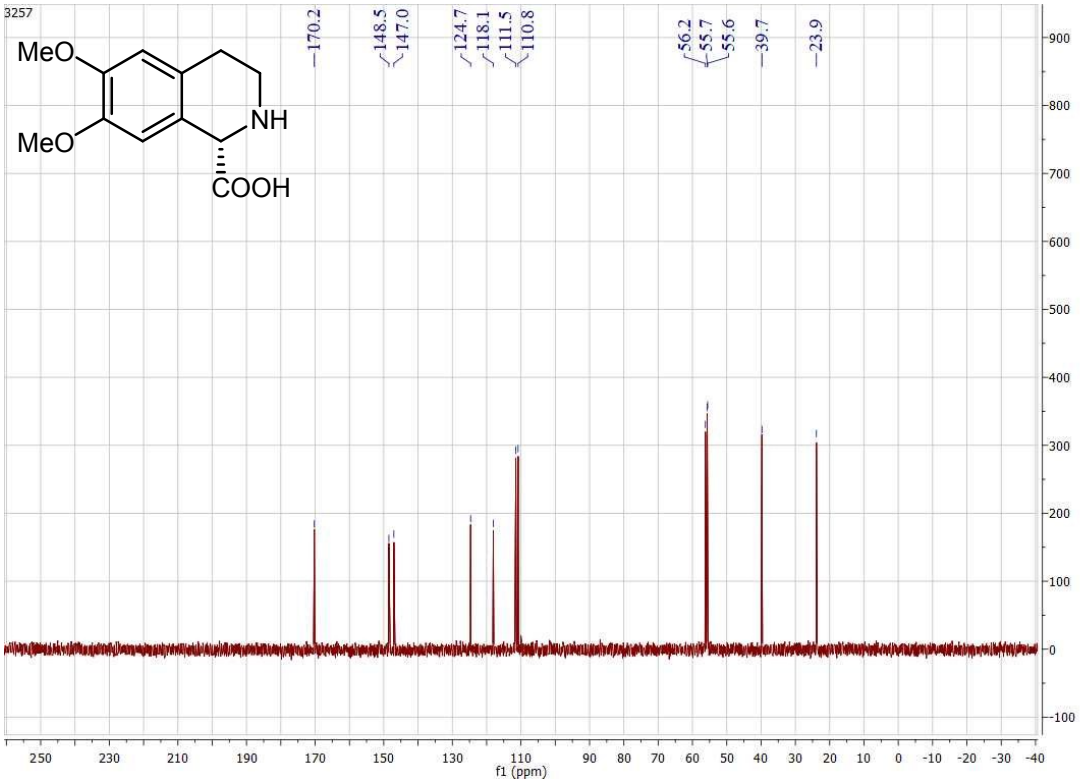


Figure S16. ^{13}C-NMR spectrum of product (*S*)-**4a**.

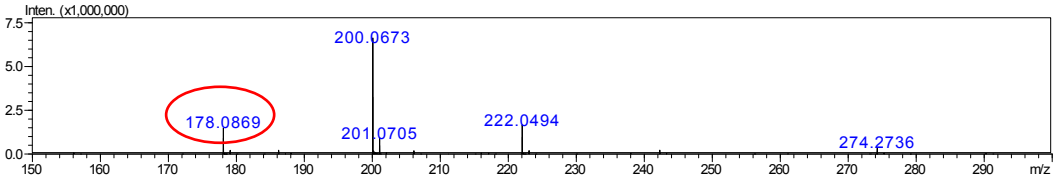


Figure S17. HRMS spectrum of product (*S*)-**1a**.

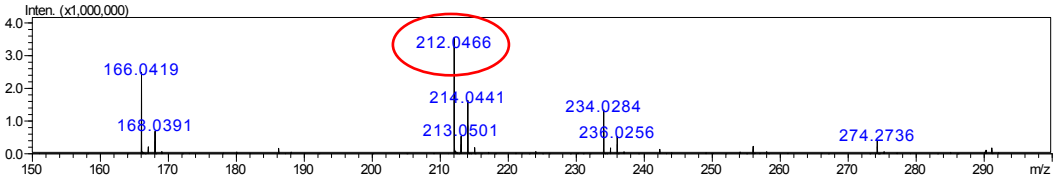


Figure S18. HRMS spectrum of product (*S*)-**2a**.

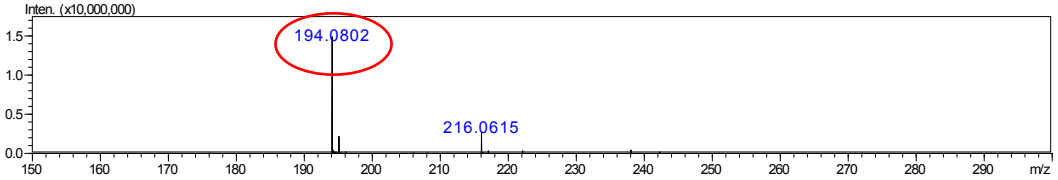


Figure S19. HRMS spectrum of product (*S*)-**3a**.

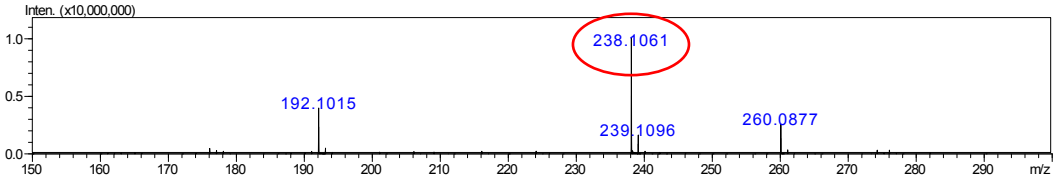
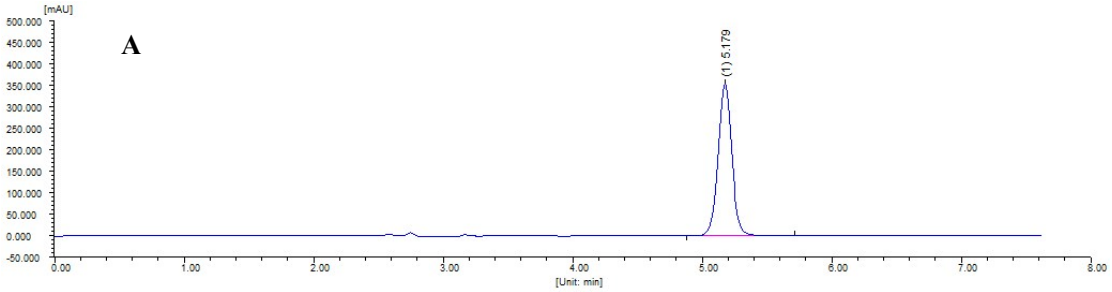
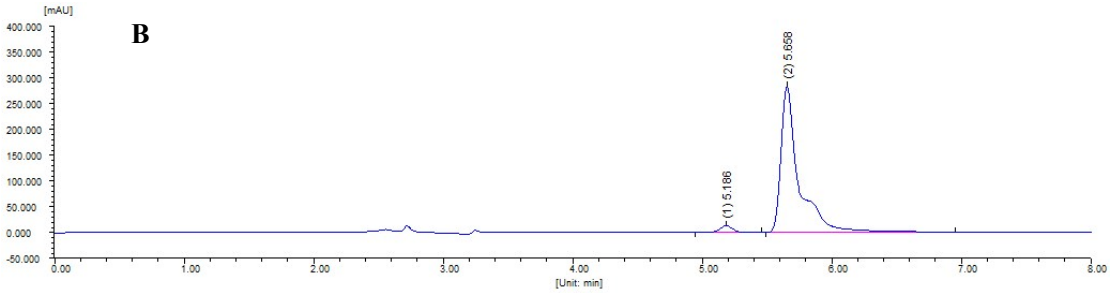


Figure S20. HRMS spectrum of product (*S*)-**4a**.

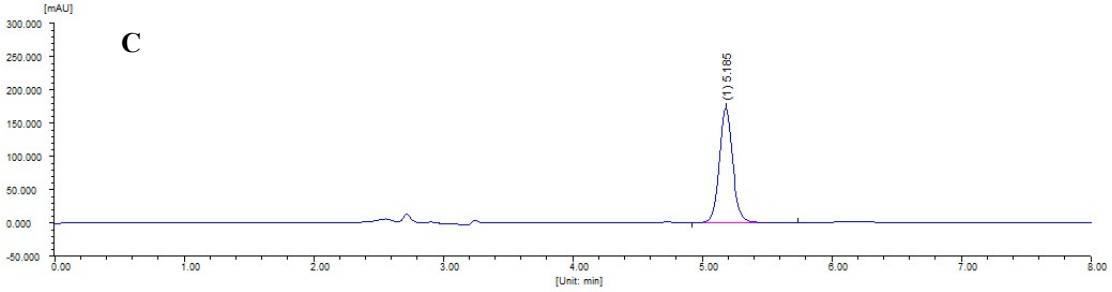
HPLC chromatograms for *Pp*DpkA-catalyzed asymmetric reduction of 1b



Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	5.179	354.6961	2617.4963	100

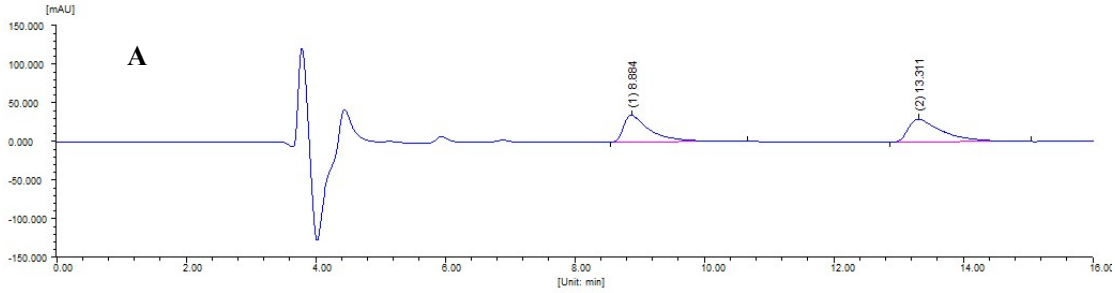


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	5.186	13.4951	93.9734	3.3392
2	5.658	283.4424	2720.2588	96.6608

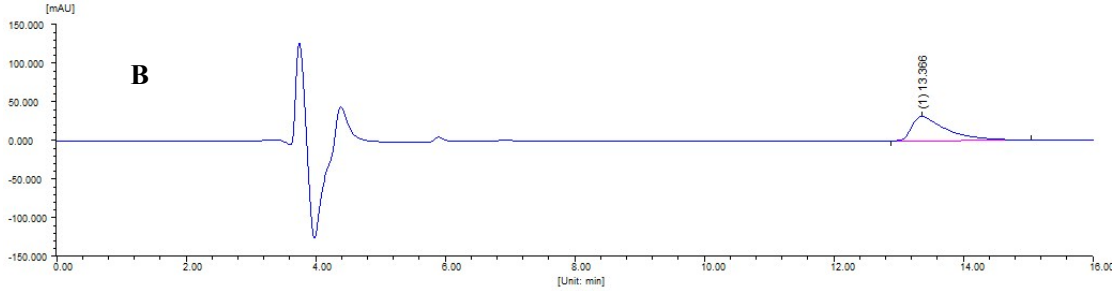


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	5.185	172.4439	1246.8274	100

Figure S21. HPLC chromatograms of (**A**) *rac*-**1a** for control, (**B**) biotransformation of substrate **1b** (reaction time=2 min), and (**C**) biotransformation of substrate **1b** (reaction time=60 min). HPLC conditions: Pntulips® QS-C18 column (5 um, 250 × 4.6 mm), 0.005% TFA in MeCN-H_2O (1 : 9 v/v), flow rate = 1.0 mL/min, 220 nm UV detector, t_R = 5.179 min (**1a**) and t_R = 5.658 min (**1b**), column temperature = 40 °C.



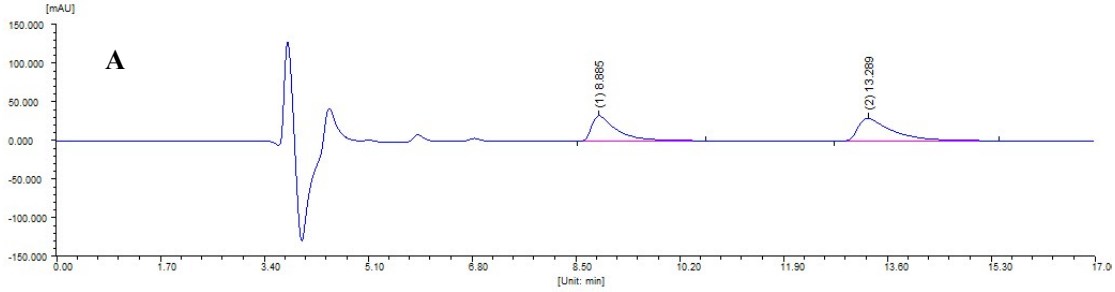
Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	8.884	39.6793	1154.3423	50.0176
2	13.311	31.2073	1153.5321	49.9824



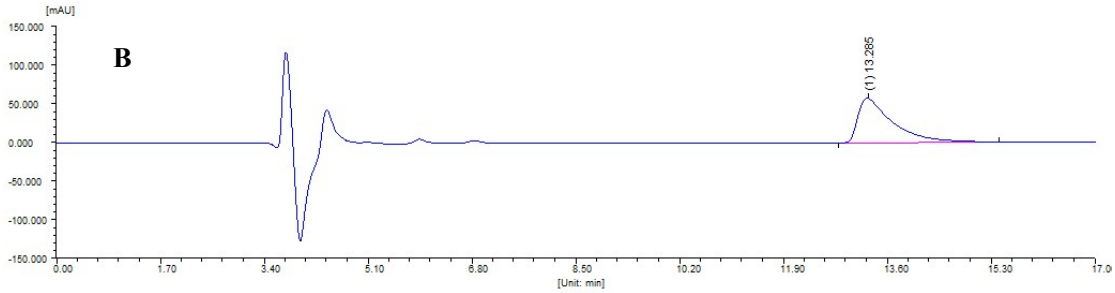
Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	13.366	31.2894	1153.8247	100

Figure S22. Chiral HPLC chromatograms of (**A**) *rac*-**1a** for control and (**B**) the product of *Pp*DpkA-catalyzed asymmetric reduction of compound **1b**. HPLC conditions: CHIRALPAK ZWIX® (-) column (150 × 4 mm, DAICEL Chiral Technologies), (50 mM FA + 25 mM DEA) in MeOH, flow rate = 0.4 mL/min, 220 nm UV detector, t_R = 8.884 min (*R*) and t_R = 13.311 min (*S*), column temperature = 25 °C.

HPLC chromatograms for multi-enzymatic deracemization of *rac*-1a–4a

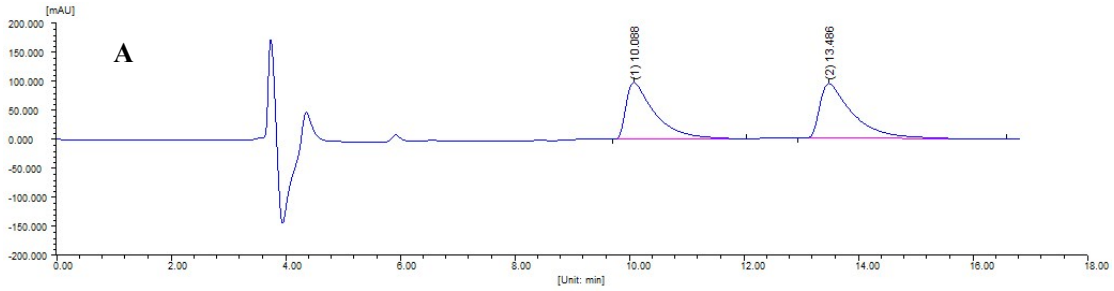


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	8.885	39.2351	1153.8419	49.9851
2	13.289	31.8177	1154.5303	50.0149

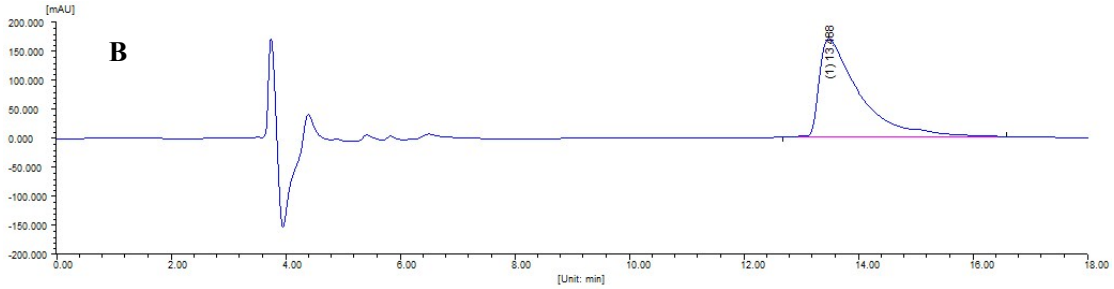


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	13.285	61.8943	2292.3505	100

Figure S23. Chiral HPLC chromatograms of (**A**) substrate *rac*-**1a** and (**B**) the product of multi-enzymatic deracemization of *rac*-**1a**. HPLC conditions: CHIRALPAK ZWIX® (-) column (150 × 4 mm, DAICEL Chiral Technologies), (50 mM FA + 25 mM DEA) in MeOH, flow rate = 0.4 mL/min, 220 nm UV detector, t_R = 8.885 min (*R*) and t_R = 13.289 min (*S*), column temperature = 25 °C.

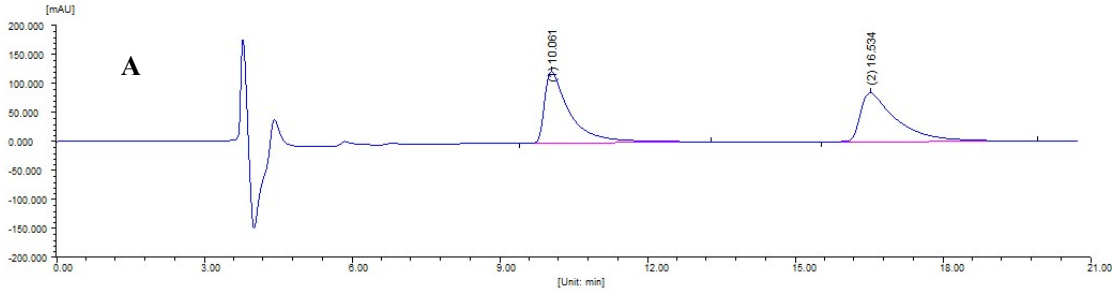


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	10.088	95.0513	4215.0925	49.8597
2	13.486	87.4921	4238.8120	50.1403

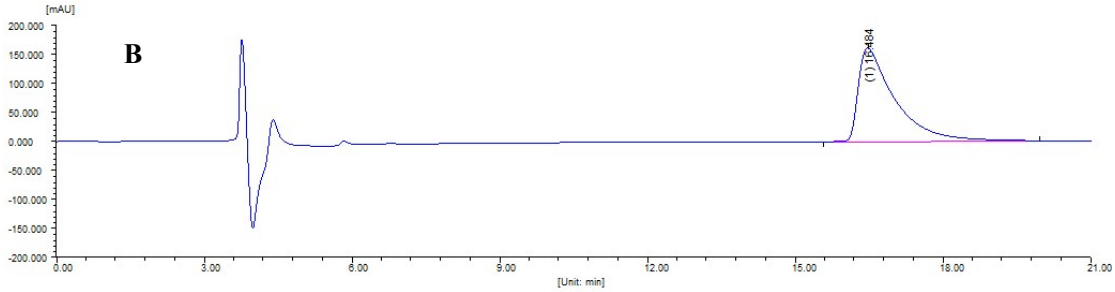


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	13.488	172.7885	8390.6270	100

Figure S24. Chiral HPLC chromatograms of (**A**) substrate *rac*-**2a** and (**B**) the product of multi-enzymatic deracemization of *rac*-**2a**. HPLC conditions: CHIRALPAK ZWIX® (-) column (150 × 4 mm, DAICEL Chiral Technologies), (50 mM FA + 25 mM DEA) in MeOH, flow rate = 0.4 mL/min, 220 nm UV detector, t_R = 10.088 min (*R*) and t_R = 13.486 min (*S*), column temperature = 25 °C.

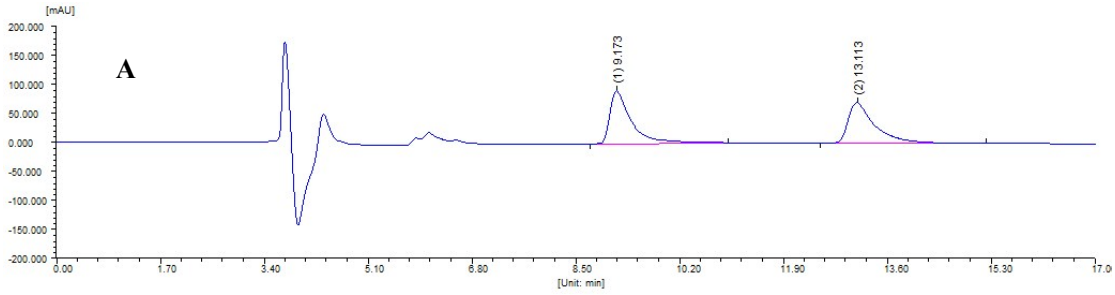


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	10.061	124.4600	4208.7396	49.7440
2	16.534	85.0963	4252.0630	50.2560

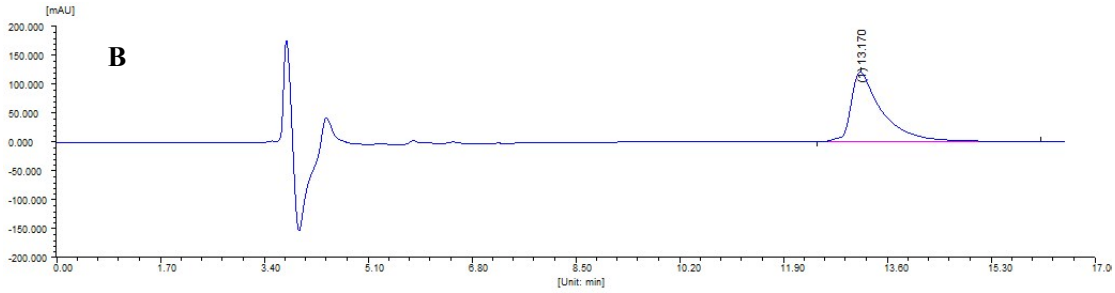


Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	16.484	161.5858	8340.7068	100

Figure S25. Chiral HPLC chromatograms of (**A**) substrate racemic *rac*-**3a** and (**B**) the product of multi-enzymatic deracemization of *rac*-**3a**. HPLC conditions: CHIRALPAK ZWIX® (-) column (150 × 4 mm, DAICEL Chiral Technologies), (50 mM FA + 25 mM DEA) in MeOH, flow rate = 0.4 mL/min, 220 nm UV detector, t_R = 10.061 min (*R*) and t_R = 16.534 min (*S*), column temperature = 25 °C.



Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	9.173	91.2306	2188.8901	50.2583
2	13.113	70.5401	2166.3931	49.7417



Peak#	Ret. Time[min]	Height[mAu]	Area[mAu*s]	Area[%]
1	13.17	120.7530	4312.5888	100

Figure S26. Chiral HPLC chromatograms of (**A**) substrate *rac*-**4a** and (**B**) the product of multi-enzymatic deracemization of *rac*-**4a**. HPLC conditions: CHIRALPAK ZWIX® (-) column (150 × 4 mm, DAICEL Chiral Technologies), (50 mM FA + 25 mM DEA) in MeOH, flow rate = 0.4 mL/min, 220 nm UV detector, t_R = 9.173 min (*R*) and t_R = 13.113 min (*S*), column temperature = 25 °C.