Supporting information

Enantioselective Hydrolysis of Racemic and *Meso*-epoxides with Recombinant *Escherichia coli* Expressing Epoxide Hydrolase from *Sphingomonas* sp. HXN-200: Preparation of Epoxides and Vicinal Diols in High *ee* and High Concentration

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Chemicals

The epoxides 2-(3-Fluorophenyl)oxirane **5**, 2-(3-Bromophenyl)oxirane **7**, *N*-phenoxycarbonyl-3,4-epoxy-piperidine **8** and *N*-benzyloxycarbonyl-3,4-epoxy-pyrrolidine **11** were synthesized according to previously reported methods.[S1, S2] All the other chemicals were obtained from commercial suppliers and used without further purification: Styrene oxide **1** (97%, Fluka), (*S*)-**1** (98%, Aldrich), (*R*)-**1** (98%, Aldrich), 2-(2-chlorophenyl)oxirane **2** (97%, Amatek Chemical), 2-(3-chlorophenyl)oxirane **3** (97%, Amatek Chemical), 2-(4-chlorophenyl)oxirane **4** (96%, Aldrich), 3-fluorostyrene (97%, Alfa Aesar), 2-(4-fluoro-phenyl)oxirane **6** (95%, Aldrich), 3-bromostyrene (97%, Alfa Aesar), cyclopentene oxide **9** (98%, Fluka), cyclohexene oxide **10** (98%, Aldrich), (1*R*, 2*R*)-1,2-cyclopentanediol **12** (98%, Fluka), (±)-*trans*-1,2-cyclopentanediol **12** (97%, Fluka), (1*R*, 2*R*)-1,2-cyclo-hexanediol **13** (99%, Aldrich), (1*S*, 2*S*)-1,2-cyclohexane-diol **13** (99%, Aldrich), THF (99.9%, Aldrich), phenyl chloroformate (99%, Aldrich), benzyl chloroformate (99%, Aldrich), 1,2,5,6-tetrahydropyridine (97%, Aldrich), *m*-CPBA (77%, Aldrich), dichloromethane (HPLC, Fisher), benzyl 3-pyrroline-1-carboxylate (90%, Aldrich), acetonitrile (HPLC grade, TEDIA), *n*-hexane (HPLC grade, TEDIA), ethyl acetate (HPLC grade, Fisher), chloroform (HPLC grade, Fisher) and isopropanol (HPLC grade, Fisher).

Strains and Biochemicals

Escherichia coli T7 expression cell, restriction enzymes (NdeI and XhoI) and Quick DNA Ligase were purchased from New England Biolabs. Oligos (primers), Tris buffer (1 M) and IPTG (inducer, >99%) were purchased from 1st BASE. Phusion DNA polymerase was from Thermo Scientific. Medium LB and components tryptone and yeast extract were purchased from Biomed Diagnostics. Antibiotics kanamycin (>99%) and NaCl (>99%) were from Sigma Aldrich.

Synthesis of 2-(3-Fluorophenyl)oxirane 5

Synthesis of 2-(3-Fluorophenyl)oxirane **5** was according to previously reported method[S1]: *m*-CPBA (0.346 g, 2 mmol) was added to a stirred solution of 3-fluorostyrene (0.244 g, 2 mmol) in CH_2Cl_2-phosphate buffer (1:1, 40 mL, pH 8.0, 0.1M K_2HPO_4-KH_2PO_4) on ice, and the mixture was stirred at room temperature for 5 h. A second equivalent of *m*-CPBA (0.346 g, 2 mmol) was then added to the mixture and stirred at room temperature overnight (12 h). NaOH (1 N, 10 mL) was added and the mixture was extracted with CH_2Cl_2 three times (3 × 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. The

solvent was then removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane: ethyl acetate = 50: 1, R_f = 0.3). 193 mg (69.9%) of **5** was obtained as colourless liquid.

Synthesis of 2-(3-Bromophenyl)oxirane 7

Synthesis of 2-(3-Bromophenyl)oxirane **7** was according to previously reported method[S1]: *m*-CPBA (0.346 g, 2 mmol) was added to a stirred solution of 3-Bromostyrene (0.366 g, 2 mmol) in CH_2Cl_2-phosphate buffer (1:1, 40 mL, pH 8.0, 0.1M K_2HPO_4-KH_2PO_4) on ice, and the mixture was stirred at room temperature for 5 h. A second equivalent of *m*-CPBA (0.346 g, 2 mmol) was then added to the mixture and stirred at room temperature overnight (12 h). NaOH (1 N, 10 mL) was added and the mixture was extracted with CH_2Cl_2 three times (3 × 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. The solvent was then removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane: ethyl acetate = 50: 1, R_f = 0.3). 255 mg (64.1%) of **7** was obtained as colourless liquid.

Synthesis of *N*-Phenoxycarbonyl-3,4-epoxy-piperidine 8

Synthesis of *N*-Phenoxycarbonyl-1,2,5,6-tetrahydropyridine (precursor for **8**) was according to previously reported method[S2]: A solution of phenyl chloroformate (0.50 mL, 4.0 mmol) in THF (2 mL) was added dropwise to a stirred mixture of 1,2,5,6-tetrahydropyridine (0.332 g, 4.0 mmol) and $NaHCO_3$ (0.43 g, 5.2 mmol) in THF/water (1:1, 4 mL) on ice, and the mixture was stirred at room temperature for 5 h. Aqueous Na_2CO_3 (5%, 5 mL) and $CHCl_3$ (10 mL) were added, the organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ twice (2 × 10 mL). The combined organic phase was washed with saturated NaCl solution and dried over Na_2SO_4 overnight. The solvent was then removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 10:1, R_f = 0.3). 551 mg (67.9%) of *N*-Phenoxycarbonyl-1,2,5,6-tetrahydropyridine was obtained as a white solid.

Synthesis of *N*-Phenoxycarbonyl-3,4-epoxy-piperidine **8** was according to previously reported method[S2]: *m*-CPBA (0.5 g, 2.23 mmol) was added to a solution of *N*-Phenoxycarbonyl-1,2,5,6-tetrahydropyridine (244 mg, 1.20 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at room temperature overnight (12 h). NaOH (1 N, 10 mL) was added and the mixture was extracted with CH_2Cl_2 three times (3 × 10 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. The solvent was then

removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 4:1, R_f = 0.3). 174 mg (66.1%) of **8** was obtained as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.32–7.38 (t, J = 8.0 Hz, 2 H, ArH), 7.17–7.21 (t. J = 7.2 Hz, 1 H, ArH), 7.08–7.11 (t, J = 6.4 Hz, 2 H, ArH), 3.98–4.09 (m, 1.5 H), 3.79–3.83 (d, J=15.2 Hz, 0.5 H), 3.66–3.71 (m, 0.5 H), 3.53–3.59 (m, 0.5 H), 3.29–3.39 (m, 3 H), 2.00–2.20 (m, 2 H).

Synthesis of *N*-Benzyloxycarbonyl-3,4-epoxy-pyrrolidine 11

Synthesis of *N*-Benzyloxycarbonyl-3,4-epoxy-pyrrolidine **11** was according to previously reported method[S2]: *m*-CPBA (2.24 g, 10.0 mmol) was added to a solution of benzyl 3-pyrroline-1-carboxylate (1.015 g, 5.0 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred at room temperature overnight (12 h). NaOH (1 N, 20 mL) was then added and the mixture was extracted with CH_2Cl_2 three times (3 × 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. The solvent was then removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 4:1, R_f = 0.3). 898 mg (82.0%) of **11** was obtained as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.26–7.37 (m, 5 H, ArH), 5.07–5.14 (m, 2 H), 3.82–3.90 (dd, J = 19.2, 12.8 Hz, 2 H), 3.66–3.69 (m, 2 H), 3.37–3.41 (m, 2 H).

Analytical Methods

The concentrations of the bioproducts (**1**–**7** and their corresponding diols) were determined using a Shimadzu prominence HPLC system (reverse phase) with an Agilent Poroshell 120 EC-C18 column (150 × 4.6 mm, 2.7 μm) and UV detection at 210 nm. Condition: 40% water: 60% acetonitrile. Flow rate: 0.5 mL min^{-1}. Retention times: 6.4 min for **1** and 3.6 min for its diol; 8.8 min for **2** and 3.9 min for its diol; 8.3 min for **3** and 3.9 min for its diol; 8.2 min for **4** and 3.9 min for its diol; 6.9 min for **5** and 3.9 min for its diol; 6.6 min for **6** and 3.6 min for its diol; 9.3 min for **7** and 4.2 min for its diol.

The *ee* values and concentrations of the bioproducts (**1**–**8** and **12**) were determined using a Shimadzu prominence HPLC system (normal phase) with a Daicel AS-H (250 × 4.6 mm, 5 μm) or OB-H chiral column (250 × 4.6 mm, 5 μm). Retention times: 10.9 min for (*R*)-**1** and 11.9 min for (*S*)-**1** (AS-H column, 10% IPA: 90% *n*-hexane, 0.5 mL min^{-1}); 22.5 min for (*R*)-**2** and 23.2 min for (*S*)-**2** (AS-H column, 0% IPA: 100% *n*-hexane, 0.5 mL min^{-1}); 11.1 min for (*R*)-**3** and 11.9 min for (*S*)-**3** (AS-H column, 10% IPA: 90% *n*-hexane, 0.5

mL min^{-1}); 11.9 min for (*R*)-**4** and 14.0 min for (*S*)-**4** (AS-H column, 10% IPA: 90% *n*-hexane, 0.5 mL min^{-1}); 10.2 min for (*R*)-**5** and 11.0 min for (*S*)-**5** (AS-H column, 10% IPA: 90% *n*-hexane, 0.5 mL min^{-1}); 13.5 min for (*R*)-**6** and 14.0 min for (*S*)-**6** (AS-H column, 10% IPA: 90% *n*-hexane, 0.5 mL min^{-1}); 11.4 min for (*R*)-**7** and 12.0 min for (*S*)-**7** (AS-H column, 10% IPA: 90% *n*-hexane, 0.5 mL min^{-1}); 56.2 min for (−)-**8** and 64.7 min for (+)-**8** (OB-H column, 40% IPA: 60% *n*-hexane, 0.5 mL min^{-1}); 46.3 min for (3*R*,4*R*)-**14** and 56.1 min for (3*S*,4*S*)-**14** (AS-H column, 5% IPA: 95% *n*-hexane, 1 mL min^{-1}).

The concentrations of the substrates (**9**–**10**) were analyzed by using an Agilent 7890A gas chromatograph with an HP-5 column (30 m × 0.32 mm × 0.25 mm). Temperature program: 45 °C for 1 min, then to 140 °C at 15 ◦C min^{-1} and finally to 280 °C at 49 °C min^{-1}. Retention times: 5.6 min for **9**, 6.7 min for **10**, 9.3 min for *n*-dodecane (internal standard). Similarly, the concentrations of the substrate **11** were analyzed by using the same Agilent GC system and HP-5 column. Temperature program: 100 °C for 1 min, then to 280 °C at 10 °C min^{-1}. Retention times: 6.7 min for *n*-dodecane (internal standard) and 14.6 min for **11**.

The *ee* values of bioproducts (**12** and **13**) were determined with Macherey-Nagel Lipodex-E chiral column (25 m × 0.25 mm) at 100 °C constant. Retention times: 31.3 min for (1*S*, 2*S*)-**12** and 34.1 min for (1*R*, 2*R*)-**12**; 32.7 min for (1*R*, 2*R*)-**13** and 33.9 min for (1*S*, 2*S*)-**13**.

The configurations of the bioproducts **1**, **12** and **13** were assigned by using authentic samples of (*S*)-**1** and (1*R*, 2*R*)-**12** and (1*R*, 2*R*)-**13**, and the bioproducts **2**, **3**, **4**, **8**, and **14** were established by comparison with our previous published HPLC data of (*S*)-**2**, (*S*)-**3**, (*S*)-**4**, (−)-**8** and (3*R*, 4*R*)-**14**.[S2–S4] The other bioproducts **5**, **6**, and **7** were established by comparison with the epoxidation products by a well known *S* selective styrene monooxygenase.[S5] The optical rotation data of preparation bioproducts (*S*)-**1**, (*S*)-**3**, (*S*)-**6**, (1*R*, 2*R*)-**12**, (1*R*, 2*R*)-**13**, and (3*R*, 4*R*)-**14** were also determined and compared with literature data:

(*S*)-Styrene oxide (*S*)-1: $[\alpha]_D^{28}$ = +25.0° (*c* 1.00, $CHCl_3$) {lit.,[S6] $[\alpha]_D^{21}$ = +24° (*c* 1.00, $CHCl_3$)}.

(*S*)-2-(3-Chlorophenyl)oxirane (*S*)-3: $[\alpha]_D^{28}$ = +12.1° (*c* 1.00, $CHCl_3$) {lit.,[S7] $[\alpha]_D^{23}$ = +11.2° (*c* 1.39, $CHCl_3$)}.

(*S*)-2-(4-Fluorophenyl)oxirane (*S*)-6: $[\alpha]_D^{28}$ = +17.2° (*c* 1.00, $CHCl_3$) {lit.,[S1] $[\alpha]_D^{20}$ = +15.6° (*c* 0.97, $CHCl_3$)}.

(1*R*, 2*R*)-1,2-cyclopentanediol 12: $[\alpha]_D^{28}$ = –28.8° (*c* 1.00, H_2O) {lit.,[S8] $[\alpha]_D^{25}$ = –24° (MeOH)}.

(1*R*, 2*R*)-1,2-cyclohexanediol 13: $[\alpha]_D^{28}$ = –39.0° (*c* 1.00, H_2O) {lit.,[S9] $[\alpha]_D^{20}$ = –38.4° (*c* 0.17, H_2O)}.

(3*R*,4*R*)-*N*-Benzyloxycarbonyl-3,4-dihydroxypyrroli-dine 14: $[\alpha]_D^{28}$ = +7.4° (*c* 1.00, $CHCl_3$) {lit.,[S2] $[\alpha]_D^{25}$ = +7.56 (*c* 1.80, $CHCl_3$)}.

His-tagged SpEH cloning, expressing and purification for kinetic data determination

For engineering of His-tagged SpEH, the similar cloning protocol applied with slightly different primers: Sp154-F2: ACTG *TCATGA* TG AAC GTC GAA CAT ATC CGC CCand Sp154-R2: AT *GGTACC* TA GTG GTG ATG ATG GTG ATG AAG ATC CAT CTG TGC AAA GGCC. The *E. coli* (His-tagged SpEH) was grown and expressed the His-tagged SpEH in the same condition of *E. coli* (SpEH). Then the cells were broken by cell homogeniser (Stansted fluid power LTD), and then subjected to centrifuge (15000 rpm, 20 min, 4 ^{o}C). The His-tagged SpEH was purified from the supernatant (cell free extract) by using Ni-NTA agarose (Qiagen) according to the standard protocol. A SDS-PAGE (12% resolving gel and 4% stacking gel) was applied to check the purity of the protein.

To determine the kinetics data, 1 μg of the purified SpEH was incubated with (*S*)-**1** (0.5–8 mM) or (*R*)-**1** (0.2–4 mM) in 1 mL of Tris buffer (50 mM, pH 7.5). The mixtures were shaken at 30 ^{o}C. 300 μL aliquots were taken out at different time points (0, 2, 4 and 8 min) and mixed with 300 μL cold acetonitrile to quench the reaction. The samples were analyzed by HPLC to quantify the diol formation immediately. The initial velocities were calculated and used to give a Lineweaker-Burk plot (1/v *vs.* 1/[S]) to determine K_m, V_{max} and k_{cat}.

Sequence alignment of SpEH with several known EHs

```
SpEH            RWPE---KETVDDWDQGIPLAYARELAIYWRDEYDWR-----------RIEARLNTWPNF 75
MgEH            RWPD---SETCKGWDQGMPLEYSRELAQYWVKDYDWR-----------RCETMLNNWPNY 105
AnEH            KIAPPTYESLQADGRFGITSEWLTTMREKWLSEFDWR-----------PFEARLNSFPQF 87
ArEH            ------------------------------------------------MTIRRPEDFKHY 12
StEH            ----------------------------------------------------MEKIEHK 7
HsEH            VFLDDIGANLKPARDLGMVTILVQDTDTALKELEKVTGIQLLNTPAPLPTSCNPSDMSHG 240
                                                                   .   :

SpEH            LATVD-GLDIHFLHIRSDNPAARPLVLTHGWPGSVLEFLDVIEPLS----------ADYH 124
MgEH            MASID-GQDIHFIHRTSTHANALPLIISHGWPGSVIEFHKIIDALAQPEQYGGDPADAFH 164
AnEH            TTEIE-GLTIHFAALFSEREDAVPIALLHGWPGSFVEFYPILQLFREEYTP---ETLPFH 143
ArEH            EVQLP-DVKIHYVREG----AGPTLLLLHGWPGFWWEWSKVIGPLA----------EHYD 57
StEH            MVAVN-GLNMHLAELG----EGPTILFIHGFPELWYSWRHQMVYLAER---------GYR 53
HsEH            YVTVKPRVRLHFVELG----SGPAVCLCHGFPESWYSWRYQIPALAQA---------GYR 287
                 . :     :*          . .: : **:*    .:   :  :             :

SpEH            LVIPSLPGFGFS-GKPTRPG--WDVEHIAAAWDALMRALGYDR---YFAQGGDWGSAVTS 178
MgEH            VVAPSLPGFGFS-SKPTTTG--TKVEKIGAMWGKLMAELGYDS---YVAQGGDWGSMVTQ 218
AnEH            LVVPSLPGYTFSSGPPLDKD--FGLMDNARVVDQLMKDLGFGSG--YIIQGGDIGSFVGR 199
ArEH            VIVPDLRGFGDSEKPDLNDLSKYSLDKAADDQAALLDALGIEKA--YVVGHDFAAIVLHK 115
StEH            AVAPDLRGYGDTTGAPLNDPSKFSILHLVGDVVALLEAIAPNEEKVFVVAHDWGALIAWH 113
HsEH            VLAMDMKGYGESSAPPEIEE--YCMEVLCKEMVTFLDKLGLSQA--VFIGHDWGGMLVWY 343
                 :  .: *:  :            :         ::  :.       .   .  .

SpEH            AIGMHHAGHCAGIHVNMVVGAPPPE--LMNDLTDEE--KLYLARFGWYQAKDNGYS--TQ 232
MgEH            SMGQTETKHCAGIHINMPIVAPDPE--TMNDLTPLE--QSALEGMAFYNDHDSGYS--KQ 272
AnEH            LLGVGFD-ACKAVHLNLCAMRAPPEGPSIESLSAAE--KEGIARMEKFMTDGLAYA--ME 254
ArEH            FIRKYSDRVIKAAIFDPIQPDFGPV---YFGLGHVH--ESWYSQFHQLDMAVEVVG--SS 168
StEH            LCLFRPDKVKALVNLSVHFSKRNPKMNVVEGLKAIYGEDHYISRFQVPGEIEAEFAPIGA 173
HsEH            MALFYPERVRAVASLNTPFIPANPNMSPLESIKANP-VFDYQLYFQEPGVAEAELEQNLS 402
                             ..       *      .:            :

SpEH            QATRPQTIGY---------------ALTDSP--AGQMAWIAEKFHGWTDCGHQPGGQSVG 275
MgEH            QSTRPQTISY---------------GLADSP--VGQMAWIVEKFYAWTDCEKN----GVK 311
AnEH            HSTRPSTIGH---------------VLSSSP--IALLAWIGEKYLQWVDKPLP------- 290
ArEH            REVCKKYFKH---------------FFDHWS--YRDELLTEEELEVHVDNCMK------- 204
StEH            KSVLKKILTYRDPAPFYFPKGKGLEAIPDAP--VALSSWLSEEELDYYANKFE------- 224
HsEH            RTFKSLFRASDESVLSMHKVCEAGGLFVNSPEEPSLSRMVTEEEIQFYVQQFK------- 455
                :                          :   .          *:

SpEH            GHPEQAVSKDAMLDTISLYWLTASAASSARLYWHSFRQFAAGE----------IDVPTGC 325
MgEH            -HPENVLSKDELLDNVMLYWLNNCAGSSARLYWESFNQPNLAP----------IDMPVGC 360
AnEH            --------SETILEMVSLYWLTESFPRAIHTYRETTPTASAPNGATMLQKELYIHKPFGF 342
ArEH            --------PDNIHGGFNYYRANIRPDAALWTDLDHTMS--------------DLPVTMIW 242
StEH            --------QTGFTGAVNYYRALPINWELTAPWTGAQVKVPTKF---------IVGEFDLV 267
HsEH            --------KSGFRGPLNWYRNMERNWKWACKSLGRKILIP----------------ALMV 491
                          :   .  *

SpEH            SLFPNEIMRLSRRWAERRYRNIVYWSEAARGGHFAAWEQPELFAAEVRAAFAQMDL---- 381
MgEH            SIFPCEIFRSSRRWAAKRFSNIVHWNELEKGGHFAAFEQPQIFIKEVSDCFRKLR----- 415
AnEH            SFFPKDLCPVPRSWIATTG-NLVFFRDHAEGGHFAALERPRELKTDLTAFVEQVWQK--- 398
ArEH            GLGDTCVPYAPLIEFVPKYYSNYTMETIEDCGHFLMVEKPEIAIDRIKTAFR-------- 294
StEH            YHIPGAKEYIHNGGFKKDVPLLEEVVVLEGAAHFVSQERPHEISKHIYDFIQKF------ 321
HsEH            TAEKDFVLVPQMSQHMEDWIPHLKRGHIEDCGHWTQMDKPTEVNQILIKWLDSDARNPPV 551
                                          .*:   ::*      :   .
```

Figure S1. Sequence alignment of SpEH with several known EHs (the multiple alignment by ClustalW2). SpEH, EH from *Sphingomonas* sp. HXN-200 (This study); MgEH, putative EH from marine *gamma proteobacterium* HTCC2148 (UniProt: EEB77043.1); AnEH, EH from *Aspergillus niger* (UniProt: Q9UR30); ArEH, EH from *Agrobacterium radiobacter* AD1 (UniProt: O31243); StEH, EH from *Solanum tuberosum* (Potato, UniProt: Q41415); HsEH, EH from *Homo sapiens* (Human, UniProt: P34913). Yellow: the conserve motif (H-G-X-P and G-X-Sm-X-S/T); Green: catalytic trial (D-H-D/E); Cyan: two conserve tyrosine residues (Y). "*": the identical amino acids; ".": similar amino acids; ":": highly similar amino acids.

Enantioselective hydrolysis of cyclohexene oxide 10 with resting cells of *E. coli* (SpEH)

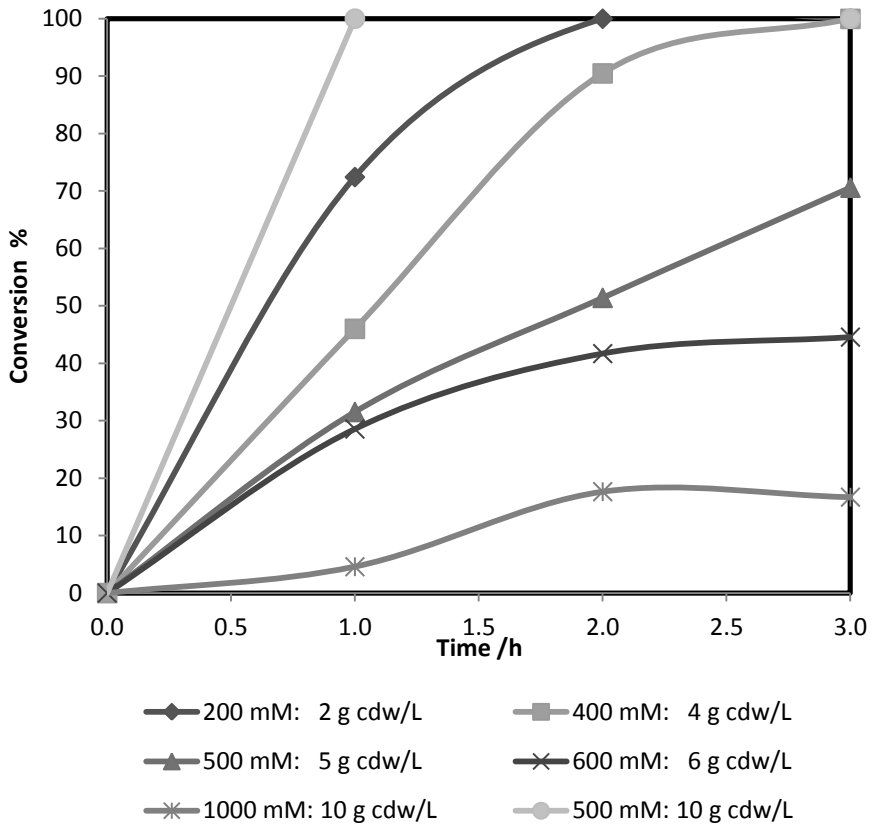


Figure S2. Enantioselective hydrolysis of cyclohexene oxide **10** with resting cells of *E. coli* (SpEH) in Tris–HCl buffer (50 mM, pH 7.5) with various substrate concentrations (mM) and cell densities (g cdw/L).

Figure S3-S19. Chiral HPLC chromatograms

Racemic **1**:

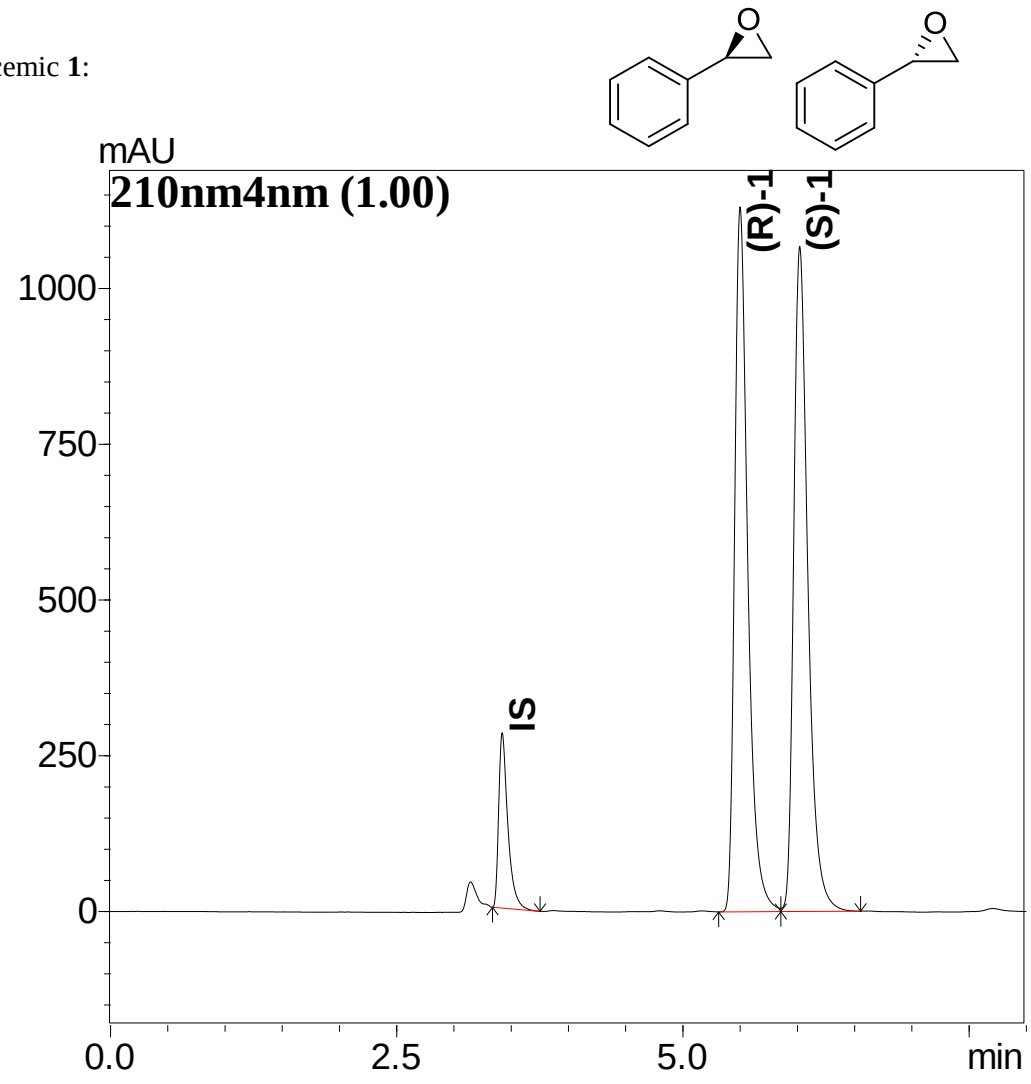


Figure S3. Chiral HPLC chromatogram of racemic substrate **1** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 1.0 mL min^{-1}).

Product (*S*)-**1**:

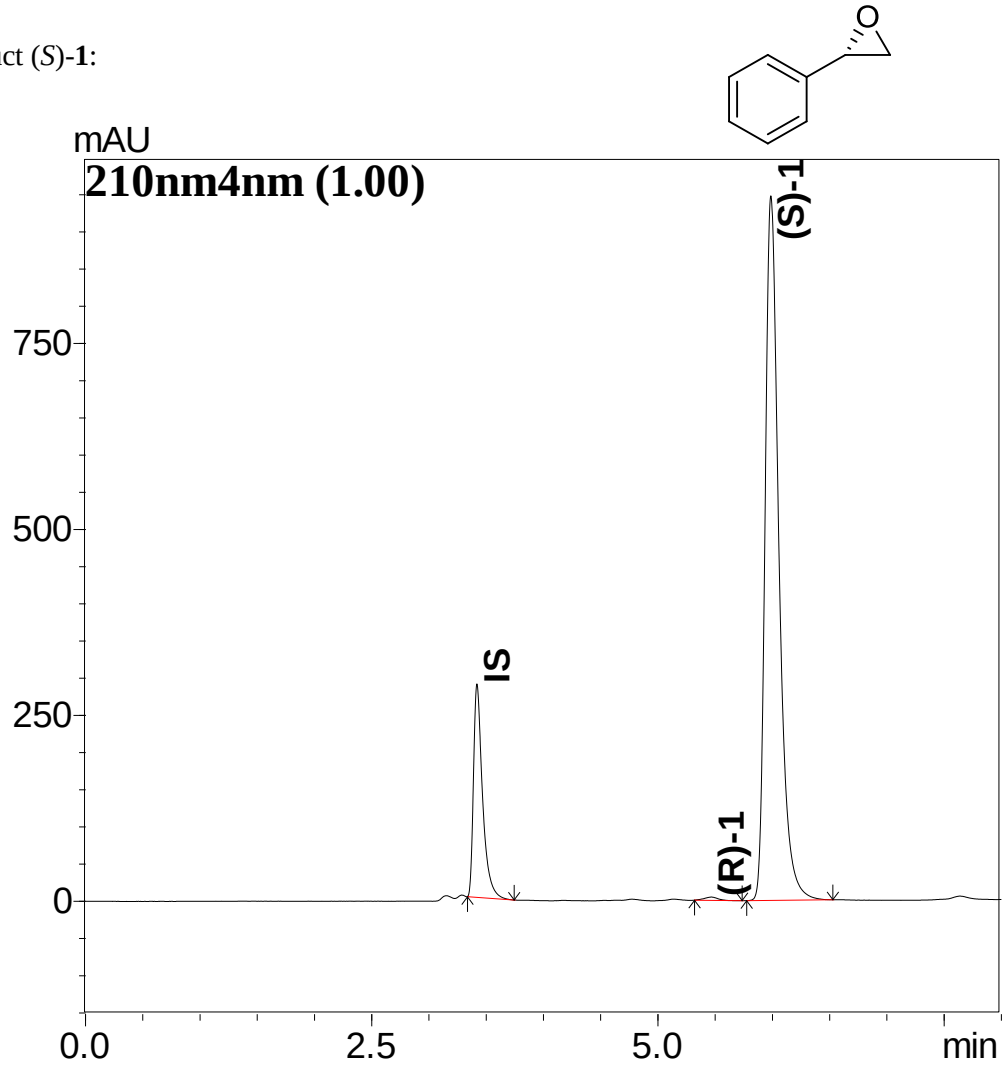


Figure S4. Chiral HPLC chromatogram of biotransformation product (*S*)-**1** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 1.0 mL min^{-1}).

Racemic **2**:

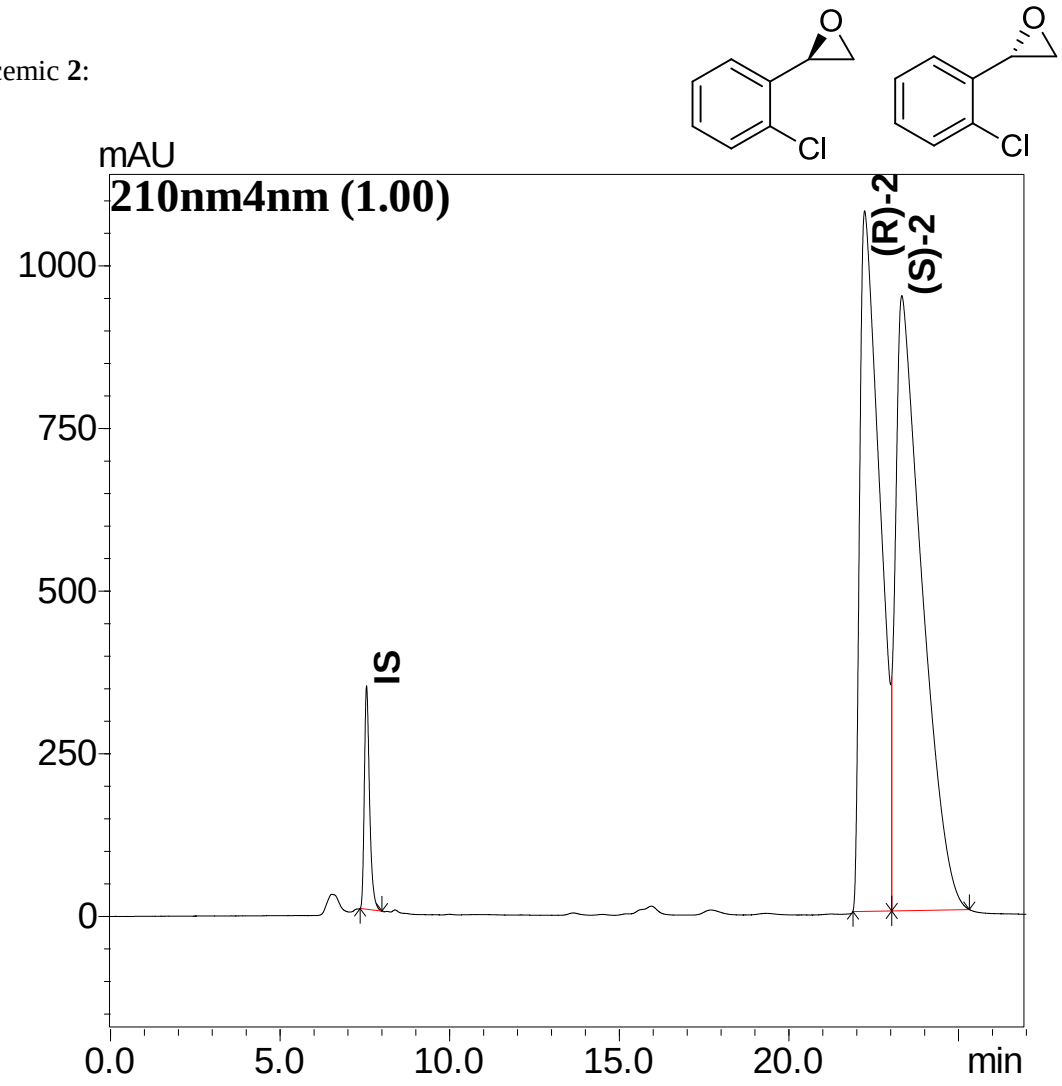


Figure S5. Chiral HPLC chromatogram of racemic substrate **2** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 0% IPA: 100% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (*S*)-**2**:

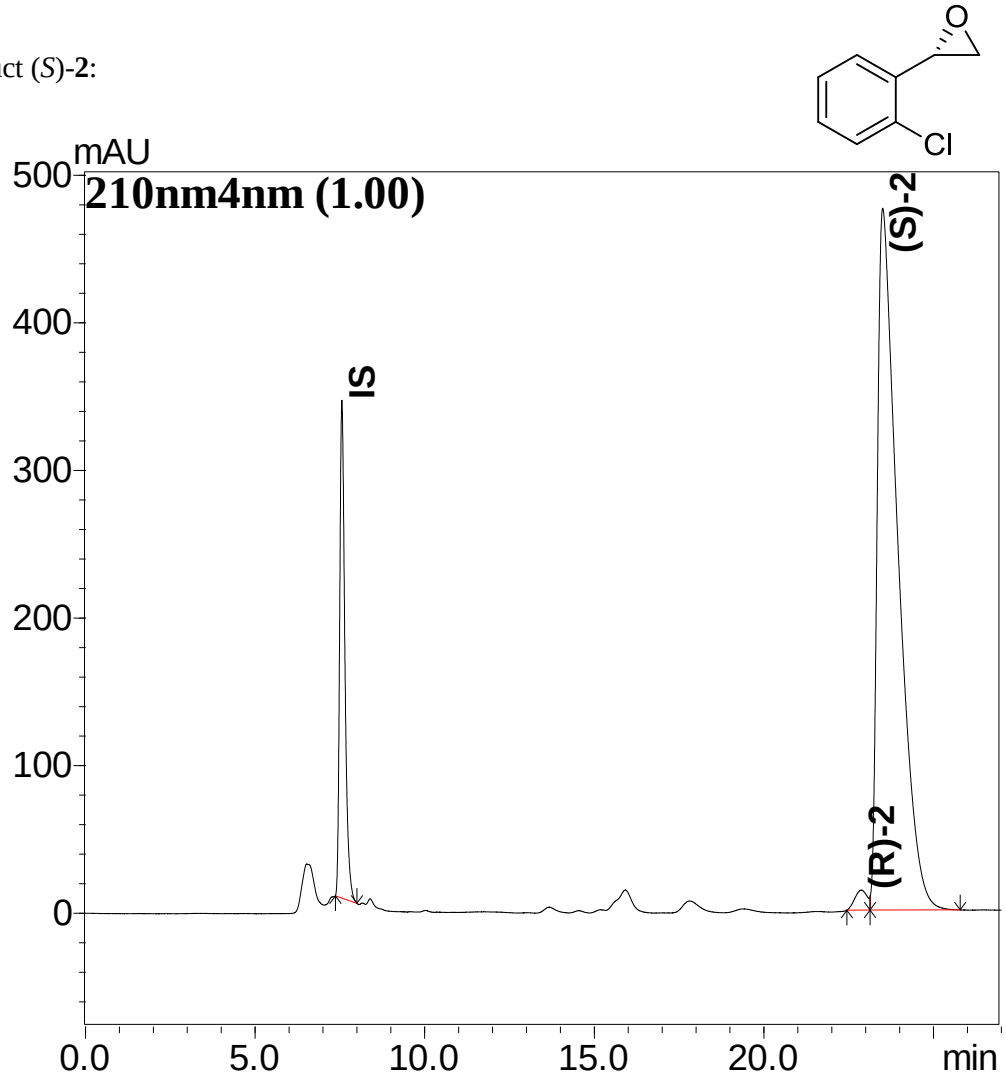


Figure S6. Chiral HPLC chromatogram of biotransformation product (*S*)-**2** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 0% IPA: 100% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Racemic **3**:

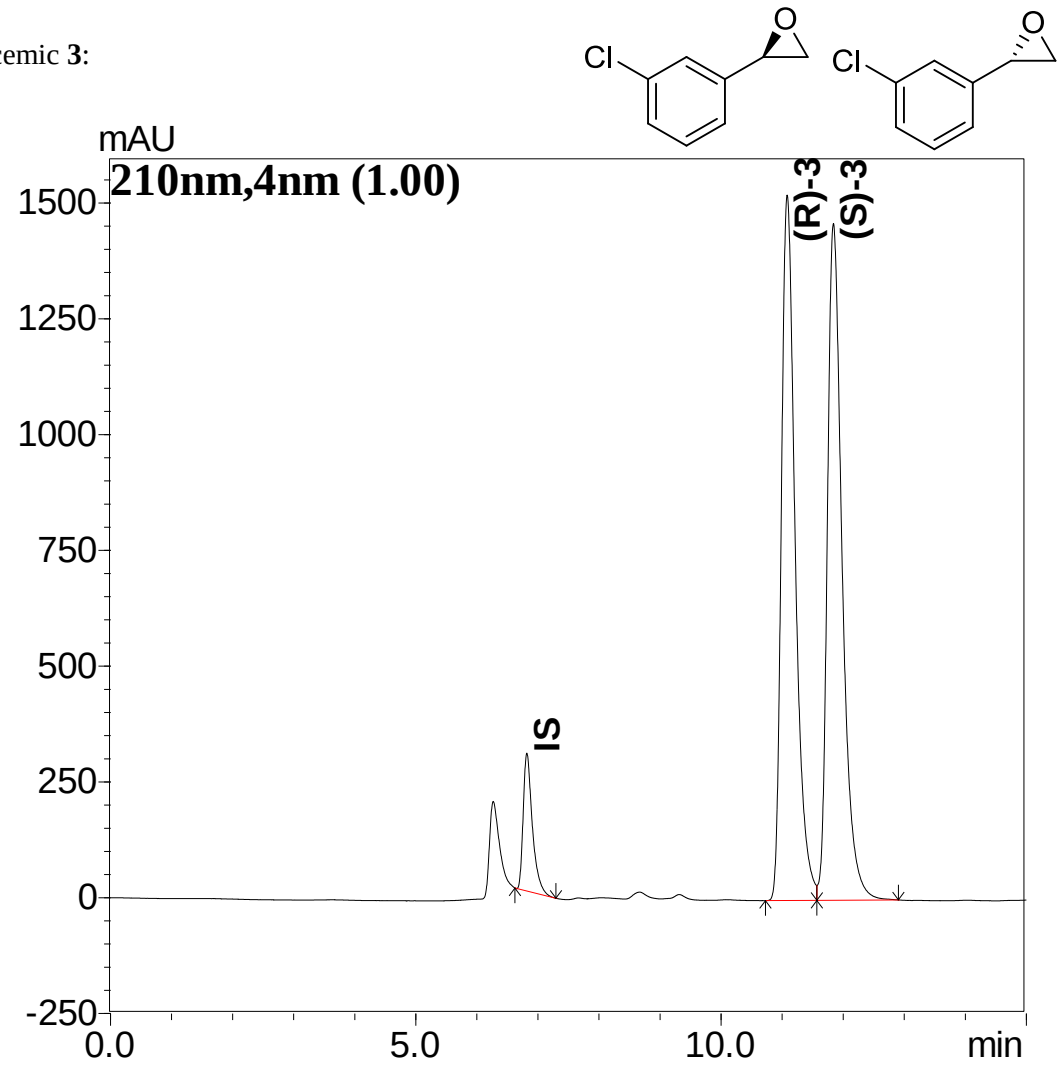


Figure S7. Chiral HPLC chromatogram of racemic substrate **3** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (*S*)-**3**:

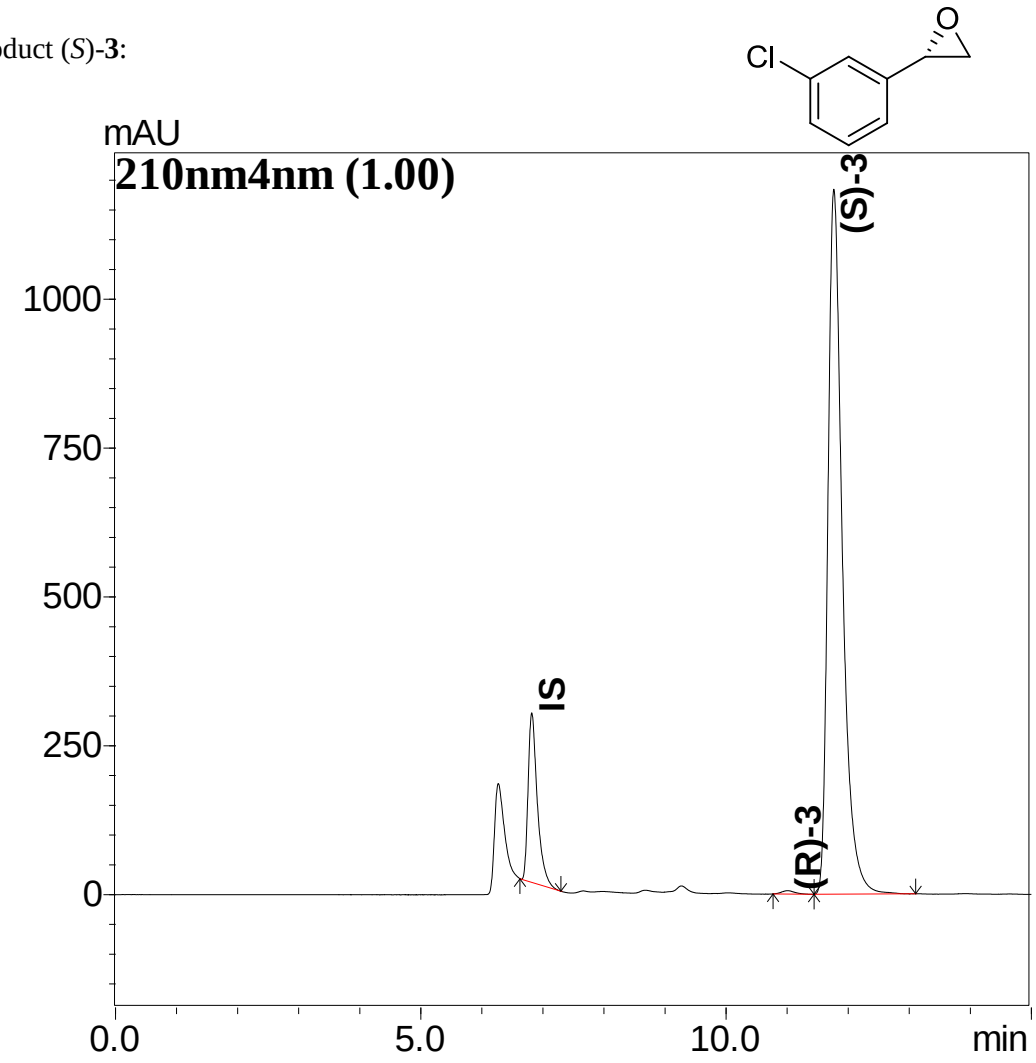


Figure S8. Chiral HPLC chromatogram of biotransformation product (*S*)-**3** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Racemic **4**:

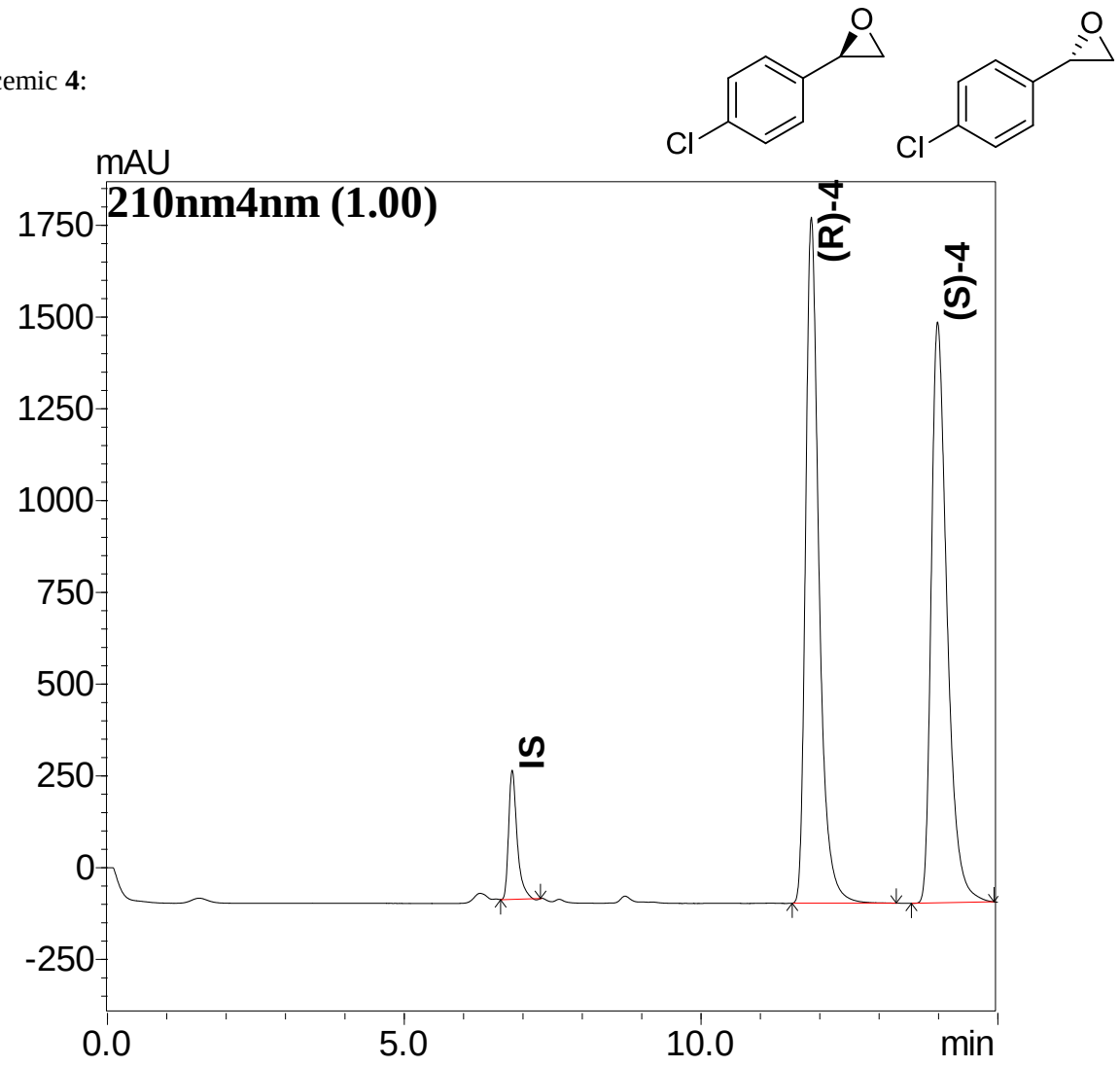


Figure S9. Chiral HPLC chromatogram of racemic substrate **4** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (*S*)-**4**:

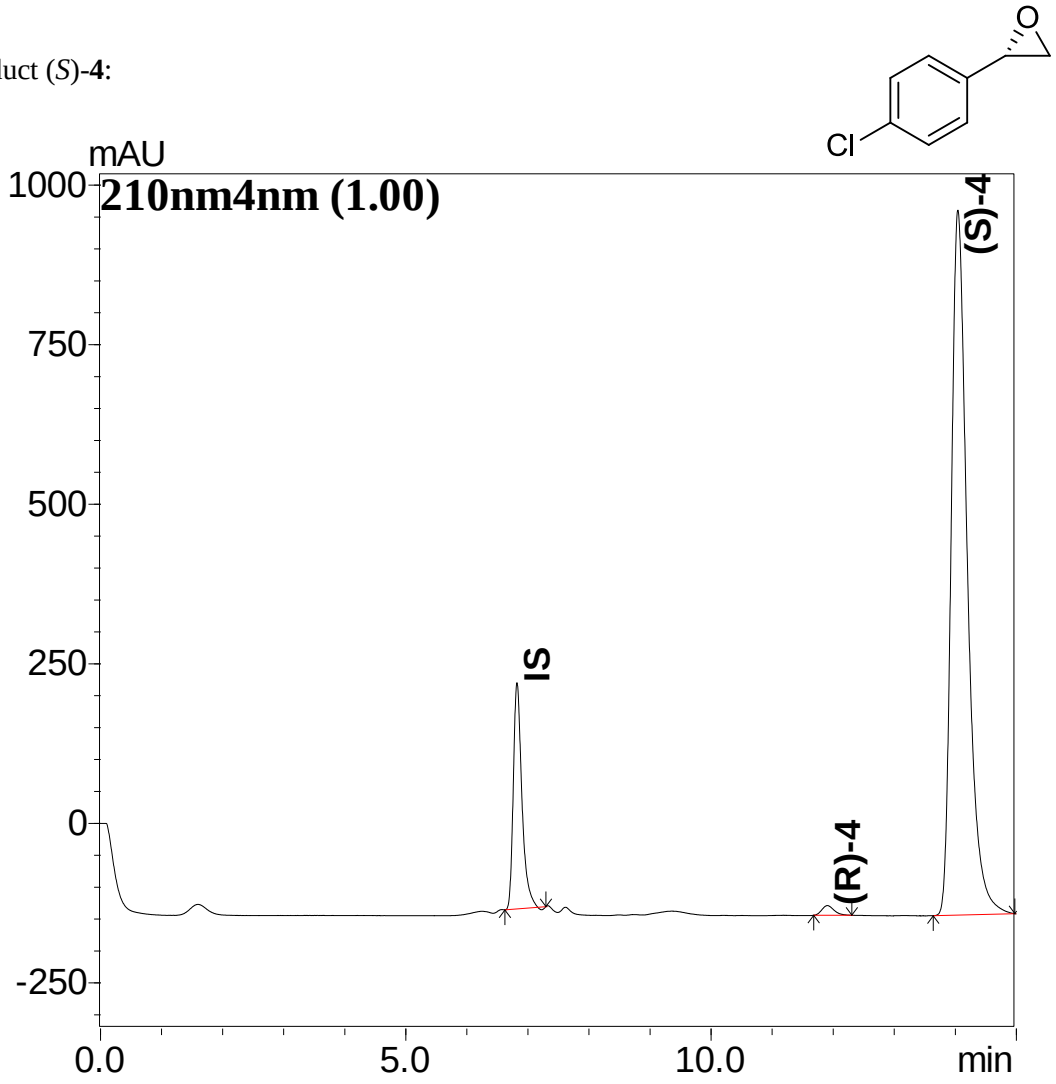


Figure S10. Chiral HPLC chromatogram of biotransformation product (*S*)-**4** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Racemic **5**:

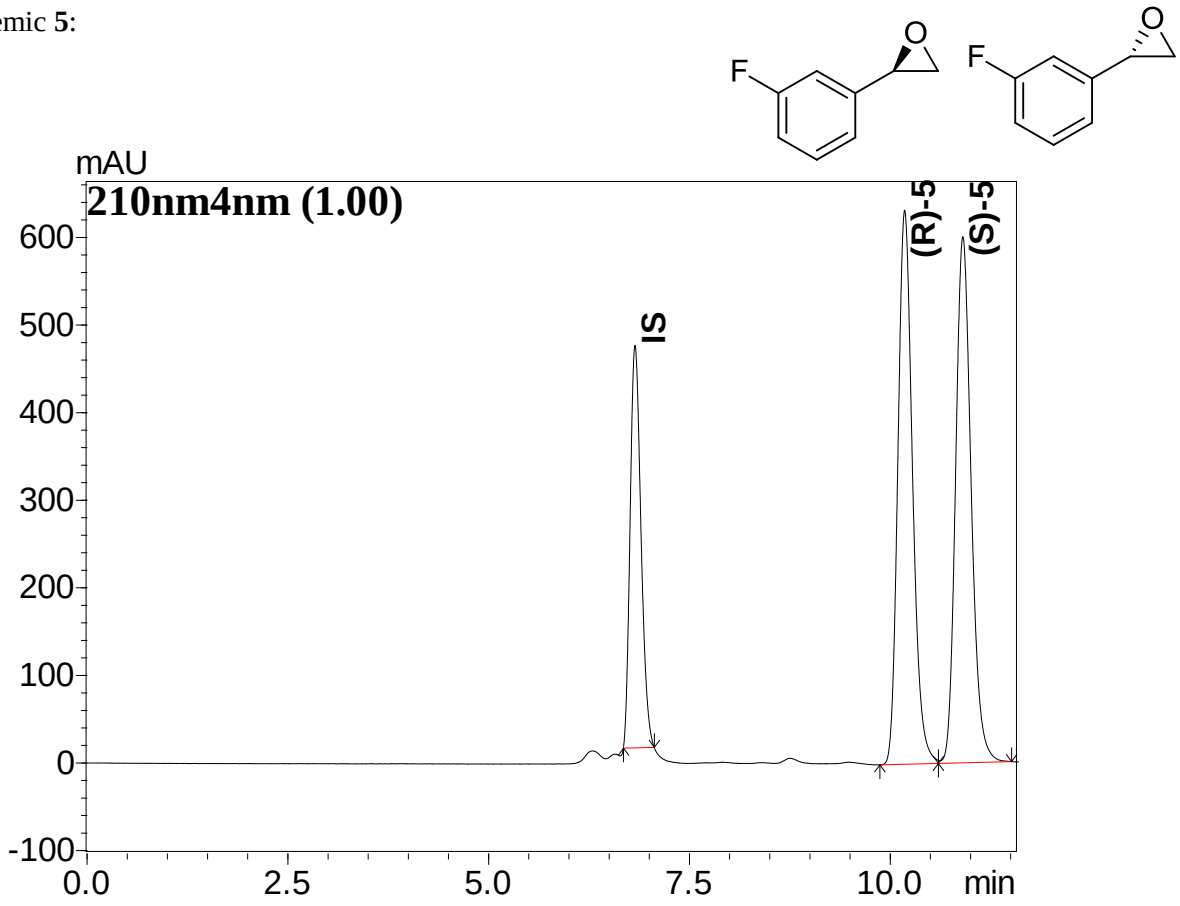
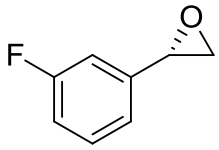


Figure S11. Chiral HPLC chromatogram of racemic substrate **5** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (*S*)-**5**:



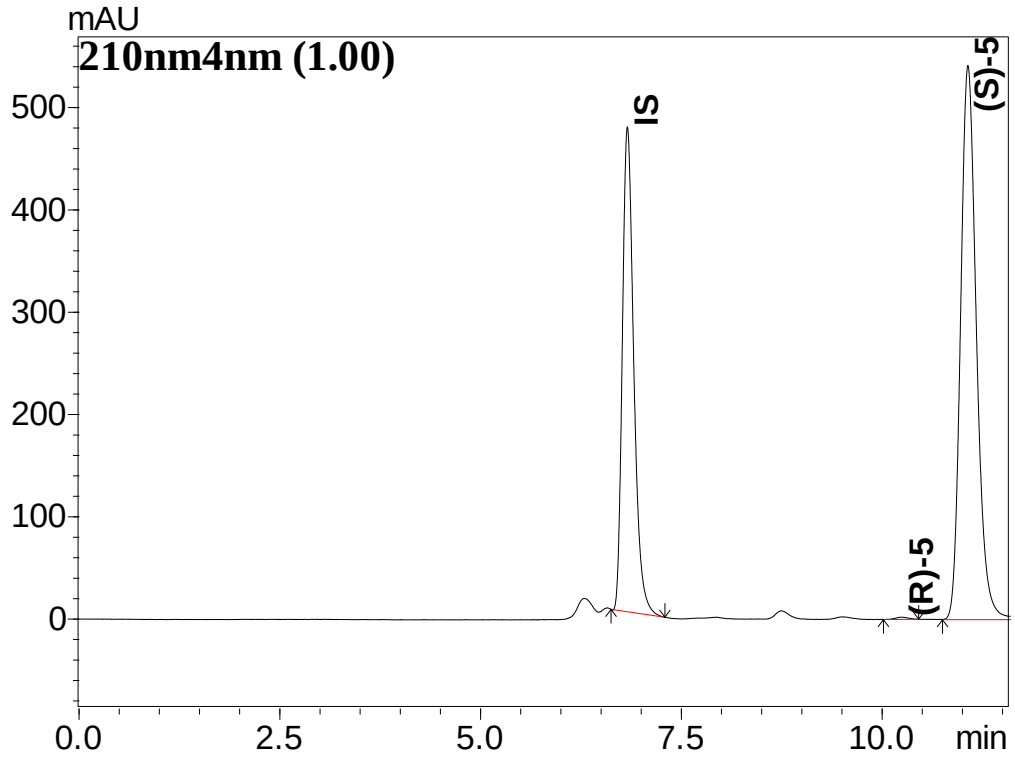
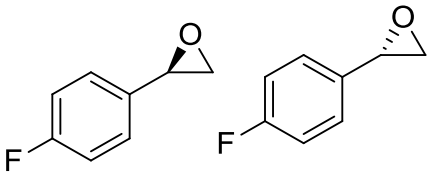


Figure S12. Chiral HPLC chromatogram of biotransformation product (*S*)-**5** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Racemic **6**:



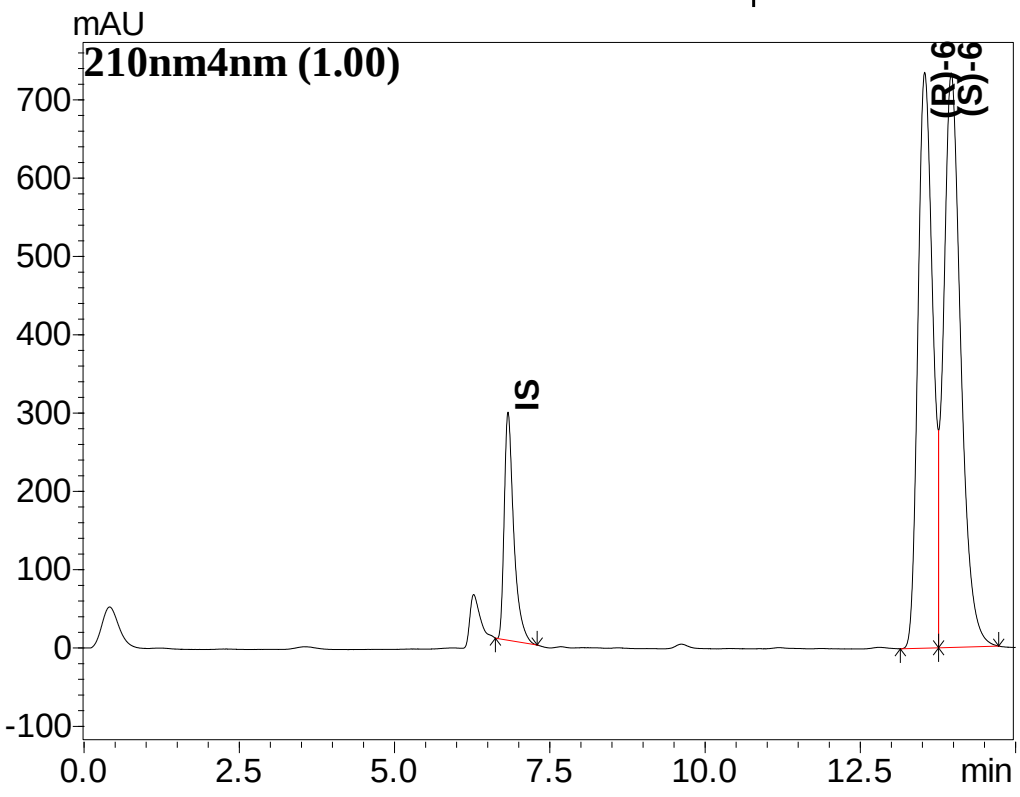


Figure S13. Chiral HPLC chromatogram of racemic substrate **6** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (*S*)-**6**:

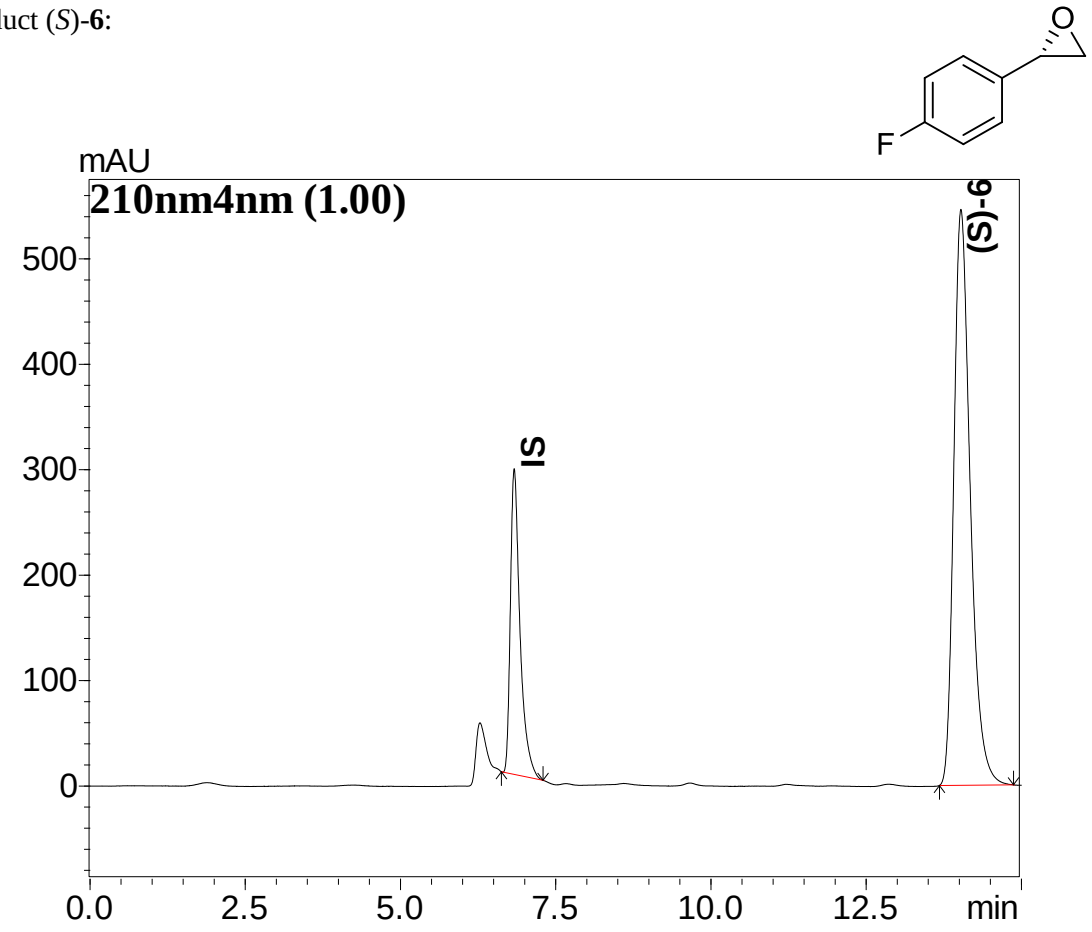


Figure S14. Chiral HPLC chromatogram of biotransformation product (*S*)-**6** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Racemic **7**:

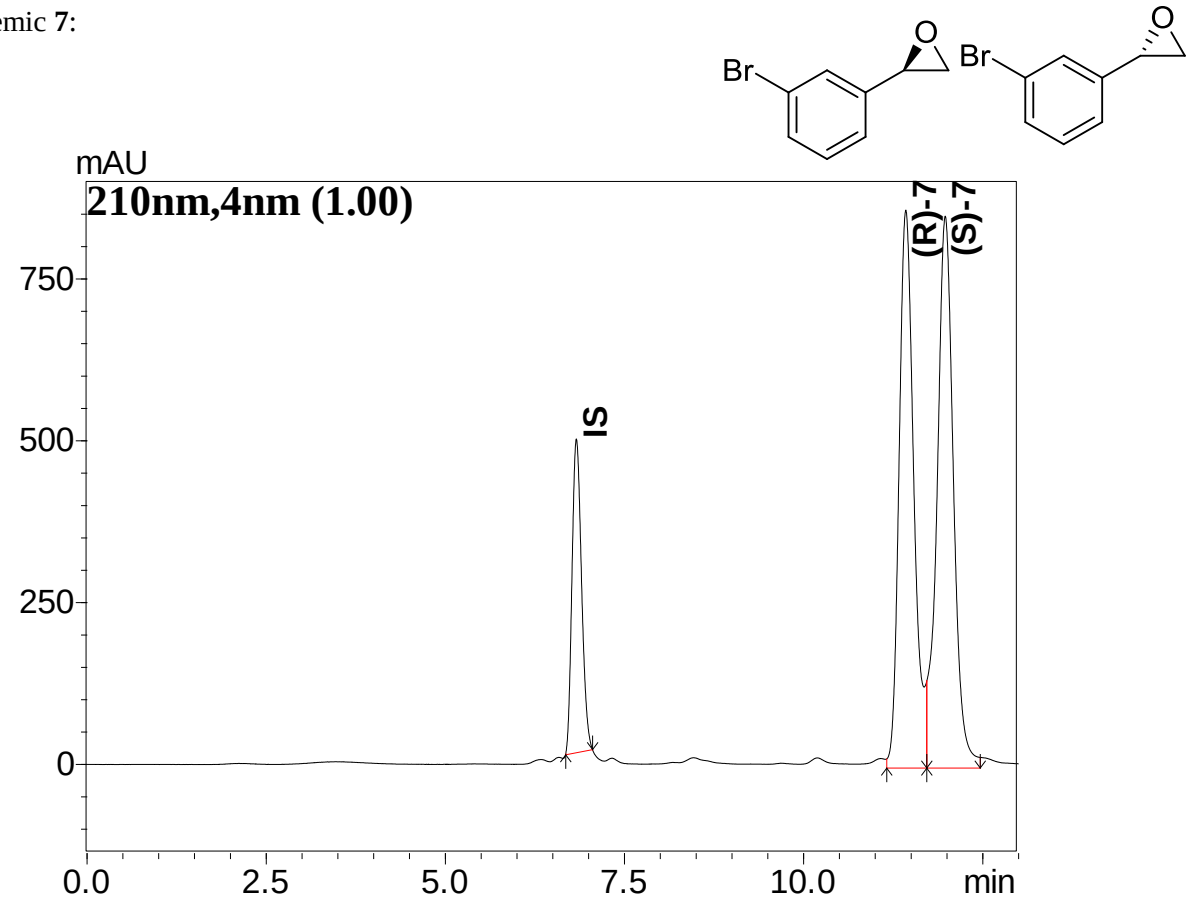
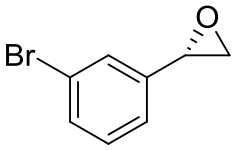


Figure S15. Chiral HPLC chromatogram of racemic substrate **7** (Column: Daicel AS-H (250 × 4.6 mm, 5μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (*S*)-**7**:



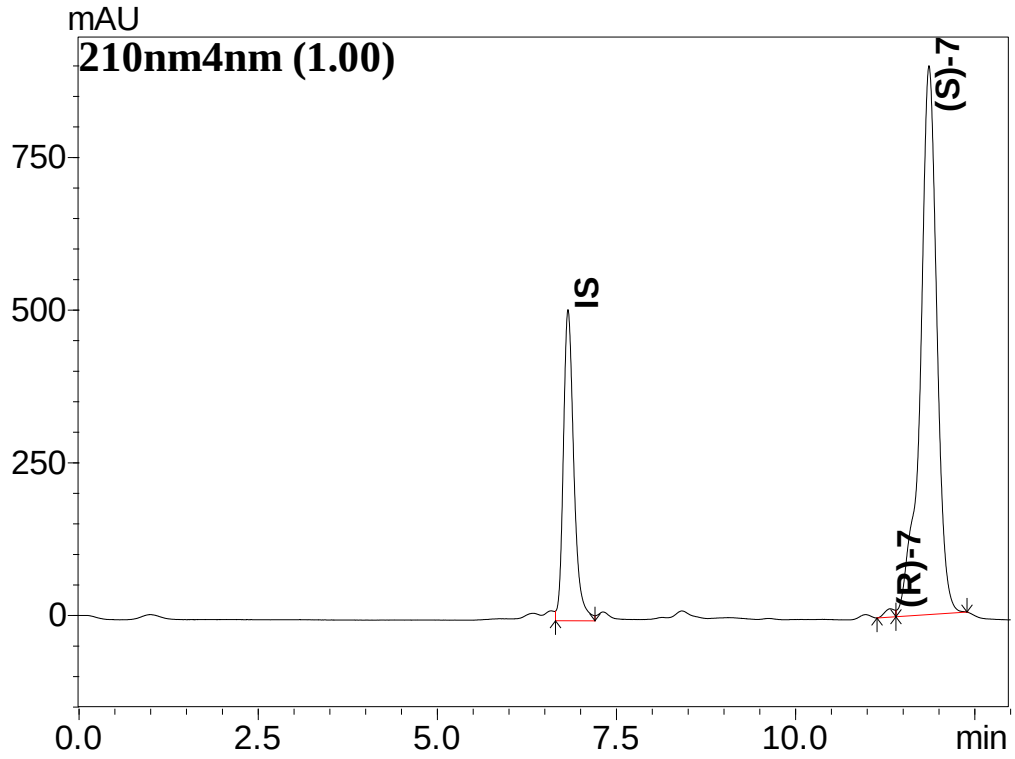


Figure S16. Chiral HPLC chromatogram of biotransformation product (*S*)-**7** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 10% IPA: 90% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Racemic **8**:

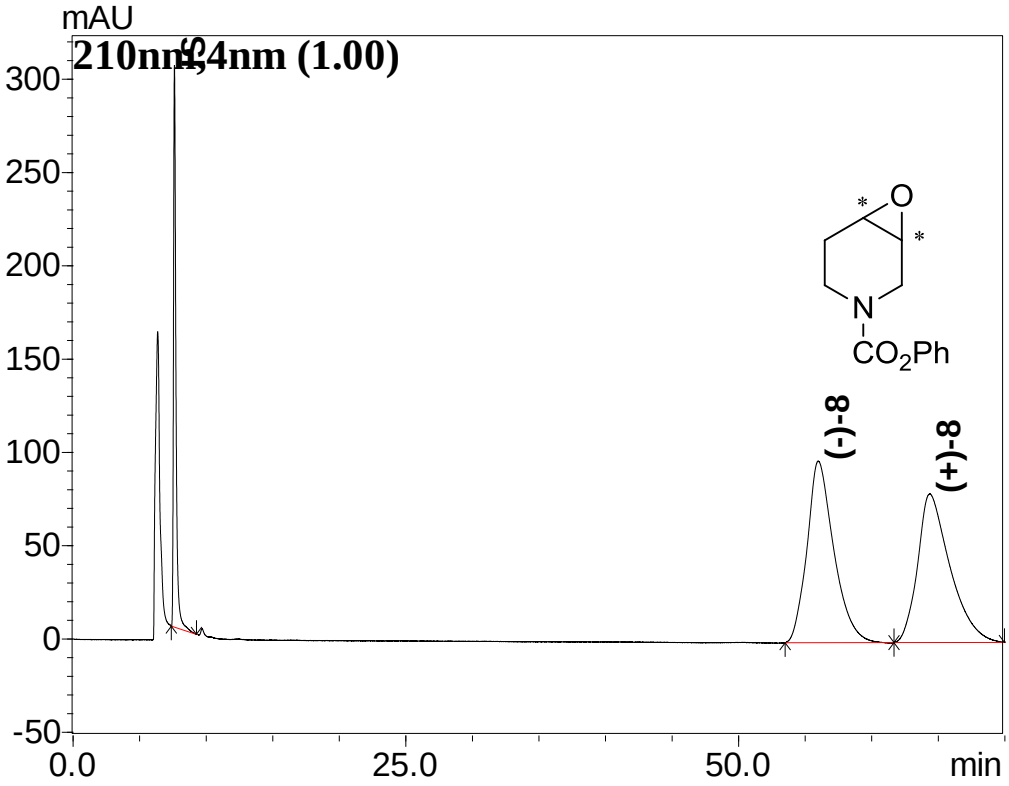
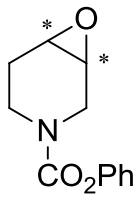


Figure S17. Chiral HPLC chromatogram of racemic substrate **8** (Column: Daicel OB-H (250 × 4.6 mm, 5 μm); eluent: 40% IPA: 60% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (–)-**8**:



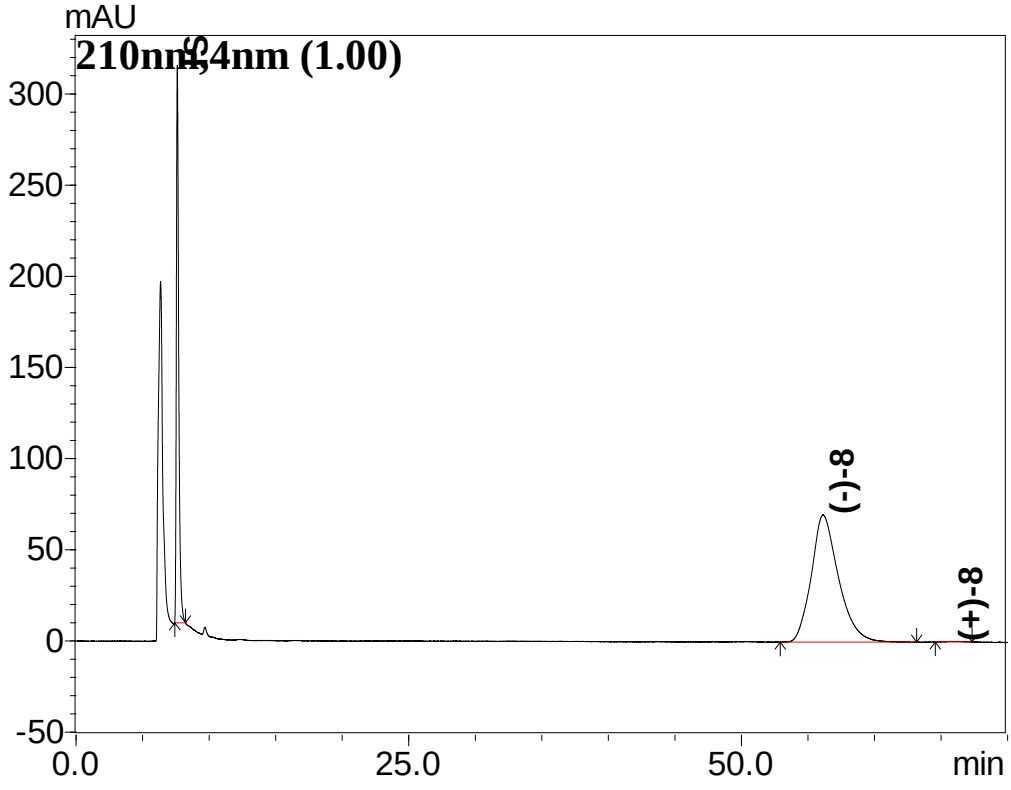
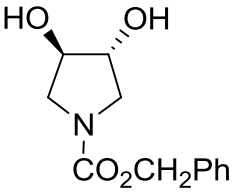


Figure S18. Chiral HPLC chromatogram of biotransformation product (–)-**8** (Column: Daicel OB-H (250 × 4.6 mm, 5 μm); eluent: 40% IPA: 60% *n*-hexane; flow rate: 0.5 mL min^{-1}).

Product (3*R*, 4*R*)-**14**:



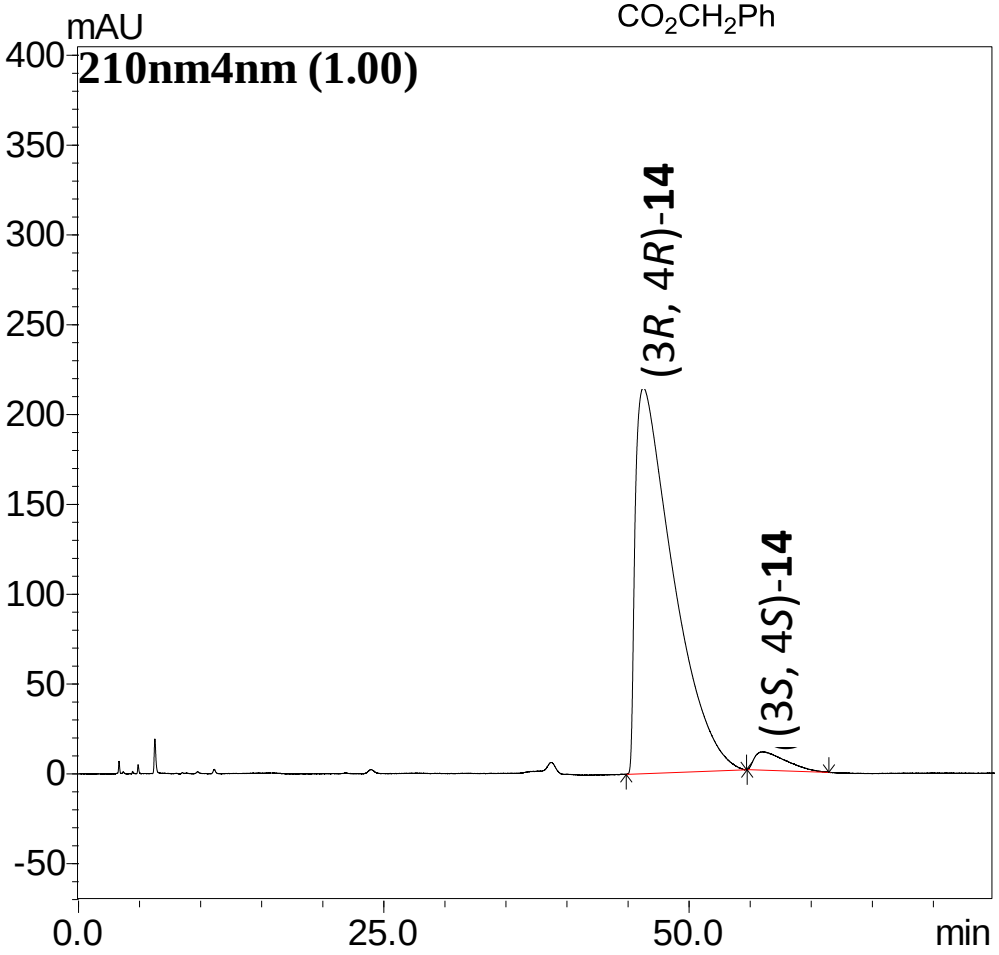


Figure S19. Chiral HPLC chromatogram of biotransformation product (3*R*, 4*R*)-**12** (Column: Daicel AS-H (250 × 4.6 mm, 5 μm); eluent: 5% IPA: 95% *n*-hexane; flow rate: 1.0 mL min^{-1}).

Figure S20-S24: Chiral GC chromatograms

trans-**12**:

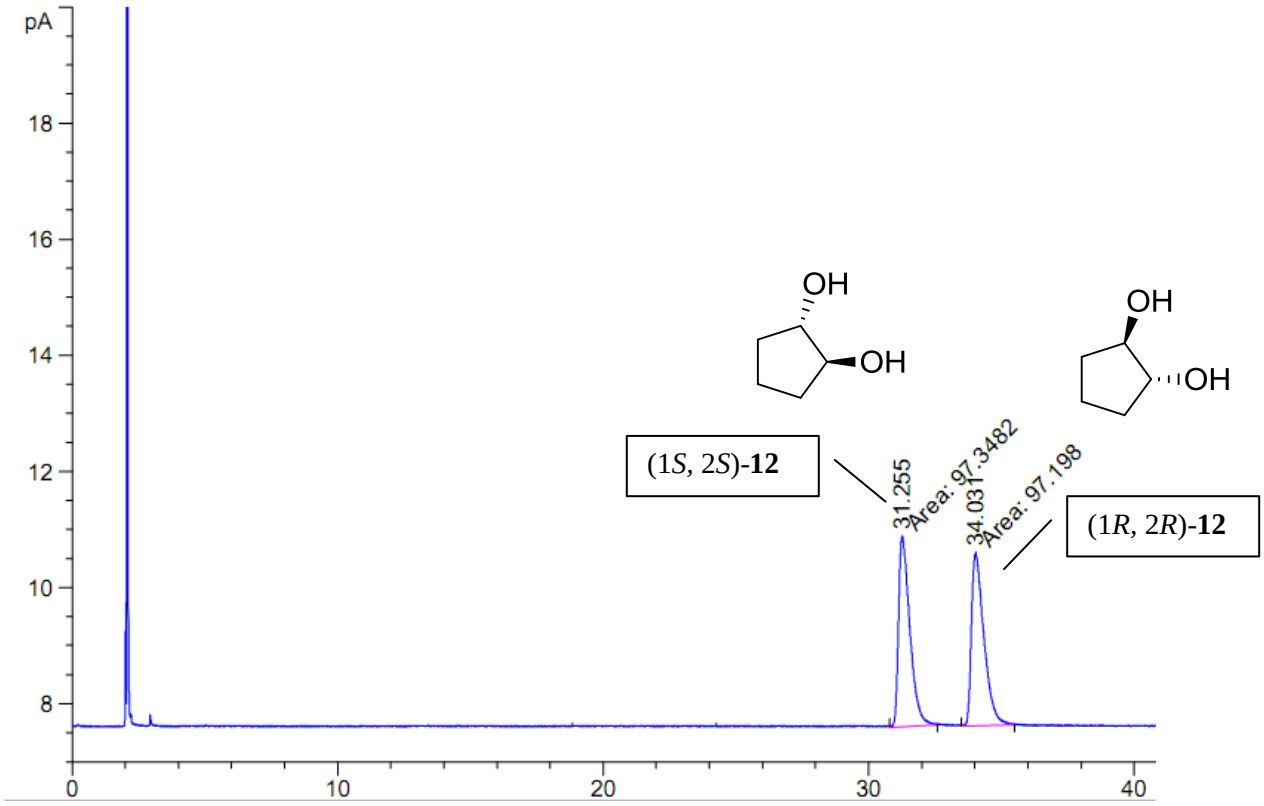


Figure S20. Chiral GC chromatogram of *trans*-**12** (Column: Macherey-Nagel Lipodex-E (25 m × 0.25 mm); temperature: 100 °C constant; pressure: 11.093 psi).

Product (1*R*, 2*R*)-**12**:

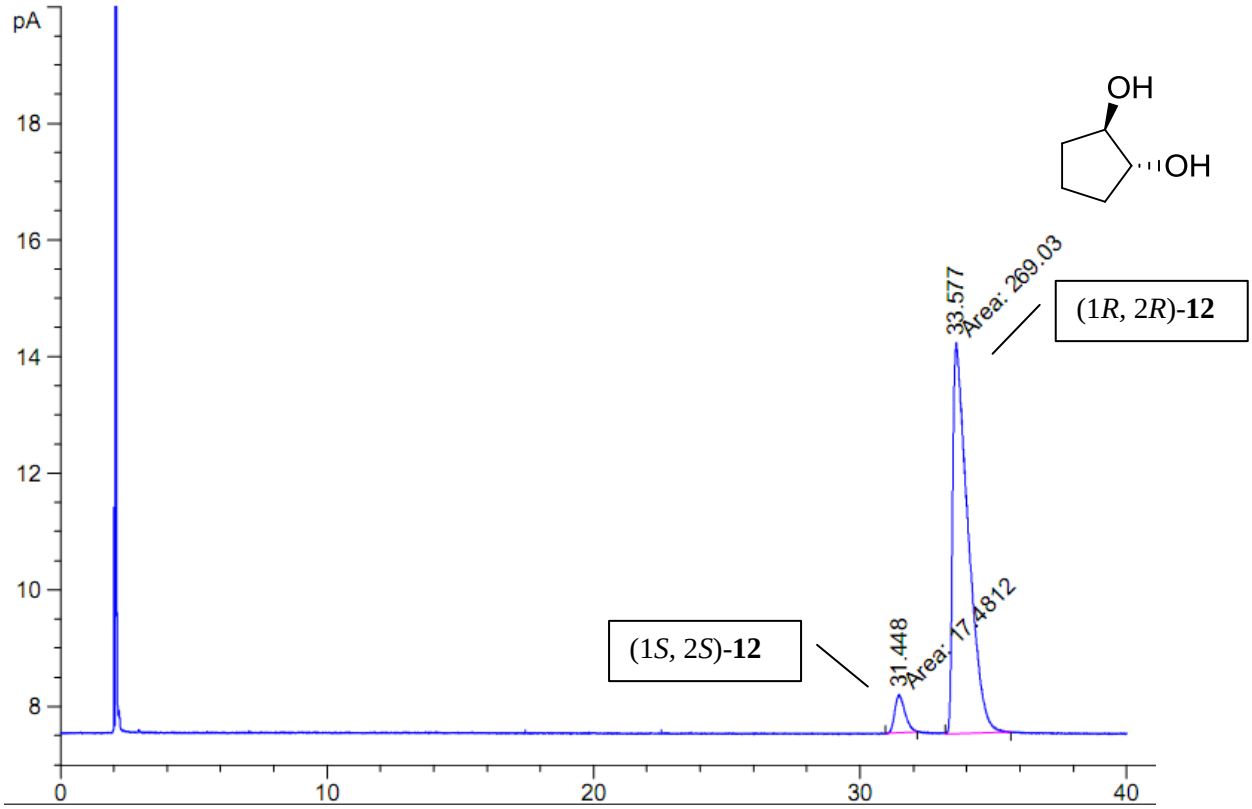


Figure S21. Chiral GC chromatogram of biotransformation product (1*R*, 2*R*)-**12** (Column: Macherey-Nagel Lipodex-E (25 m × 0.25 mm); temperature: 100 °C constant; pressure: 11.093 psi).

trans-**13**:

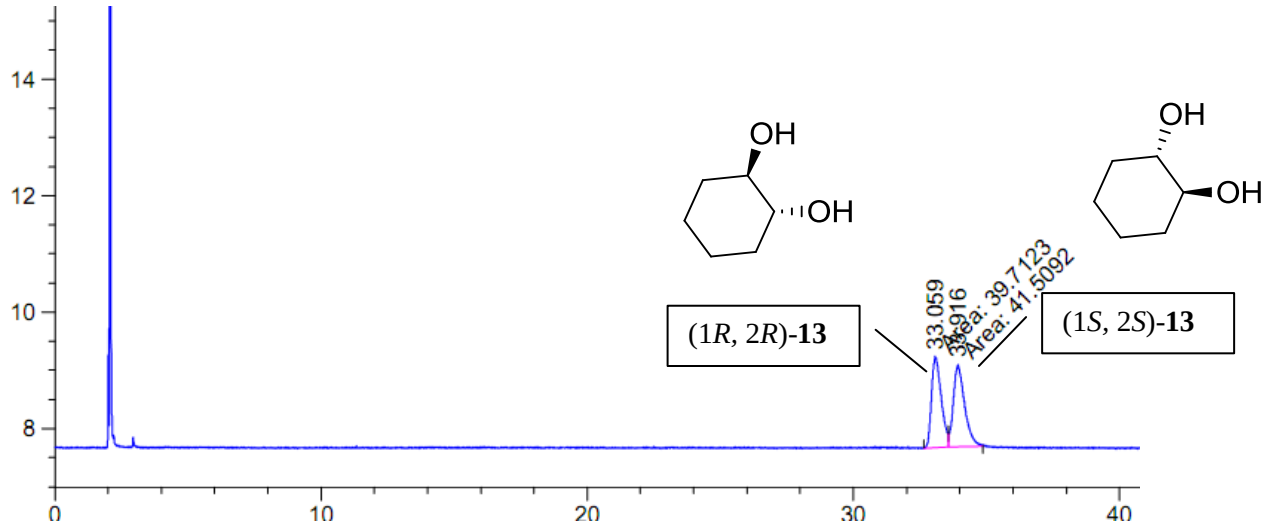


Figure S22. Chiral GC chromatogram of *trans*-**13** (Column: Macherey-Nagel Lipodex-E (25 m × 0.25 mm); temperature: 100 ^{o}C constant; pressure: 11.093 psi).

Product (1*R*, 2*R*)-**13**:

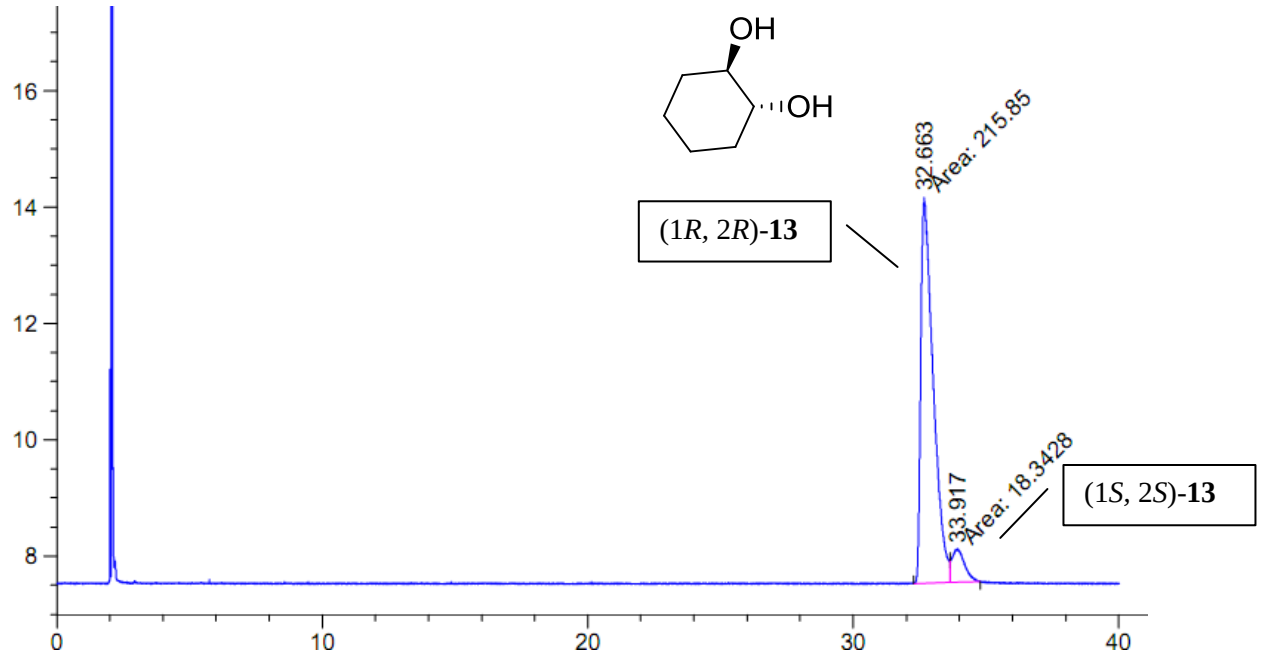


Figure S23. Chiral GC chromatogram of biotransformation product (1*R*, 2*R*)-**13** (before crystallization). (Column: Macherey-Nagel Lipodex-E (25 m × 0.25 mm); temperature: 100 ^{o}C constant; pressure: 11.093 psi)

Product (1*R*, 2*R*)-**13** after crystallization:

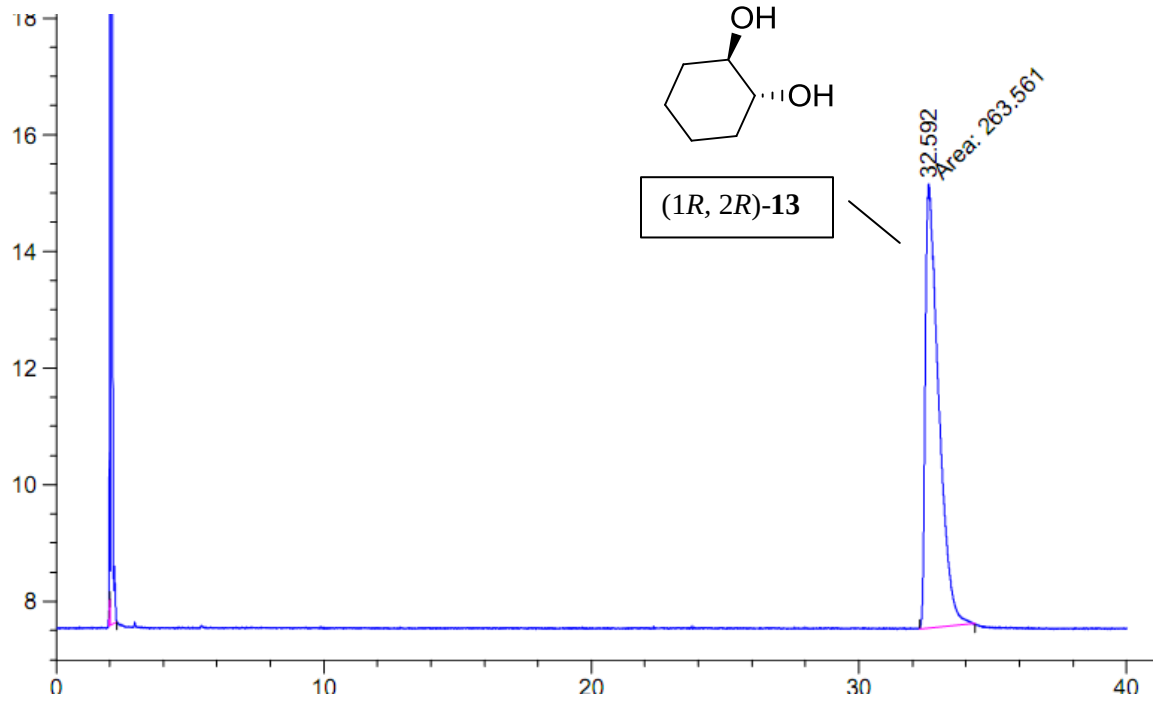


Figure S24. Chiral GC chromatogram of biotransformation product (1*R*, 2*R*)-**13** (after crystallization). (Column: Macherey-Nagel Lipodex-E (25 m × 0.25 mm); temperature: 100 ^{o}C constant; pressure: 11.093 psi)

Figure S25-S32. ^{1}H NMR spectra

Product (*S*)-**1**:

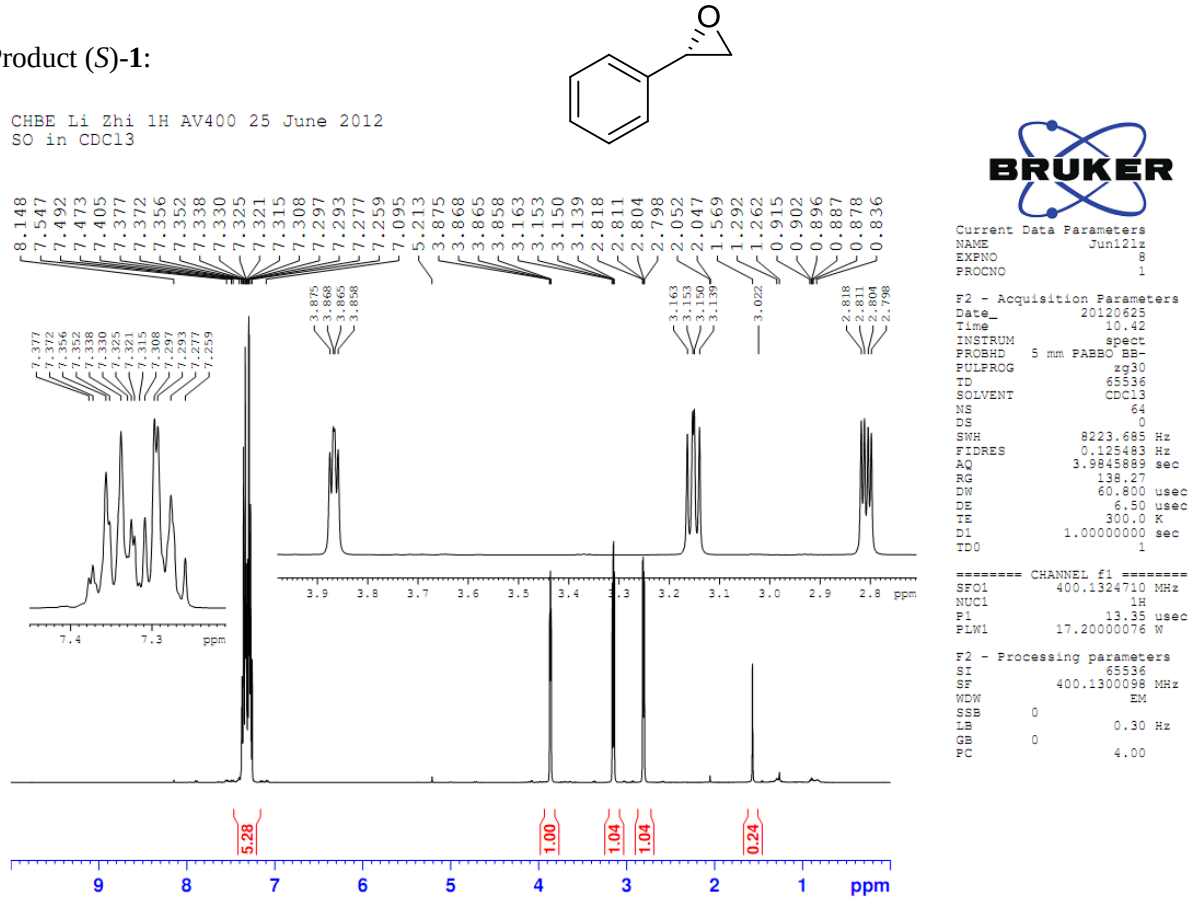
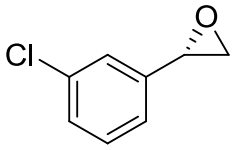


Figure S25. ^{1}H NMR spectrum of biotransformation product (*S*)-**1** (400 MHz, $CDCl_3$, TMS).

Product (*S*)-**3**:



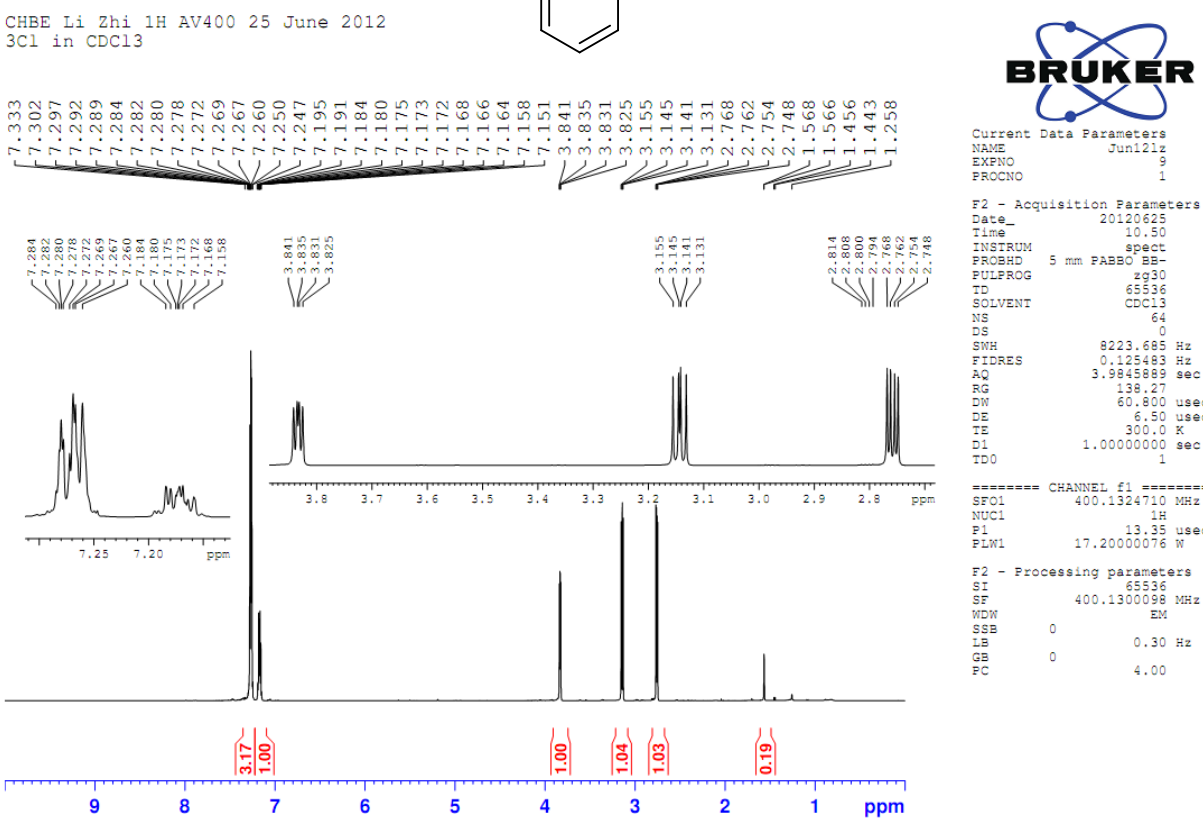


Figure S26. ^{1}H NMR spectrum of biotransformation product (*S*)-**3** (400 MHz, $CDCl_3$, TMS).

Product (*S*)-**6**:

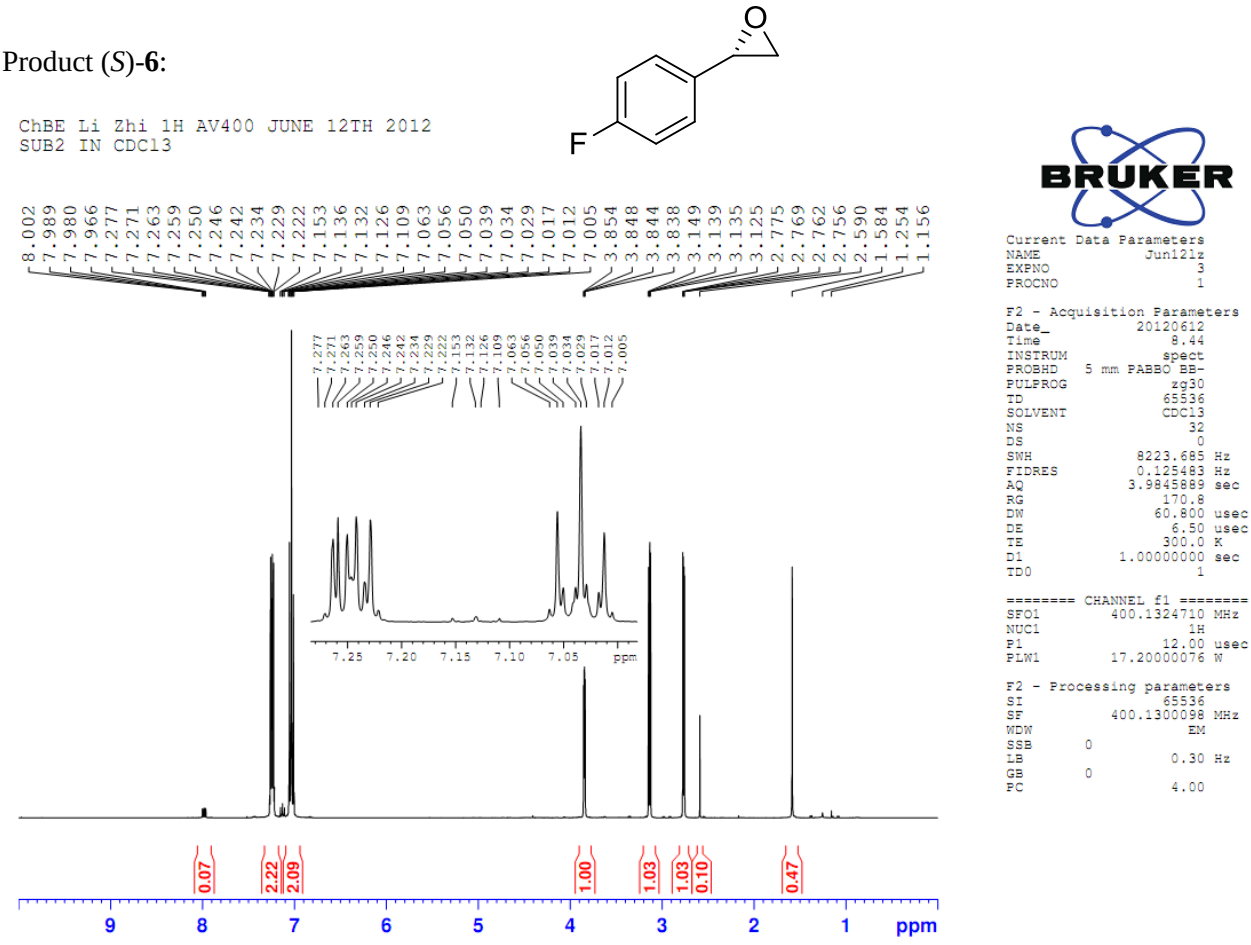


Figure S27. ^{1}H NMR spectrum of biotransformation product (*S*)-**6** (400 MHz, $CDCl_3$, TMS).

Product (1*R*, 2*R*)-**12**:

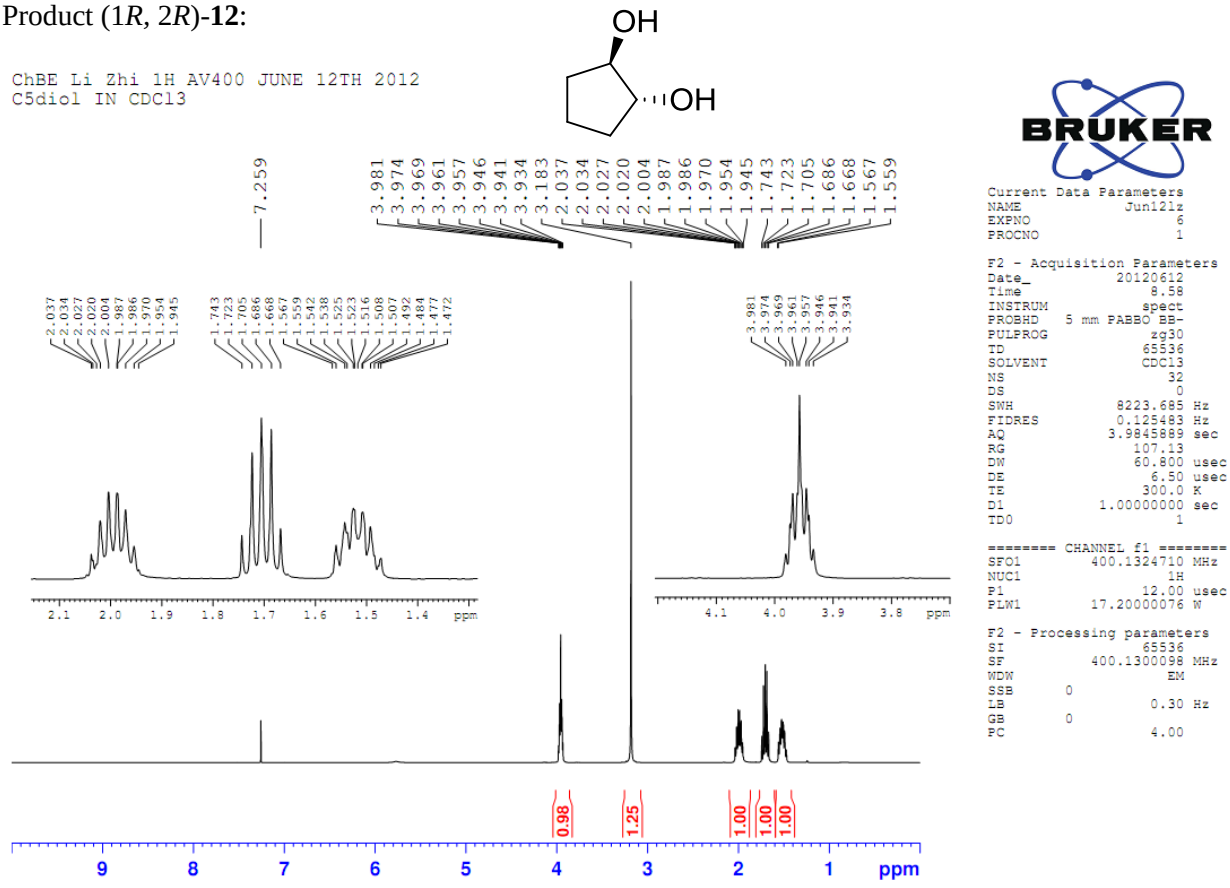
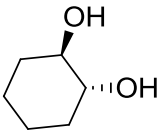


Figure S28. ^{1}H NMR spectrum of biotransformation product (1*R*, 2*R*)-**12** (400 MHz, $CDCl_3$, TMS).

Product (1*R*, 2*R*)-**13**:



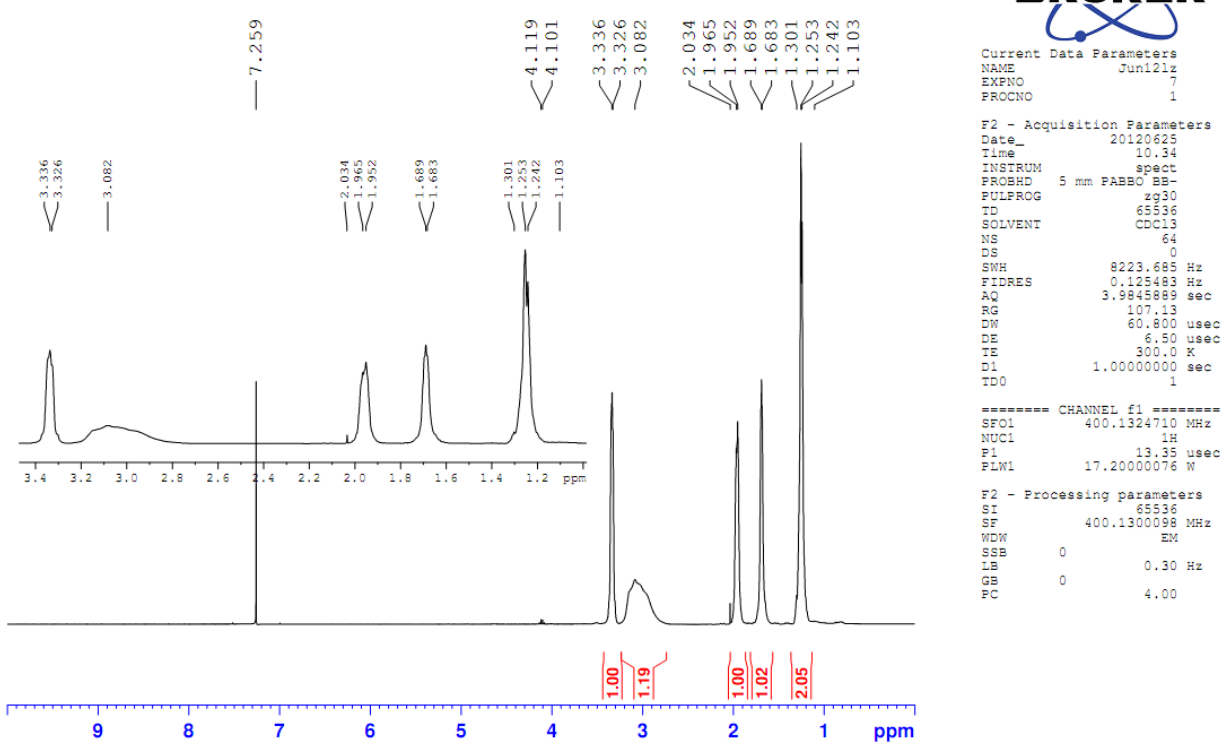


Figure S29. ^{1}H NMR spectrum of biotransformation product (1*R*, 2*R*)-**13** (400 MHz, $CDCl_3$, TMS).

Product (3*R*, 4*R*)-**14**:

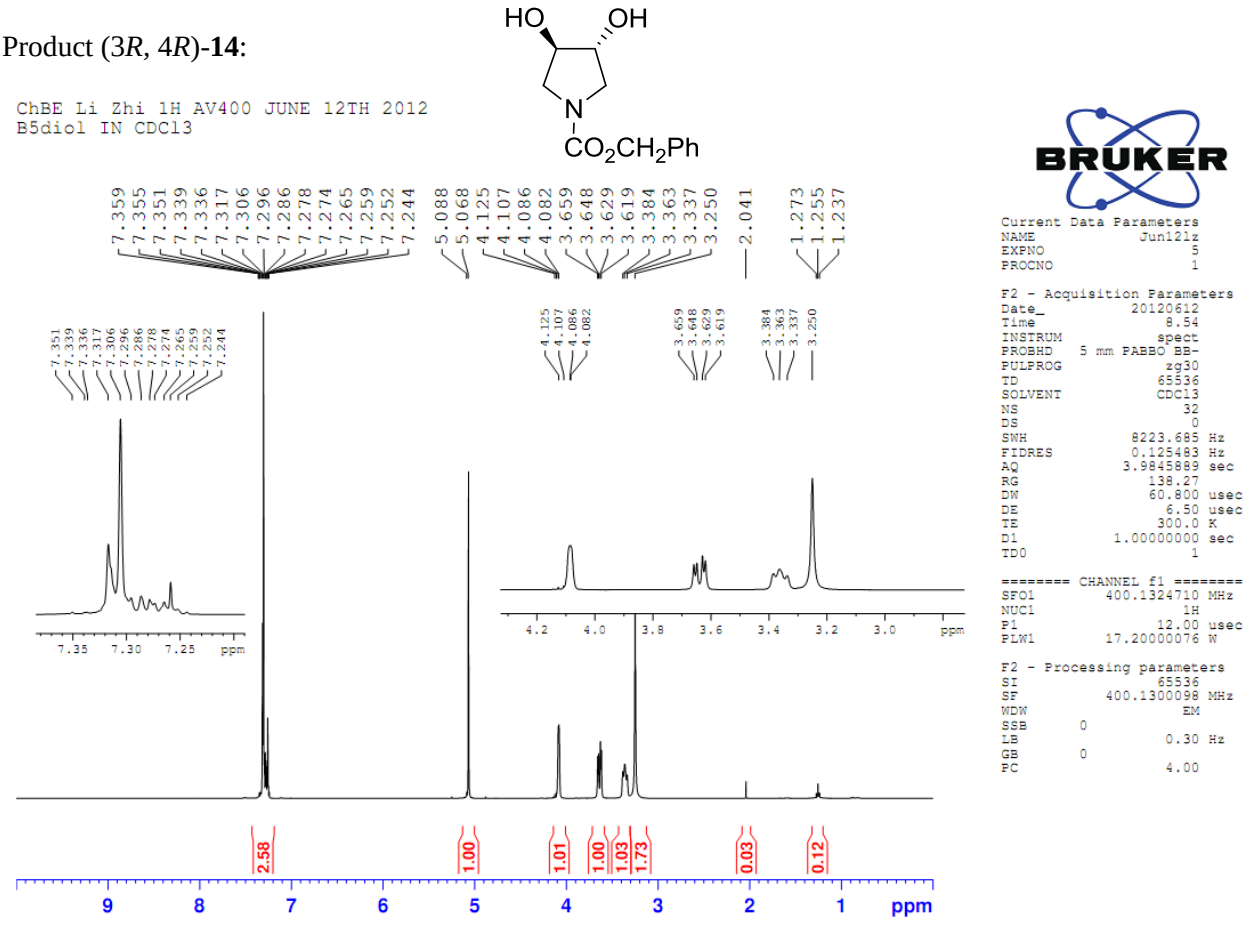


Figure S30. ^{1}H NMR spectrum of biotransformation product (3*R*, 4*R*)-**14** (400 MHz, $CDCl_3$, TMS).

Substrate **8**:

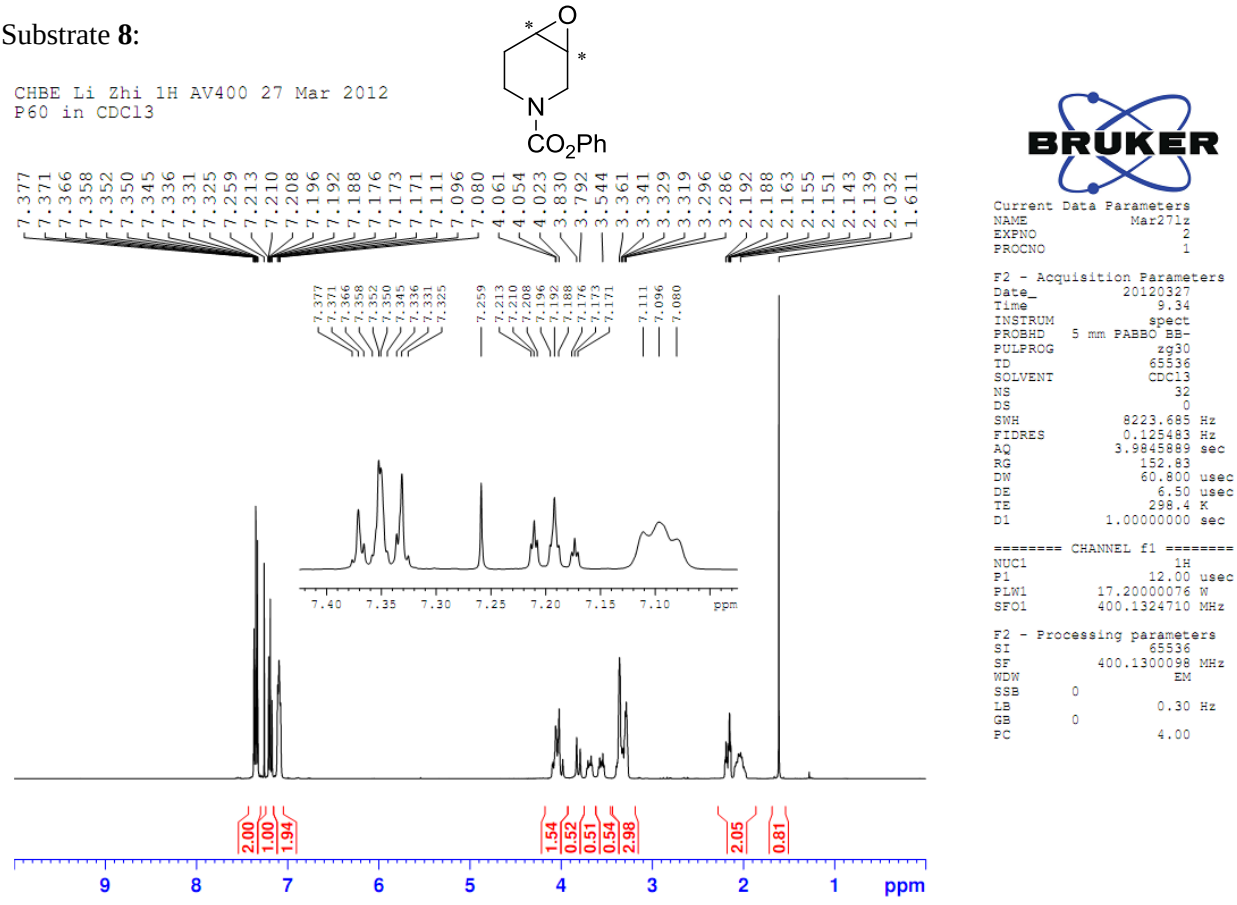


Figure S31. ^{1}H NMR spectrum of substrate **8** (400 MHz, $CDCl_3$, TMS).

Substrate **11**:

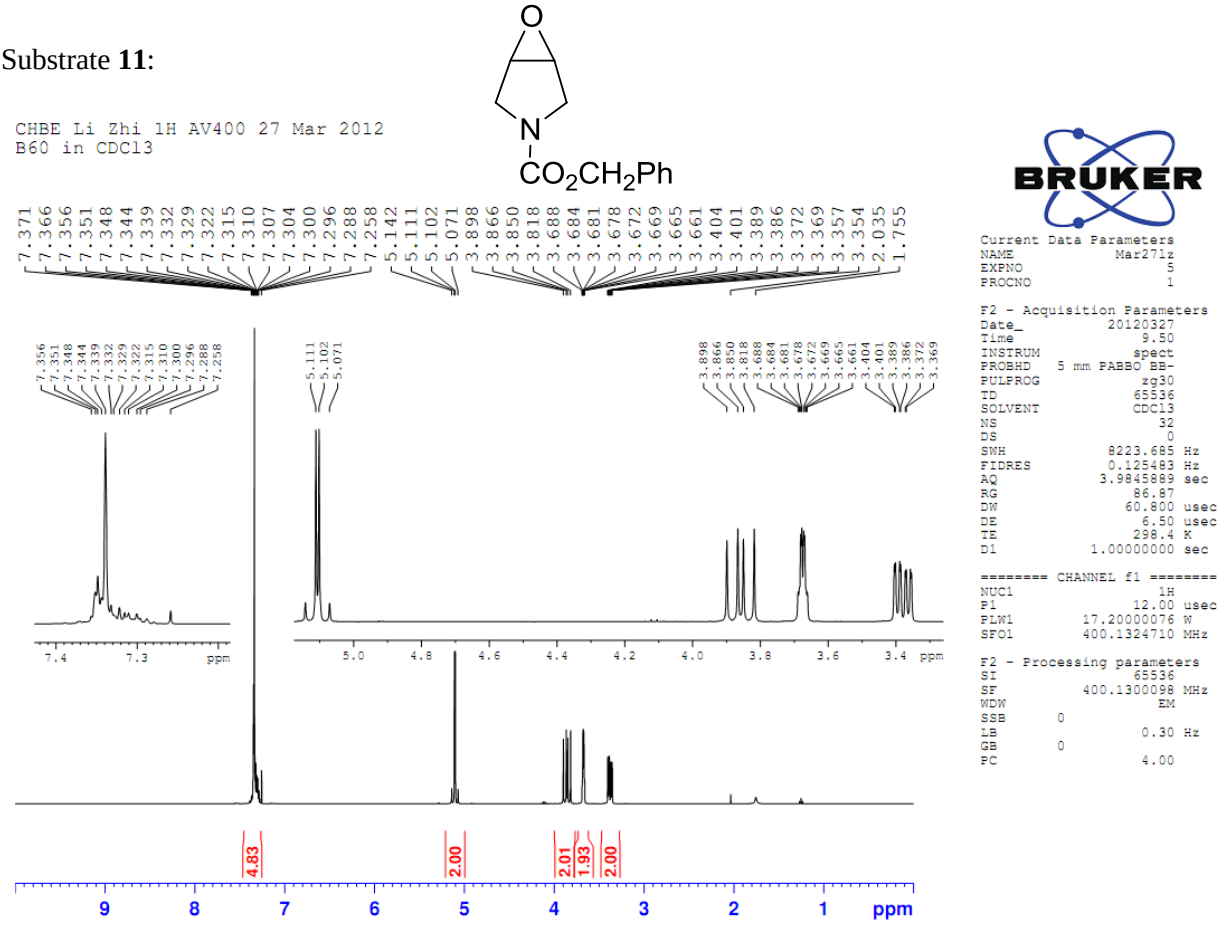


Figure S32. ^{1}H NMR spectrum of substrate **11** (400 MHz, $CDCl_3$, TMS).

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