Electronic Supplementary Information

Engineered P450pyr Monooxygenase for Asymmetric Epoxidation of Alkenes with Unique and High Enantioselectivity

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1 Materials

1.1 Chemicals and strains

Ampicillin sodium salt (>99%), kanamycin disulfate salt (>99%), 4-fluorostyrene **1** (98%), 2-(4-fluorophenyl)oxirane **2** (98%), 4-chlorostyrene **3** (98%), 2-(4-chlorophenyl)oxirane **4** (98%), 4-bromostyrene **5** (98%), 2-(4-bromophenyl)oxirane **6** (98%), 4-nitrostyrene **7** (>98%), 4-cyanostyrene **11** (98%), 2-fluorostyrene **19** (98%), 2-chlorostyrene **13** (98%), 2-(2-chlorophenyl)oxirane **14** (98%), 2-bromostyrene **15** (98%), 2-trifluorostyrene **17** (98%), *alpha*-methylstyrene **23** (98%), *δ*-aminolevulinic acid hydrochloride (ALA, >99%) and *meta*-chloroperoxybenzoic acid (*m*-CPBA, 77%) were purchased form Sigma-Aldrich. 4-trifluorostyrene **9** (98%) and 2-methyl-3-phenyl-1-propene **21** (97%) were purchased from Alfa-Aesar. 2-(4-trifluorophenyl)oxirane **10** (95%), 4-(oxiran-2-yl)benzonitrile **12** (97%) and 2-(2-trifluorophenyl)oxirane **18** (95%) were obtained from Enamine (Kiev, Ukraine). 2-phenylpropylene oxide **24** (98%) was purchased from TCI. Hydrophobic resin XAD 16 was bought from Sigma-Aldrich. Isopropyl *β*-D-1-thiogalactopyranoside (ITPG, >99%) was obtained from Calbiochem. Medium components LB premix, tryptone and yeast extract were purchased from Biomed Diagnostics.

The *Escherichia coli* (*E. Coli*) BL21 (DE3) was used as host for enzyme expression. The recombinant *E. coli* (P450pyr) or *E. coli* (P450pyrTM) with dual plasmids, pETDuet containing P450pyr or its triple mutant I83H/M305Q/A77S (P450pyrTM) and ferredoxin reductase (FdR) genes, and pRSFDuet containing glucose dehydrogenase (GDH) and ferredoxin (Fdx) genes, were constructed as described previously. [S1]

1.2 Synthesis of 2-(4-nitrophenyl)oxirane 8

The 2-(4-nitrophenyl)oxirane **8** was prepared according to previously reported method [S2]: *m*-CPBA (1.43 g, 8.34 mmol) was added to a stirred solution of 4-nitrostyrene **7** (0.426 g, 2.67 mmol) in dichloromethane (CH_2Cl_2) (20 mL) on ice, and the mixture was stirred at room temperature for 5 h. After reaction, sodium hydroxide (1N, 20 mL) was added to the mixture to neutralize the unreacted *m*-CPBA, followed by extraction with CH_2Cl_2 (3 x 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation.

The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 20/1, R_f = 0.2). 0.244 g pure product (50.0% yield) of **8** was obtained as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): 2.77 (dd, *J* = 2.8, 5.6 Hz, 1H), 3.22 (dd, *J* = 4.0, 5.2 Hz, 1H), 3.96 (dd, *J* = 2.8, 4.0 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 2H), 8.2 (d, *J* = 8.8 Hz, 2H).

1.3 Synthesis of 2-(2-bromophenyl)oxirane 16

The 2-(2-bromophenyl)oxirane **16** was prepared according to previously reported method [S2]: *m*-CPBA (1.43 g, 8.34 mmol) was added to a stirred solution of 2-bromostyrene **15** (0.357 g, 1.95 mmol) in CH_2Cl_2 (20 mL) on ice, and the mixture was stirred at room temperature for 5 h. After reaction, sodium hydroxide (1N, 20 mL) was added to the mixture to neutralize the unreacted *m*-CPBA, followed by extraction with CH_2Cl_2 (3 x 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 50/1, R_f = 0.2). 0.316 g pure product (79.8% yield) of **16** was obtained as colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): 2.65 (dd, *J* = 2.4, 5.6 Hz, 1H), 3.19 (dd, *J* = 4.4, 6.0 Hz, 1H), 4.15 (dd, *J* = 2.8, 6.4 Hz, 1H), 7.15-7.32 (m, 3H), 7.54 (dd, *J* = 0.8, 8 Hz, 1H).

1.4 Synthesis of 2-(2-fluorophenyl)oxirane 20

The 2-(2-fluorophenyl)oxirane **20** was prepared according to previously reported method [S2]: *m*-CPBA (1.43 g, 8.34 mmol) was added to a stirred solution of 2-fluorostyrene **19** (0.488 g, 3.99 mmol) in CH_2Cl_2 (20 mL) on ice, and the mixture was stirred at room temperature for 5 h. After reaction, sodium hydroxide (1N, 20 mL) was added to the mixture to neutralize the unreacted *m*-CPBA, followed by extraction with CH_2Cl_2 (3 x 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 50/1, R_f = 0.2). 0.232 g pure product (42.0% yield) of **20** was obtained as colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): 2.79 (dd, *J* = 2.4, 5.6 Hz, 1H), 3.18 (dd, *J* = 4.0, 5.6 Hz, 1H), 4.14-4.16 (m, 1H), 7.03-7.28 (m, 4H).

1.5 Synthesis of 2-benzyl-2-methyloxirane 22

The 2-benzyl-2-methyloxirane **22** was prepared according to previously reported method [S2]: *m*-CPBA (1.43 g, 8.34 mmol) was added to a stirred solution of 2-methyl-3-phenyl-1-propene **21** (0.48 g, 3.63 mmol) in CH_2Cl_2 (20 mL) on ice, and the mixture was stirred at room temperature for 5 h. After reaction, sodium hydroxide (1N, 20 mL) was added to the mixture to neutralize the unreacted *m*-CPBA, followed by extraction with CH_2Cl_2 (3 x 20 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 50/1, R_f = 0.2). 0.392 g pure product (73.0% yield) of **22** was obtained as colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (s, 3H), 2.77 (dd, *J*=4.8, 18 Hz, 2H), 2.87 (dd, *J*=14, 30.4 Hz, 2H), 7.22-7.33 (m, 5H).

1.6 Synthesis of *N*-phenoxycarbonyl-1, 2, 5, 6-tetrahydropyridine 25

N-phenoxycarbonyl-1,2,5,6-tetrahydropyridine **25** was synthesized according to previously described method.[S3] A solution of phenyl chloroformate (0.563 g, 3.6 mmol) in THF (1.5 mL) was added drop wise to a stirred mixture of 1,2,5,6-tetrahydropyridine (0.299 g, 3.6 mmol) and $NaHCO_3$ (0.39 g, 1.68 mmol) in THF-water (1:1, 3.6 mL) on ice, and the mixture was stirred at room temperature for 3 h. $CHCl_3$ (6 mL) and 5% aqueous Na_2CO_3 (3 mL) were added, the organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 5 mL). The combined organic phase was washed with saturated NaCl solution and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 4/1, R_f = 0.4). 0.496 g pure product (67.9% yield) of product **25** was obtained as a white solid. ^{1}H NMR (400MHz, $CDCl_3$): δ 2.25 (s, 2H), 3.63-3.66 (m, 1H), 3.73-3.75 (m, 1H), 4.04 (s, 1H), 4.15 (s, 1H), 5.72 (s, 1H), 5.90 (s, 1H,), 7.11-7.25 (m, 3H), 7.34-7.38 (m, 2H).

1.7 Synthesis of *N*-phenoxycarbonyl-3, 4-epoxypiperidine 26

N-phenoxycarbonyl-3,4-epoxypiperidine **26** was synthesized according to previously described method.[S3] *m*-CPBA (0.715 g, 4.17 mmol) was added to a solution of *N*-phenoxycarbonyl-1,2,5,6-tetrahydropyridine **25** (0.37 g, 1.82 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at room temperature overnight (12 h). Sodium hydroxide (1 N, 10 mL) was added to neutralize the unreacted *m*-

CPBA, followed by extraction with CH_2Cl_2 (3 × 10 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation. The crude product was purified by flash chromatography on a silica gel column (*n*-hexane/ethyl acetate = 4/1, R_f = 0.2). 0.264 g (66.1% yield) of **26** was obtained as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.00-2.09 (m, 1H), 2.17 (dt, *J*= 4.8, 15.2 Hz, 1H), 3.29-3.36 (m, 3H), 3.53-3.59 (m, 0.5 H), 3.66-3.71(m, 0.5H), 3.81 (d, *J*=15.2Hz, 0.5H), 3.98-4.09 (m, 1.5H), 7.08-7.11 (m, 2H), 7.19 (t, *J*=7.6Hz, 1H) 7.35 (t, *J*=8.4 H, 2H).

2. Analytic methods

2.1 Analysis of the conversion for asymmetric epoxidation

The concentrations of the alkenes and their corresponding epoxides were analyzed *via* Shimadzu Prominence HPLC (Japan) system (reverse phase) on an Agilent Poroshell 120 EC-C18 column (150 mm × 4.6 mm, Agilent) at UV detection 210 nm and temperature at 25°C. The analysis conditions (flow rate and eluent) and retention times for each substrate and product were shown in Table S1.

Table S1. HPLC analysis of alkenes and their corresponding epoxides products.

Eluent (acetonitrile:water, *v/v*)	Flow rate (mL/min)	Substrate	Retention time for substrate (min)	Product	Retention time for product (min)
60:40	0.5	**1**	11.3	**2**	6.5
60:40	0.5	**3**	16.2	**4**	8.4
60:40	0.4	**5**	23.3	**6**	11.5
60:40	0.4	**7**	11.2	**8**	7.5
60:40	0.4	**9**	21.2	**10**	11.4
60:40	0.5	**11**	8.1	**12**	5.6
60:40	0.4	**13**	20.4	**14**	11.2
60:40	0.4	**15**	22.3	**16**	12.2
60:40	0.4	**17**	20.5	**18**	2.6
60:40	0.4	**19**	14.8	**20**	8.6
60:40	0.5	**21**	21.6	**22**	7.9
60:40	0.5	**23**	15.8	**24**	7.6
60:40	0.5	**25**	8.6	**26**	5.3

2.2 Analysis of *ee* of the epoxides from biotransformations

The product *ee* was determined on a Shimadu Prominence HPLC (Japan) using a chiral column (250 × 4.6 mm, 5 μm) at 25°C and UV detection was at 210 nm. The analysis conditions (flow rate, eluent and

chiral columns) and retention times of two enantiomers for each product were summarized in Table S2 below (all the chiral columns were bought from Daicel Corporation, Japan).

Table S2. HPLC analysis of *ee* of chiral epoxides.

Products	Chiral column	Eluent (hexane: isopropyl alcohol, *v/v*)	Flow rate (ml/min)	Retention time for (*R*)-enantiomer (min)	Retention time for (*S*)-enantiomer (min)
2 [a]	AS-H	90:10	0.5	13.2	13.7
4 [a]	AS-H	90:10	0.5	12.0	14.0
6 [b]	AS-H	90:10	0.5	12.0	14.4
8 [c]	IA-3	90:10	0.5	17.1	18.5
10 [c]	AS-H	90:10	0.5	9.0	9.9
12 [d]	AS-H	90:10	1.0	18.8	20.7
14 [a]	AS-H	100:0	0.5	22.0	22.8
16 [b]	AS-H	100:0	0.5	20.8	23.1
18 [d]	IA-3	100:0	0.5	13.1	14.1
20 [b]	AS-H	100:0	0.5	20.7	23.2
22 [b]	OD-H	90:10	0.5	9.1	9.9
24 [b]	OD-H	90:10	0.5	8.7	9.5
26 [a]	OB-H	90:10	0.5	55.0 [e]	62.8 [f]

[a] The absolute configuration were established in our previous study. [S4]

[b] The absolute configuration were established by comparing the products with that from the known (*S*)-selective epoxidations catalyzed by styrene monooxygenase (SMO). [S5, S6, S7]

[c] The absolute configuration were deduced from the elution order on the chiral HPLC (Chiralpak AS-H column). *R* enantiomer was eluted before the *S* enantiomer. [S8]

[d] The absolute configuration were further verified by chiral Gas Chromatography (GC) analysis (Chiral *β*-cyclodextrin column) according to the elution order. *R* enantiomer was eluted before the *S* enantiomer. [S2, S9]

[e] Retention time for (-)-**26**. [f] Retention time for (+)-**26**.

3. Cell cultivation

The recombinant *E. coli* BL21 (DE3) strains expressing P450pyrTM [*E. coli* (P450pyrTM)] or P450pyr [*E. coli* (P450pyr)] were inoculated into 3 mL LB medium containing 50 mg/L of kanamycin and 100 mg/L ampicillin. The overnight inoculum (2 mL) was transferred to 50 mL TB medium supplemented with 50 mg/L of kanamycin and 100 mg/L ampicillin in a 250 mL Erlenmeyer flask. Cells were grown at 37°C and 250 rpm to OD_{600} about of 0.6~0.8 and then induced by adding Isopropyl *β*-D-1-

thiogalactopyranoside (IPTG) to a final concentration of 0.25 mM. At the same time, δ-Aminolevulinic acid hydrochloride (0.5 mM) as the heme precursor was added to increase heme-incorporated P450 enzyme expression. Cells were then grown at 22°C for another 12 h and harvested by centrifugation at 5, 000 × g for 5 min, washed with water followed by centrifugation to give the cell pallets as wet-cell biocatalysts.

4. Biotransformations

4.1 Epoxidation of alkenes in single aqueous system

General procedure of asymmetric epoxidation of aromatic alkenes ***1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21,*** *and* ***23***: To 4 mL cell suspension (10 g cdw/L) of *E. coli* (P450pyrTM) or *E. coli* (P450pyr) in potassium phosphate buffer (100 mM, pH 8.0) were added 80 μL glucose stock solution (50% w/v, final concentration of 1% w/v) and 20 μL 0.4 M alkene substrate in ethanol (final concentration of 2 mM). The reaction was conducted at 30^{o}C and 250 rpm in a 100-mL flask with screw cap. For following the reaction process, at different reaction time points, acetonitrile containing 2 mM benzyl alcohol as internal standard was added to the reaction mixture with equivalent volume. The mixture was centrifuged at 21, 000 × g for 10 min and the supernatnat was subjected to reverse HPLC analysis for the determination of substrate/product concentration. For the analysis of product *ee*, the reaction mixture was extracted with equivalent volume of *n*-hexane and centrifuged at 21, 000 × g for 10 min, the upper layer was subjected to chiral HPLC analysis.

Asymmetric epoxidation of cyclic alkene ***25***: To 4 mL cell suspension (10 g cdw/L) of the *E. coli* (P450pyrTM) or *E. coli* (P450pyr) in potassium phosphate buffer (100 mM, pH 8.0) were added 80 μL glucose stock solution (50% w/v, final concentration of 1% w/v) and 40 μL 0.5 M substrate in ethanol (final concentration of 5 mM). The reaction was conducted at 30^{o}C and 250 rpm in a 100-mL flask with screw cap. For following the reaction process, at different reaction time points, ethanol containing 2 mM benzylacetone as internal standard was added to the reaction mixture with equivalent volume. The mixture was centrifuged at 21, 000 × g for 10 min and the supernatant was subjected to HPLC analysis

for the determination of substrate/product concentration. The product *ee* was determined according to the above mentioned procedure for epoxidation of aromatic alkenes.

4.2 Epoxidation of alkenes in resin/water biphasic system

*General procedure of asymmetric epoxidation of alkenes **1**, **3**, **5**, **7**, **9**, and **21** in resin/water biphasic system:* To 5 mL cell suspension (10 g cdw/L) of the *E. coli* (P450pyrTM) in potassium phosphate buffer (100 mM, pH 8.0) were added 100 μL glucose stock solution (50% w/v, final concentration of 1% w/v) and different amount of resins and substrates (with different ratios of resin to substrtae, g/m mole). The reactions were conducted at 30^{o}C and 250 rpm in 100-mL flask with screw cap. After reaction, for determination of product concentration in aqueous phase, acetonitrile containing 2 mM benzyl alcohol as internal standard was added to the reaction mixture with equivalent volume, followed by centrifugation at 21, 000 × g for 10 min, and the supernatant was subjected to HPLC analysis. For the determination of the amount of product absorbed by the resin, the resin was seperated by sedimentation and the product was extracted by adding 5 mL methanol then shaking at 30^{o}C and 250 rpm for 10 min, the same procedure was repeated 4 times. The four portions of methanol were combined, acetonitrile containing 2 mM benzyl alcohol as internal standard was added with equivalent volume, the mixture was centrifuged at 21, 000 × g for 10 min and the supernatant was subjected to HPLC analysis. To determine the product *ee*, the epoxide product in aqueous phase was extracted with equivalent volume of *n*-hexane, the mixture was centrifuged at 21, 000 × g for 10 min and the upper layer was subjected to chiral HPLC analysis.

The general procedure for product isolation from the resin/waster biphasic system: The product isolation consists of two parts. For product recovery from resin, after reaction, the reaction flask was allowed to stand for 5 min to settle the resin at the bottom of the flask. The resin was obtained by removing the cell suspension, the product was extracted by adding equivalent volume of methanol and then shaking at 30 ^{o}C and 250 rpm for 10 min, the same procedure was repeated for 4 times. For product recovery from cell suspension, the cell suspension was centrifuged at 8, 000 × g for 10 min to remove the cells and the supernatant was extracted by adding equivalent volume of EtOAc for two times. The two parts of product were then combined, washed with saturated NaCl solution, dried over Na_2SO_4 overnight

After filtration, the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography.

5. Preparation of enantiopure epoxides by asymmetric epoxidations with recombinant *E. coli* (P450pyrTM)

*Preparation of enantiopure epoxides (R)-**2**, (R)-**4**, (R)-**6**, (R)-**8**, (R)-**10**, and (S)-**22**:* To 120 mL cell suspension (10 g cdw/L) of the *E. coli* (P450pyrTM) were added 600 µL glucose stock solution (50% w/v, final concentration of 1% w/v) and different alkene substrates (alkenes **1**, **3**, **5**, **7** and **9**, 2 mM; alkene **21**, 5 mM). The reaction was conducted at 30°C and 300 rpm in a 300-mL flask with screw cap for 5 h. After reaction, the reaction mixture was centrifuged at 8, 000 × g for 10 min to remove the cells and the supernatant was extracted with equivalent volume of EtOAc for three times (3 × 120 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the solvent was removed by evaporation. The crude product was purified by flash chromatography with *n*-hexane and ethyl acetate (EtOAc) (20:1, R_f= 0.2~0.3) as elution solvent, yielding enantiopure epoxides in high purity. The enantiopure epoxides produced were identified by ^{1}H NMR analysis.

(*R*)-2-(4-fluorophenyl)oxirane (*R*)-**2:** obtained as colorless oil after column chromatography; yield: 39.2%; *ee*: 97.6%(*R*); $[\alpha]_D^{25}$ -16.5 (*c* 1.0, $CHCl_3$) {lit.[S2] $[\alpha]_D^{25}$ -15.1 (*c* 0.45, $CHCl_3$), *ee* 96.0%(*R*)}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.77 (dd, *J* =2.4, 5.2 Hz, 1H), 3.14 (dd, *J* =4.0, 5.6 Hz, 1H), 3.85 (dd, *J* =2.4, 4.0 Hz, 1H), 7.01-7.06 (m, 2H), 7.22-7.28 (m, 2H).

(*R*)-2-(4-chlorophenyl)oxirane (*R*)-**4:** obtained as colorless oil after column chromatography; yield: 46.9%; *ee*: 97.1%(*R*); $[\alpha]_D^{25}$ -21.8 (*c* 0.75, $CHCl_3$) {lit.[S10] $[\alpha]_D^{21}$ -17.2 (*c* 1, $CHCl_3$), *ee* >99.0%(*R*)}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.75 (dd, *J* =2.8, 5.6 Hz, 1H), 3.14 (dd, *J* =4.0, 5.6 Hz, 1H), 3.83 (dd, *J* =2.8, 4.0 Hz, 1H), 7.19-7.23 (m, 2H), 7.30-7.35 (m, 2H).

(*R*)-2-(4-bromophenyl)oxirane (*R*)**-6:** Obtained as colorless oil after column chromatography; yield: 64.1%; *ee*: 98.5%(*R*); $[\alpha]_D^{25}$ -20.3 (*c* 1.2, $CHCl_3$) {lit.[S2] $[\alpha]_D^{20}$ -12.9 (*c* 1.04, $CHCl_3$), *ee* 96.0%(*R*)}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.75 (dd, *J* =2.8, 5.6 Hz, 1H), 3.14 (dd, *J* =4.0, 5.6 Hz, 1H), 3.82 (dd, *J* =2.8, 4.0 Hz, 1H), 7.07-7.20 (m, 2H), 7.46-7.50 (m, 2H).

(*R*)-2-(4-nitrophenyl)oxirane (*R*)**-8:** Obtained as colorless oil after column chromatography; yield: 59.6%; *ee*: 97.4%(*R*); $[\alpha]_D^{25}$ -38.9 (*c* 0.75, $CHCl_3$) {lit.[S2] $[\alpha]_D^{20}$ +37.6 (*c* 1.99, $CHCl_3$), *ee* >98.0%(*S*). The spectroscopic data is the same as the one for **8** reported above.

(*R*)-2-(4-trifluorophenyl)oxirane (*R*)**-10:** Obtained as colorless oil after column chromatography; yield: 41.6%; *ee*: 99.7%(*R*); $[\alpha]_D^{25}$ -19.1 (*c* 1.1, $CHCl_3$) {lit.[S9] $[\alpha]_D^{22}$ +18 (*c* 1.13, $CHCl_3$), *ee* 97.9%(*S*)}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.77 (dd, *J* =2.4, 5.6 Hz, 1H), 3.19 (dd, *J* =4.0, 5.6 Hz, 1H), 3.92 (dd, *J* =2.8, 4.0 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.61 (d, *J* = 8.4 Hz, 2H).

(*S*)-2-benzyl-2-methyloxirane (*S*)**-22**: obtained as a colorless oil after column chromatography; yield: 37.2%; *ee*: 90.1%(*S*); $[\alpha]_D^{25}$ +10.9 (*c* 1.1, $CHCl_3$) {lit.[S11] $[\alpha]_D^{25}$ -8.2 (*c* 1, $CHCl_3$), *ee* 81%(*R*)}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (s, 3H), 2.77 (dd, *J*=4.8, 18 Hz, 2H), 2.87 (dd, *J*=14, 30.4 Hz, 2H), 7.22-7.33 (m, 5H).

*Preparation of enantiopure epoxide (+)-**26***: To 30 mL cell suspension (10 g cdw/L) of the *E. coli* (P450pyrTM) in 100 mM KP buffer (pH8.0) were added 150 µL glucose stock solution (50% w/v, final concentration of 1% w/v) and 600 µL 0.5 M substate **25** in ethanol (final concentration, 10 mM). The reaction was conducted at 30°C and 250 rpm in a 300-mL flask with screw cap for 5 h. Atfer reaction, the reaction mixture was centrifuged at 8, 000 × g for 10 min to remove the cells and the supernatant was extract by addition of dichloromethane with equivalent volume for three times (3 × 30 mL). The organic phase was separated, washed with saturated NaCl solution, and dried over Na_2SO_4 overnight. After filtration, the crude product was purified by flash chromatography with *n*-hexane and EtOAc (4:1, R_f = 0.2) as elution solvent, yielding the pure product (+)-**26**. The enantiopure (+)-**26** produced was identified by ^{1}H NMR analysis.

(+)-***N*-phenoxycarbonyl-3, 4-epoxypiperidine** (+)-**26**: obtained as a colourless oil after column chromatography; yield: 65.9%; *ee*: 90.5%, $[\alpha]_D^{25}$ +16.4 (*c* 1.0, $CHCl_3$) {lit.[S3] $[\alpha]_D^{25}$ -18.05 (*c* 1.23, $CHCl_3$) for (-)-**26**, *ee* >99.9%}. The ^{1}H NMR spectrum is the same as that prepared from section 1.7.

6. Apparent kinetic parameters of P450pyrTM and P450pyr catalyzed epoxidation of *para*-fluorostyrene 1.

The apparent kinetic parameters (K_m and V_{max}) of whole-cells of *E. coli* (P450pyrTM) and *E. coli* (P450pyr) catalyzed epoxidation of fluorostyrene **1** were determined at 30^{o}C with the cell concentration of 4 g cdw/L. The initial reaction rates were measured at different substrate concentrations. The K_m and V_{max} for asymmetric epoxidation of *para*-fluorostyrene **1** were calculated from Lineweaver–Burk equation, as follows:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Where V is the initial rate of reaction at different substrate concentrations ([S]), K_m is the Michaelis constant, V_{max} is the maximum reaction rate.

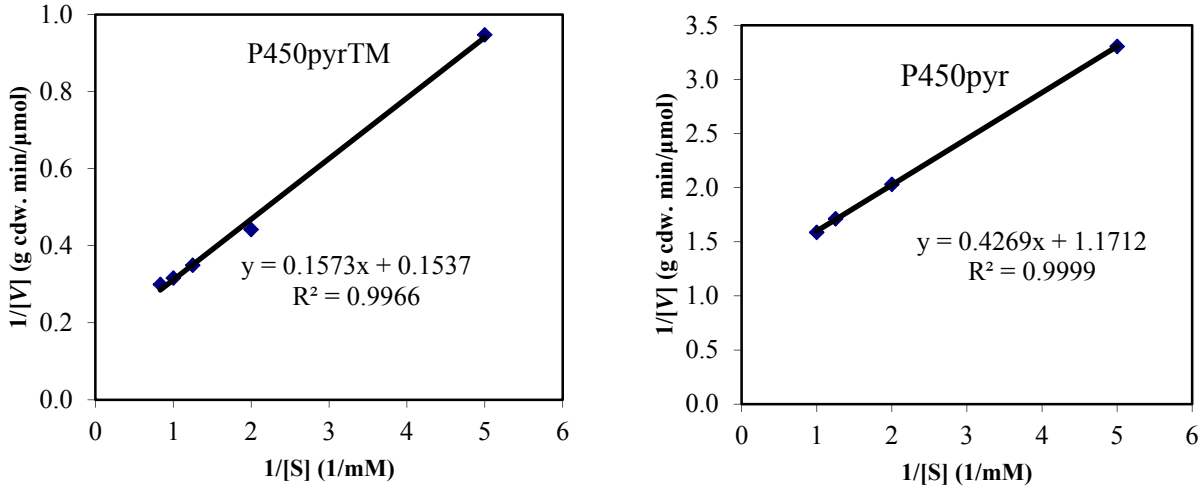


Figure S1. The Lineweaver–Burk plots for P450pyrTM and P450pyr catalyzed asymmetric epoxidation of *para*-fluorostyrene **1**.

Lineweaver–Burk plots for the P450pyrTM and P450pyr catalyzed asymmetric epoxidation of *para*-fluorostyrene **1** were obtained and shown in the Figure S1. The Michaelis constant (K_m), maximal initial

rate (V_{max}) and other related kinetic parameters for P450pyrTM and P450pyr catalyzed epoxidation of *para*-fluorostyrene **1** are presented in Table S3. V_{max} value of P450pyrTM was almost 8-fold higher than that of P450pyr. Compared with P450pyr, the *K*m value of P450pyrTM increased to 1.02 mM from 0.36 mM and implied a lower enzyme-substrate affinity. The catalytic efficiency (V_{max} /K_m) of P450pyrTM was still higher than that of P450pyr (6.38 vs 2.36).

Table S3. Comparison of apparent kinetic parameters between *E. coli* (P450pyrTM) and *E. coli* (P450pyr) catalyzed epoxidation of *para*-fluorostyrene **1**.

Enzyme	V_{max} (μmol/min/g cdw)	K_m (mM)	Catalytic efficiency (V_{max} /K_m)
P450pyrTM	6.51	1.02	6.38
P450pyr	0.85	0.36	2.36

7. Docking of *para*-substituted styrene derivatives in P450pyrTM and P450pyr

The docking of substrate to P450pyrTM and P450pyr were accomplished with Autodock Vina software.[S12] The receptor structures were prepared from the X-ray structure of P450pyr and the molecular dynamics (MD) structure of P450pyrTM.[S13] The heme was set as ferryl-oxo-heme known as CpdI complex.[S14] Substrate structures were gained from the PubChem Compound database. The catalytic active poses of substrates were screened from the docking results with the reported geometric criteria.[S15, S16] The enantioselectivity was then deduced from the substrate's binding poses related to the heme (Fig. S2).

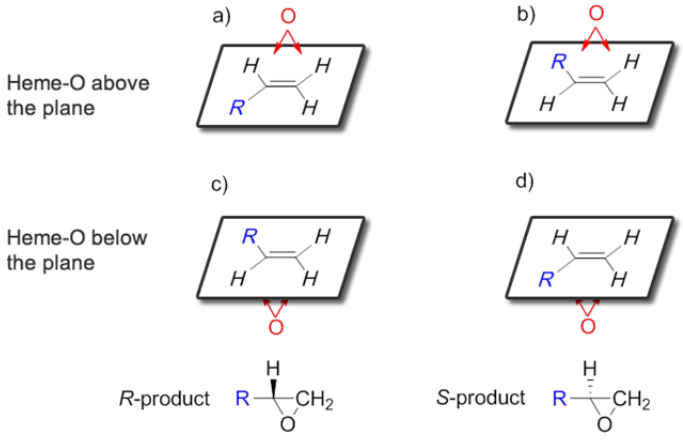


Figure S2. Geometric criteria for the predition of enantioselectivity of P450-catalyzed epoxidation.

Table S4. Prediction of the active poses of the *para*-substituted styrene derivatives in P450pyrTM and P450pyr with molecular docking

Entry	Substrate	Enzyme	Product	Prediction	Experimental
1	**1**	P450pyrTM	**2**	*R*	*R*
2	**3**	P450pyrTM	**4**	*R*	*R*
3	**5**	P450pyrTM	**6**	*R*	*R*
4	**7**	P450pyrTM	**8**	*R*	*R*
5	**9**	P450pyrTM	**10**	- [a]	*R*
6	**11**	P450pyrTM	**12**	- [a]	*R*
7	**1**	P450pyr	**2**	*S*	*S*
8	**3**	P450pyr	**4**	*S*	*S*
9	**5**	P450pyr	**6**	*R/S* [b]	*R*
10	**7**	P450pyr	**8**	- [a]	*R*
11	**9**	P450pyr	**9**	- [a]	*R*
12	**11**	P450pyr	**10**	- [a]	*R*

[a] No active pose was obtained.
[b] Active poses leading to both (*R*)- and (*S*)- product were recorded.

As shown in the Table S4, active poses were resolved in 7 out of 12 docking cases in P450pyrTM and P450pyr. The obtained active poses successfully predicted the enantioselectivity of both enzymes. Based on the obtained substrate docking poses, the molecular basis of the distinct enantioselectivity of P450pyrTM and P450pyr was further investigated.

The modeling failed to predict the active poses for those substrates with bulky side chain (**9** and **11**) due to the tight fitting of the substrates in the enzymes. As shown in the Fig. S3, the binding of the substrates was contributed by the hydrophobic interaction between substrates and a group of non-polar amino acids (Ile102, Val254 (α-helix), Leu302, Phe403 and Val404) in both enzymes. In P450pyr, the substrates posed within the narrow binding site giving (*S*)-products for the substrates with small side chains (**1** and **3**). In the P450pyrTM, the mutations A77S and I83H gave 3 additional hydrogen bonds (H-bonds) compared with P450pyr. [S11] The H-bonds increased the rigidity of the 'big loop' (Ser74-Asp105) and pulled the C-terminal of the loop 1Å inwards the pocket (Fig. S3). This led to the narrowed space between Ile102 and Val254 (from 6.6Å to 5.7Å), which casted steric clash to the substrates and turned over the C-C double bond in a vertical pose to the heme plane.

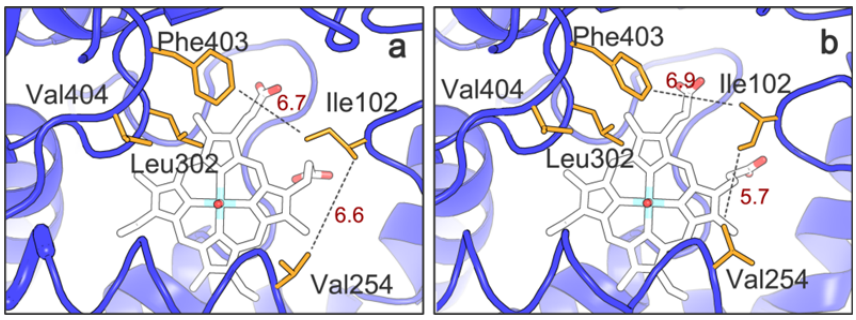


Figure S3. (a) Binding pocket of P450pyr; (b) binding pocket of P450pyrTM

As shown in the Fig. 1 (main text) and Fig. S4, the active poses of **1**, **3** and **5** in P450pyrTM shared high similarity with root-mean-square deviation (RMSD) below 0.2 Å, giving exclusively (*R*)-products. Different substrate poses were found with **7** (Fig. S4c) due to the unfavored clash between its bulky side chain and the protein. The C-C double bond oriented in parallel to the heme plane and located behind the heme-bound oxygen atom, resulting in the product (*R*)-**8**. This shed a light on the molecular basis of the enantioselectivity of the mutated enzyme and would help further understanding of the protein engineering on P450 enzymes.

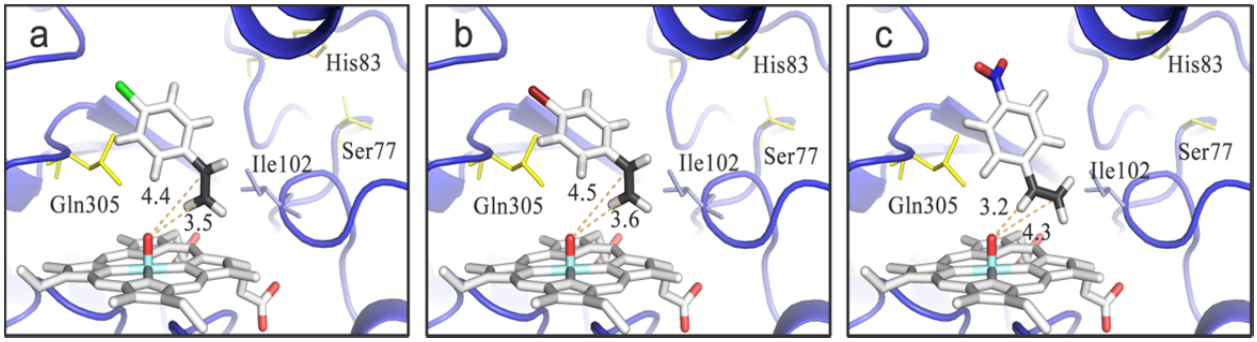


Figure S4. (a) Active pose of 4-chlorostyrene **3** in P450pyrTM. (b) Active pose of 4-bromostyrene **5** in P450pyrTM. (c) Active pose of 4-nitrostyrene **7** in P450pyrTM.

The docking of the substrate *ortho*-fluorostyrene **19** was successful in both P450pyr and P450pyrTM, showing the correct stereometric conformation of the corresponding products (Figure S5).

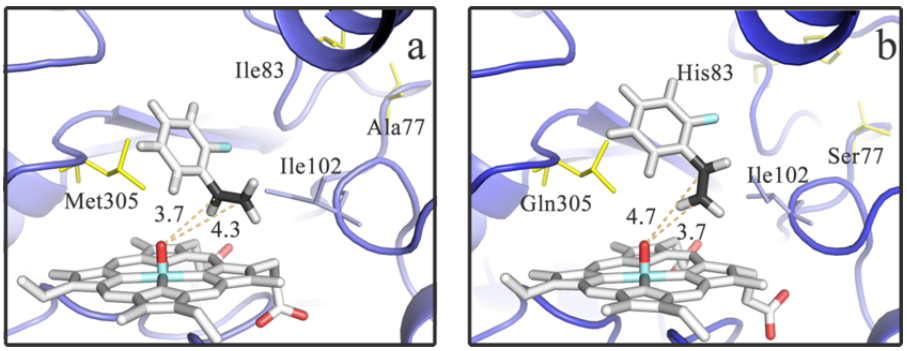


Figure S5. a) Catalytically active pose of **19** in P450pyr giving (*S*)-**20**; b) Catalytically active pose of **19** in P450pyrTM resulting in (*R*)-**20**.

8. HPLC chromatograms and ^{1}H NMR spectra

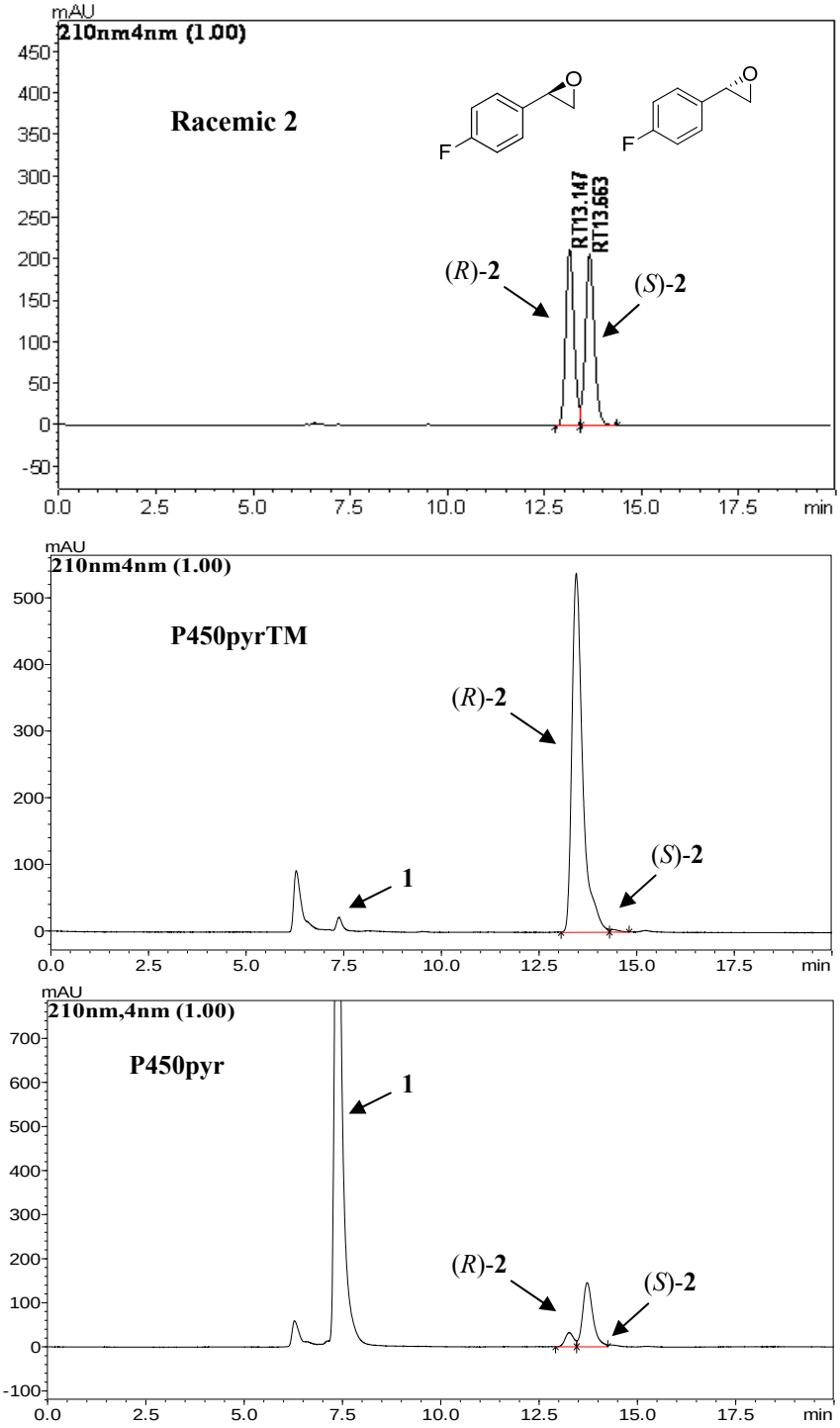
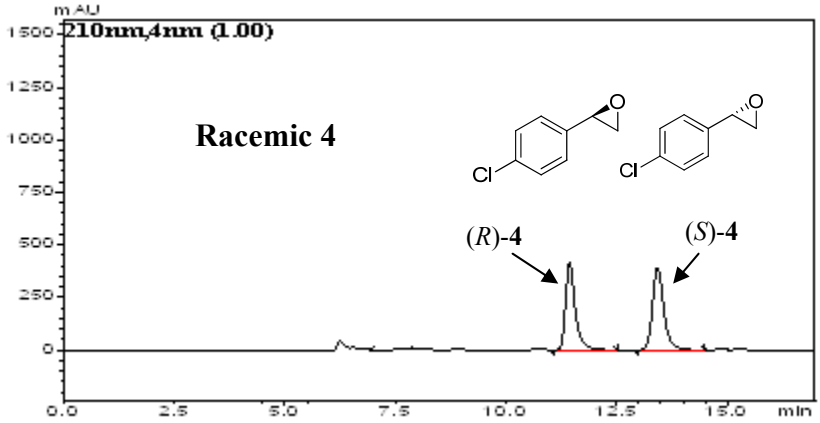
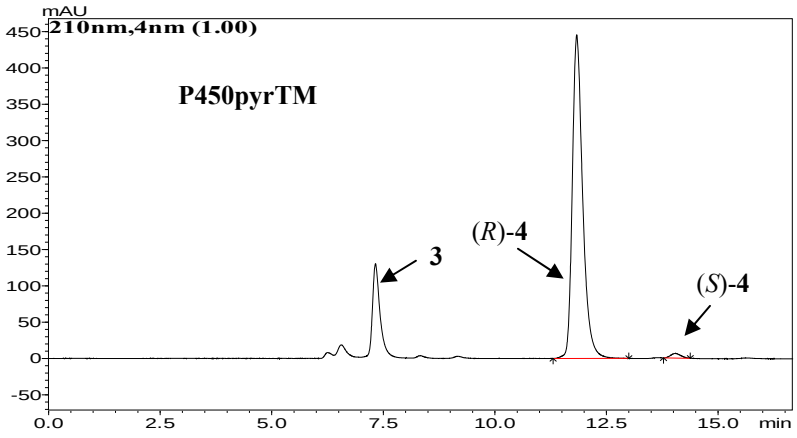


Figure S6. Chiral HPLC chromatograms for the analysis of 2-(4-fluorophenyl)oxirane **2**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.





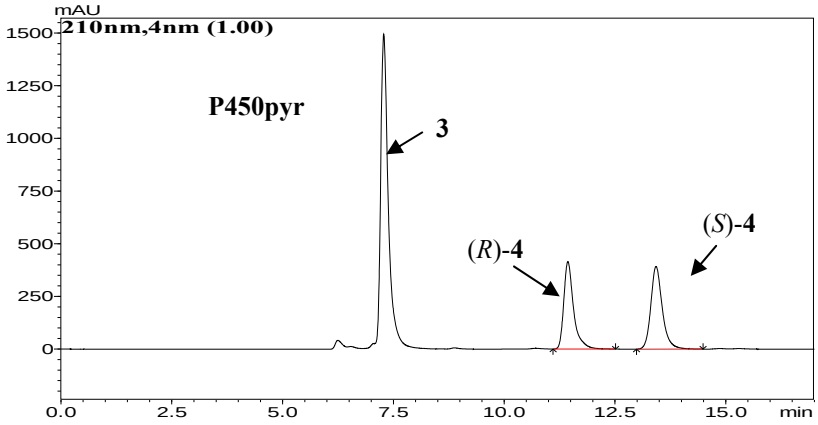


Figure S7. Chiral HPLC chromatograms for the analysis of 2-(4-chlorophenyl)oxirane **4**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

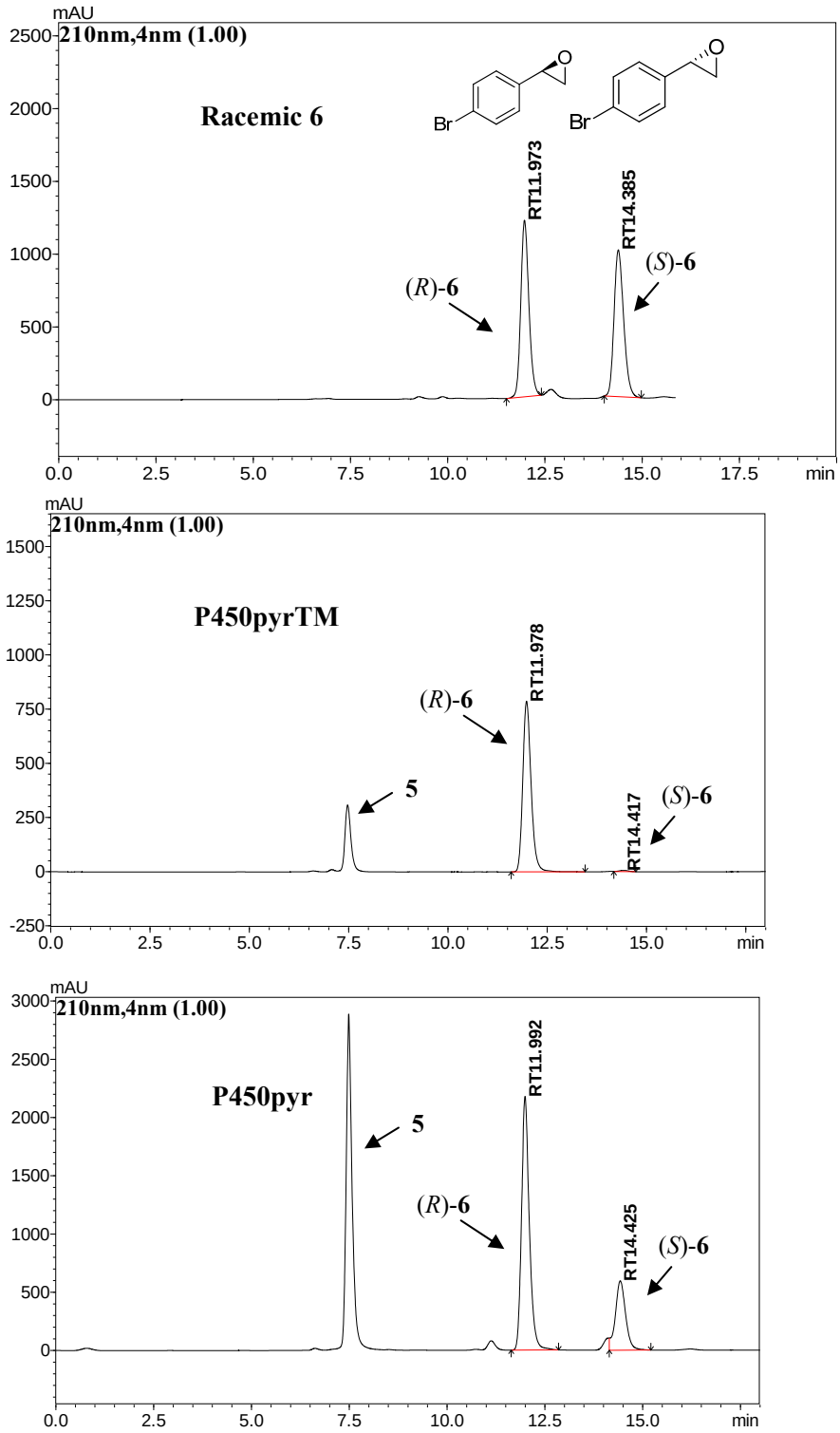
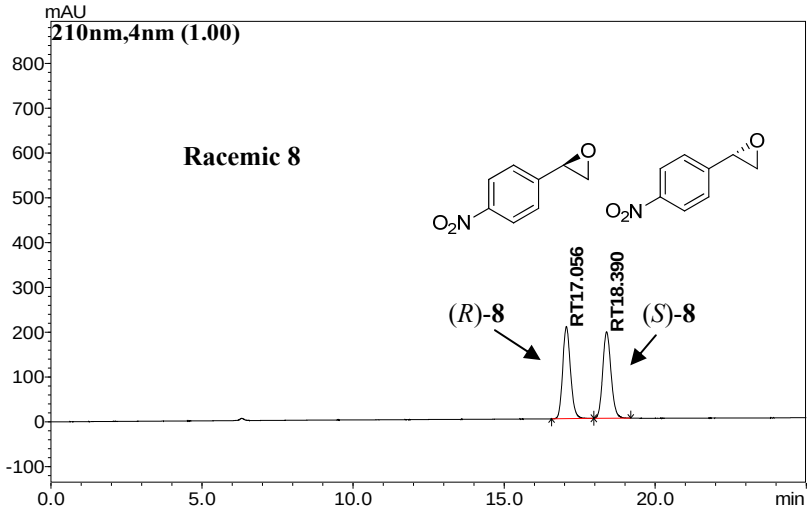
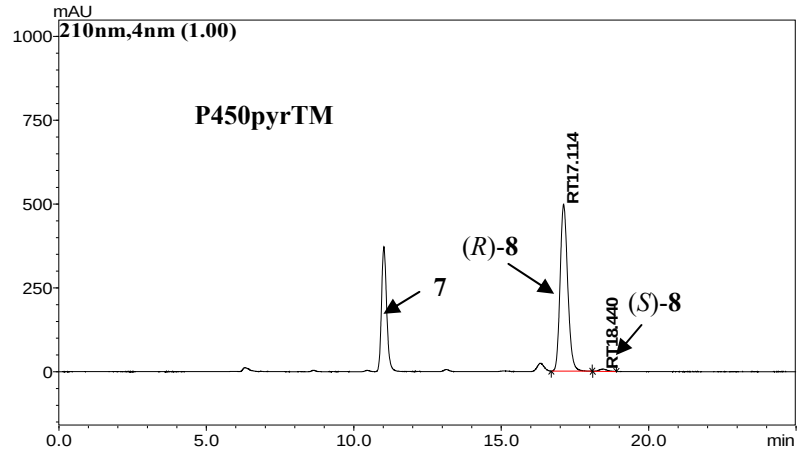


Figure S8. Chiral HPLC chromatograms for the analysis of 2-(4-bromophenyl)oxirane **6**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.





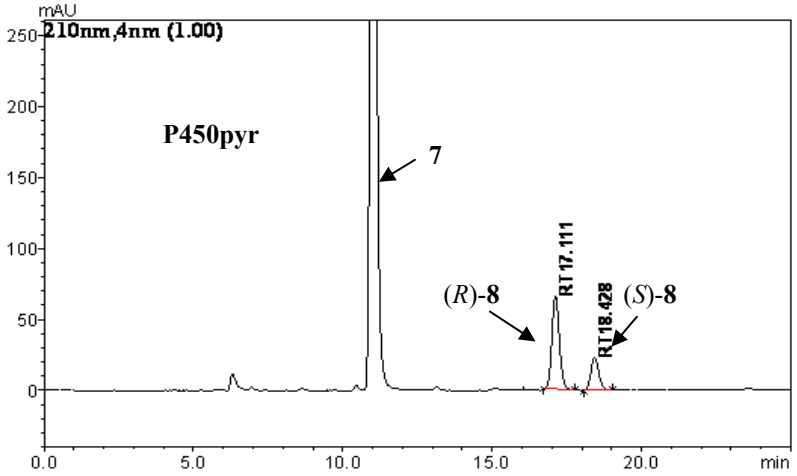


Figure S9. Chiral HPLC chromatograms for the analysis of 2-(4-nitrophenyl)oxirane **8**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

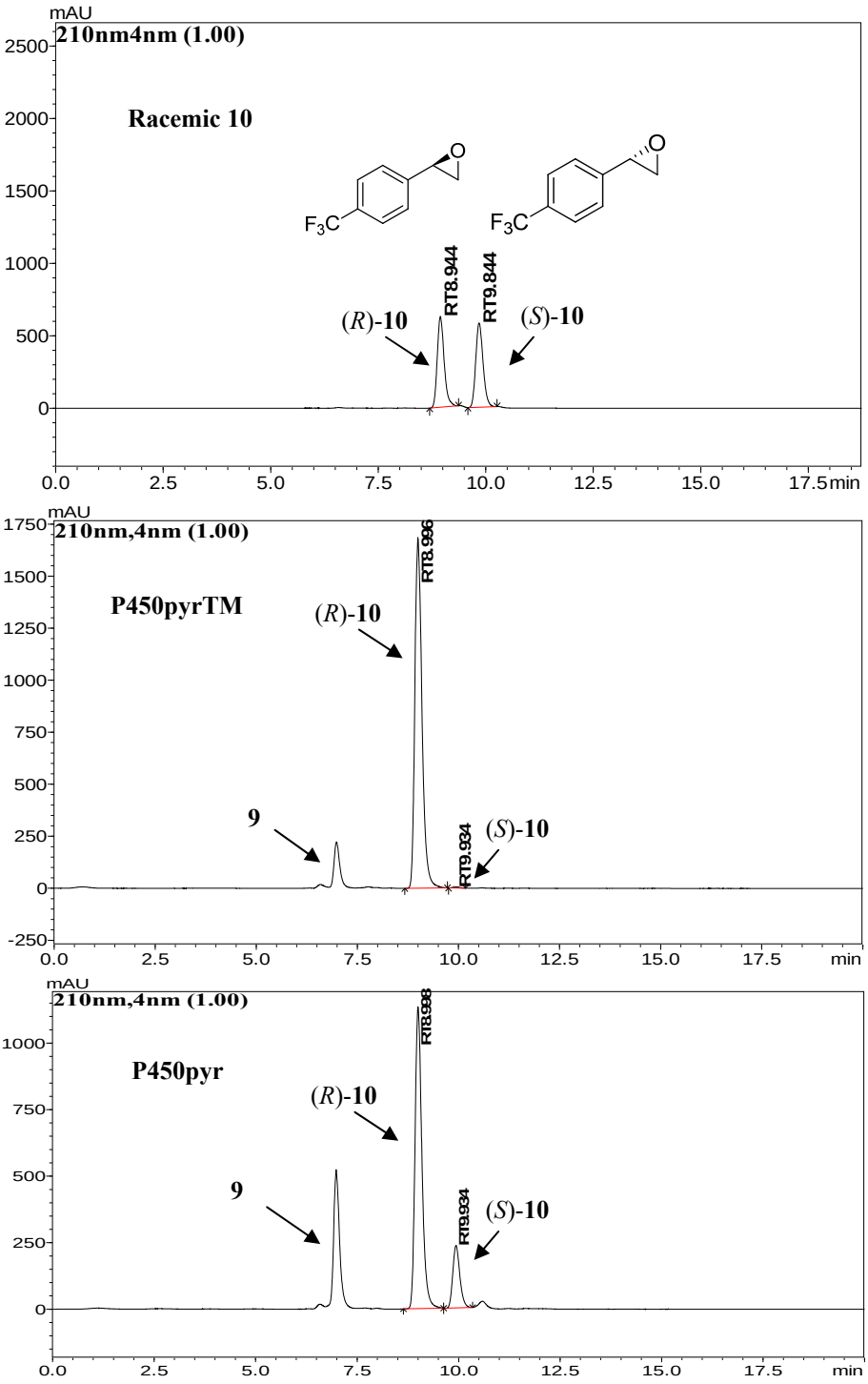


Figure S10. Chiral HPLC chromatograms for the analysis of 2-(4-trifluorophenyl)oxirane **10**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

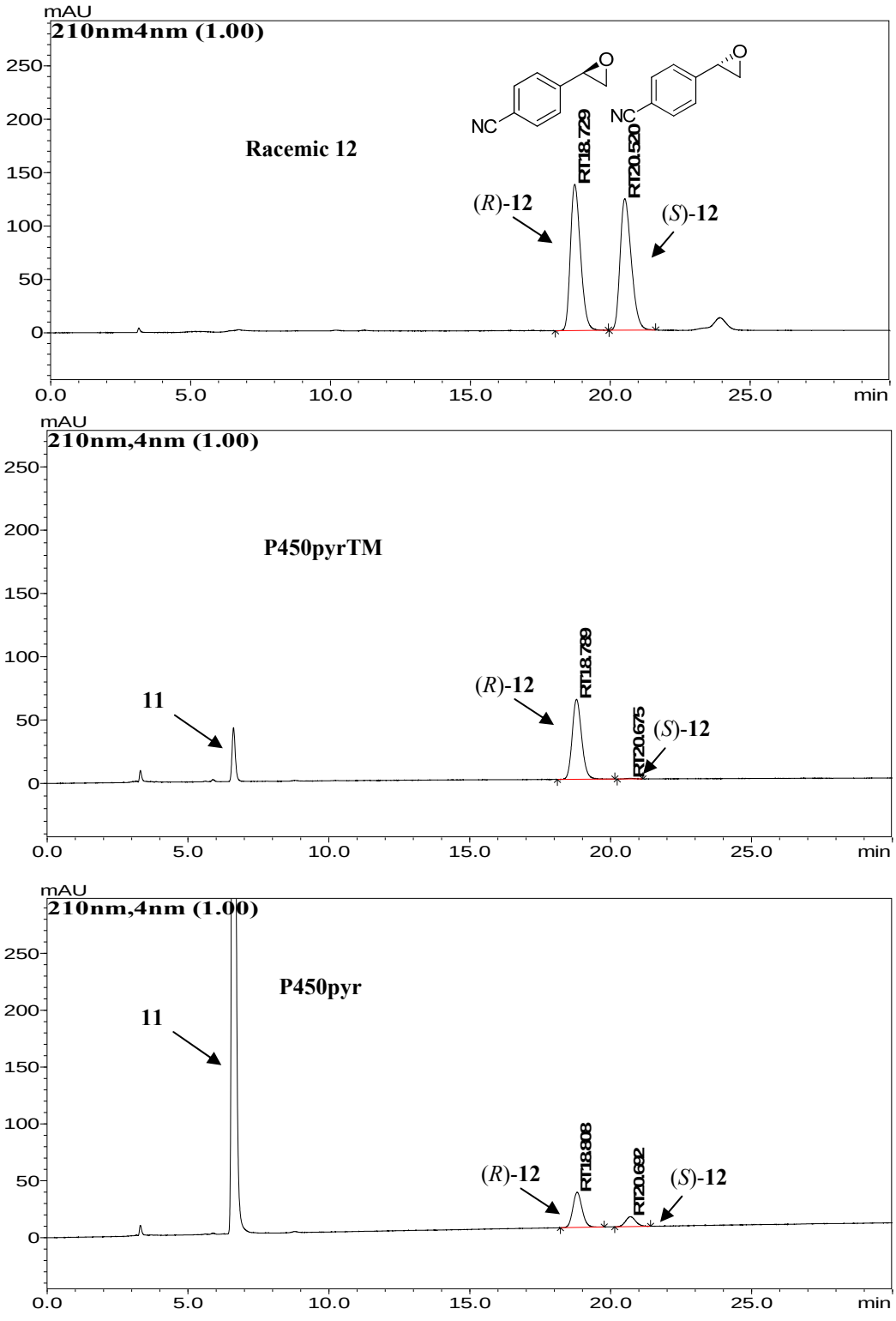
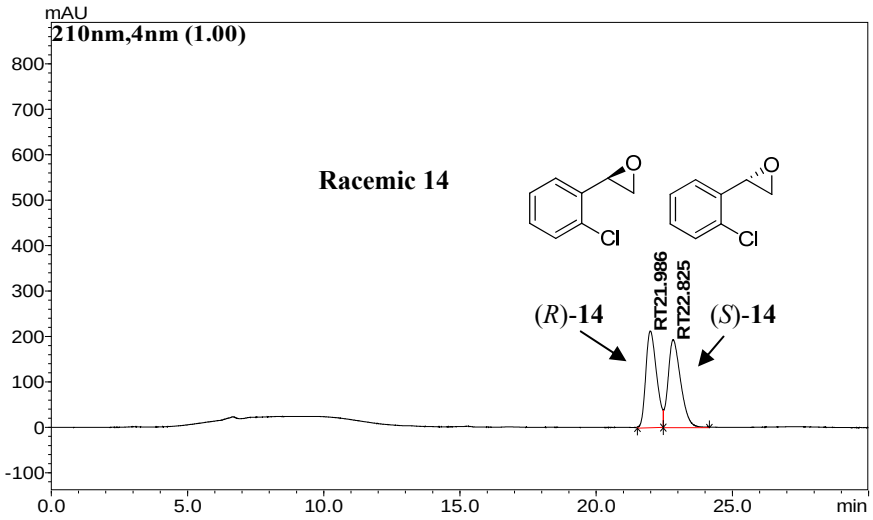
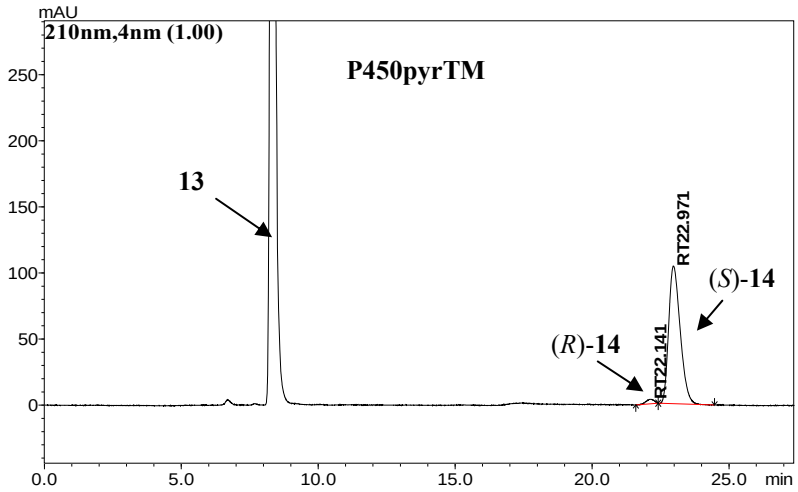


Figure S11. Chiral HPLC chromatograms for the analysis of 4-(oxiran-2-yl)benzonitrile **12**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.





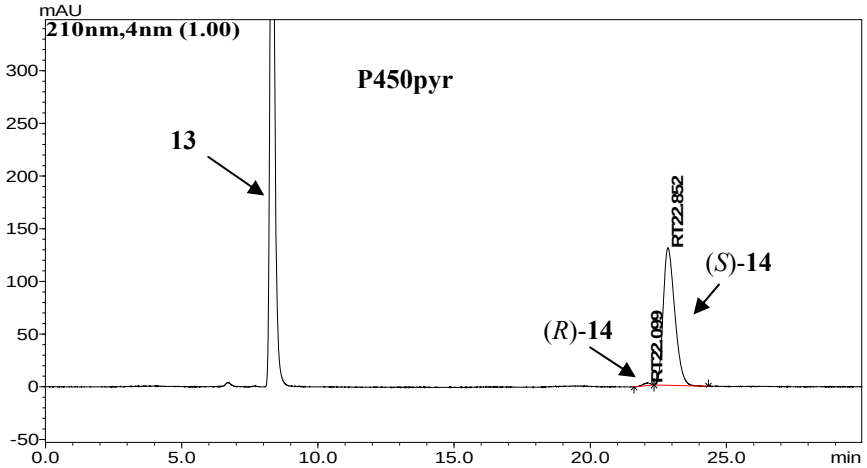


Figure S12. Chiral HPLC chromatograms for the analysis of 2-(2-chlorophenyl)oxirane **14**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

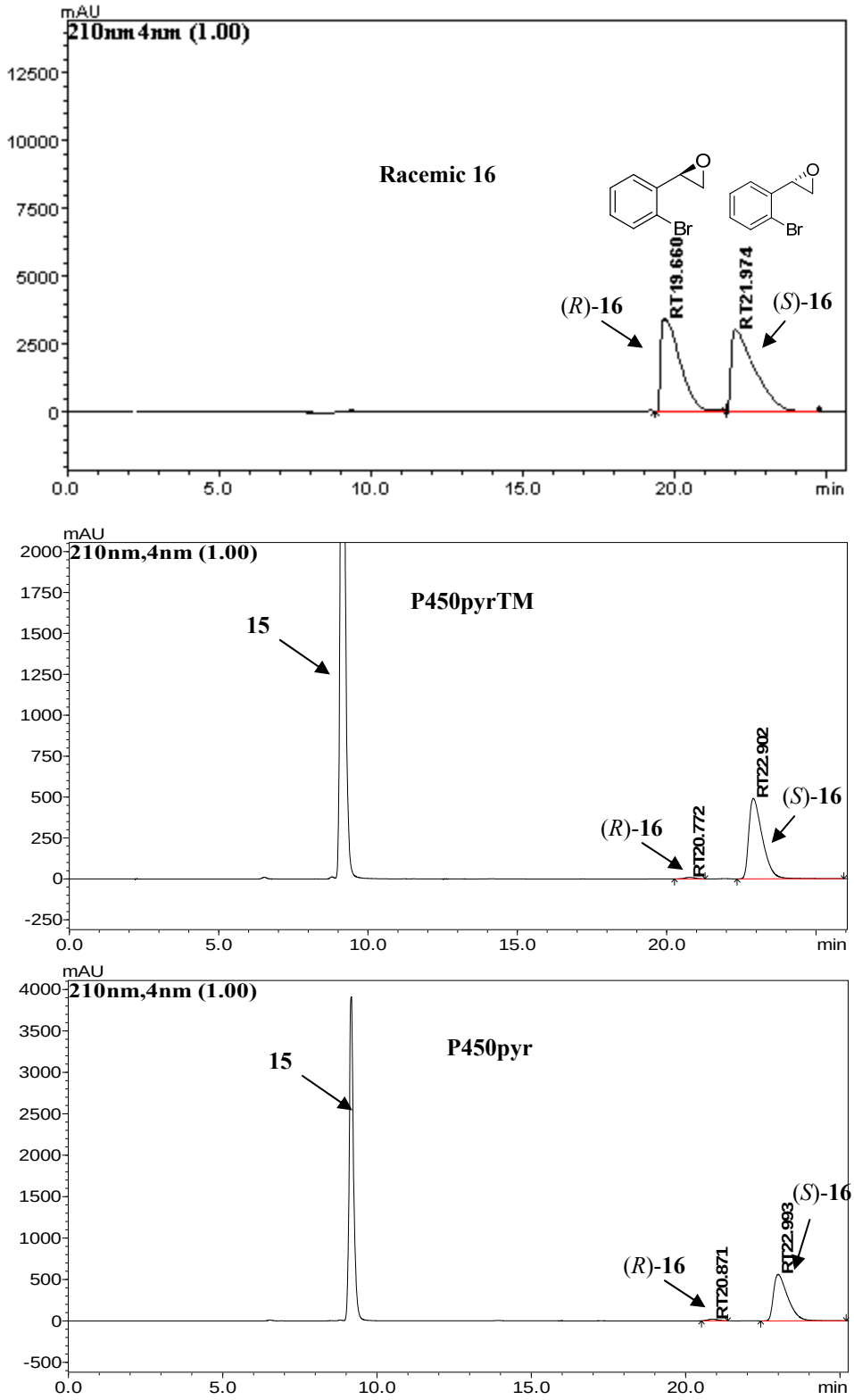


Figure S13. Chiral HPLC chromatograms for the analysis of 2-(2-bromophenyl)oxirane **16**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

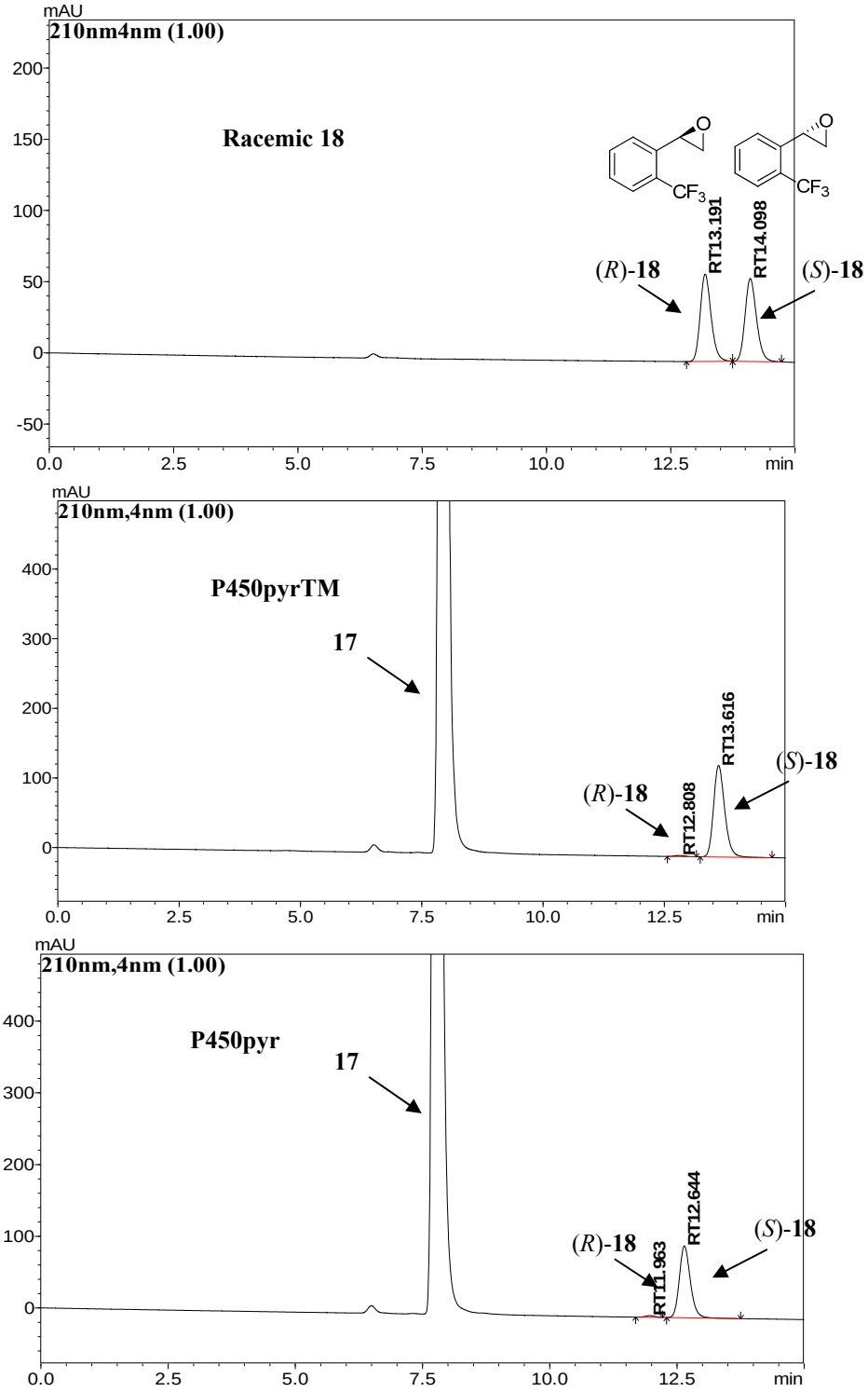
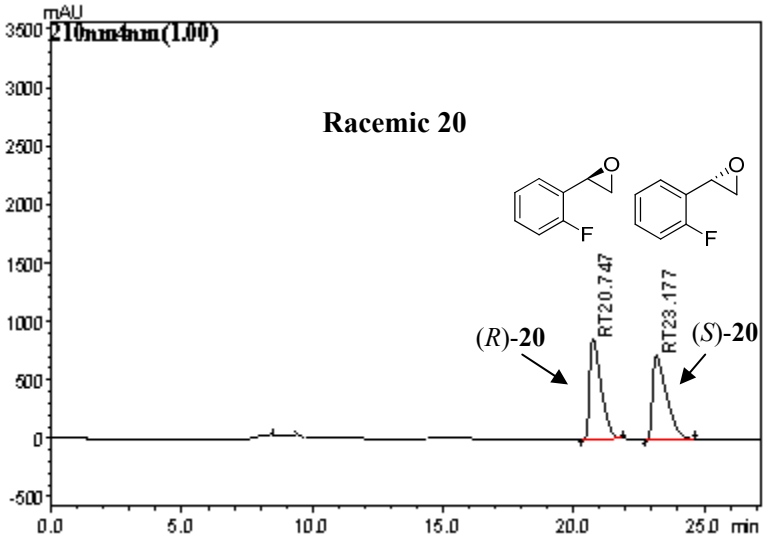
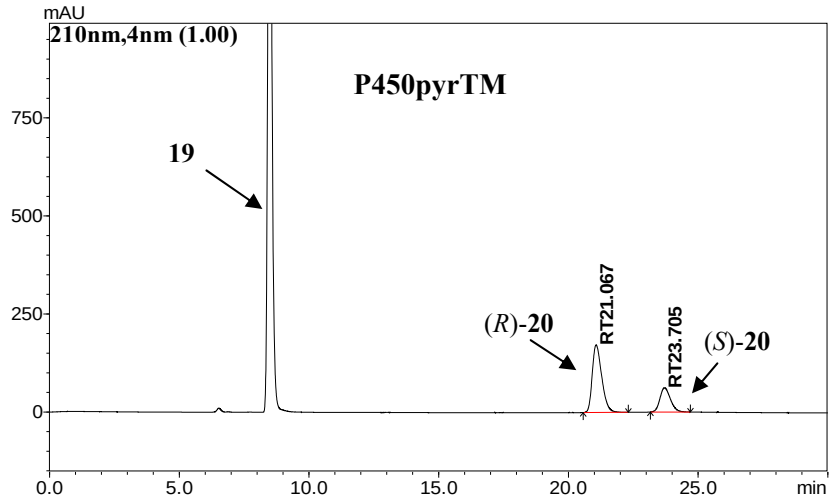


Figure S14. Chiral HPLC chromatograms for the analysis of 2-(2-trifluorophenyl)oxirane **18**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.





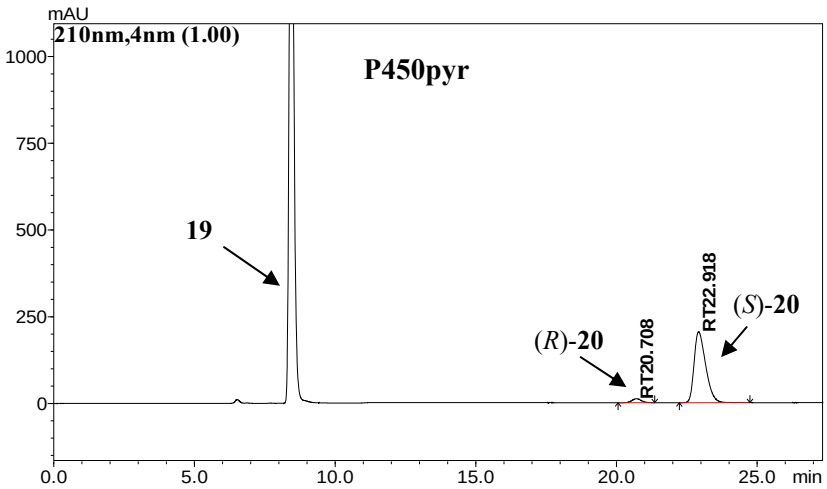


Figure S15. Chiral HPLC chromatograms for the analysis of 2-(2-fluorophenyl)oxirane **20**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

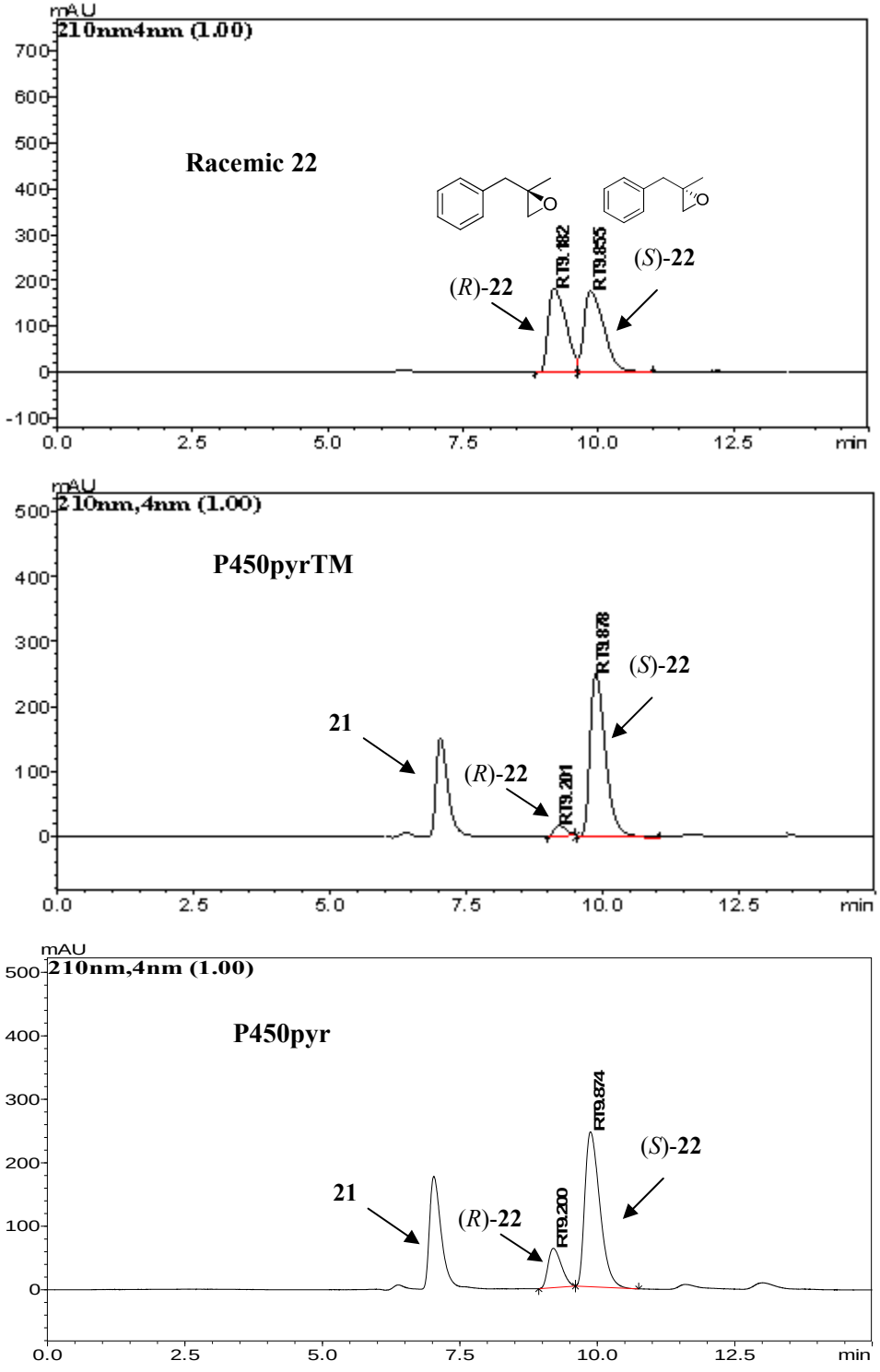


Figure S16. Chiral HPLC chromatograms for the analysis of 2-benzyl-2-methyloxirane **22**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

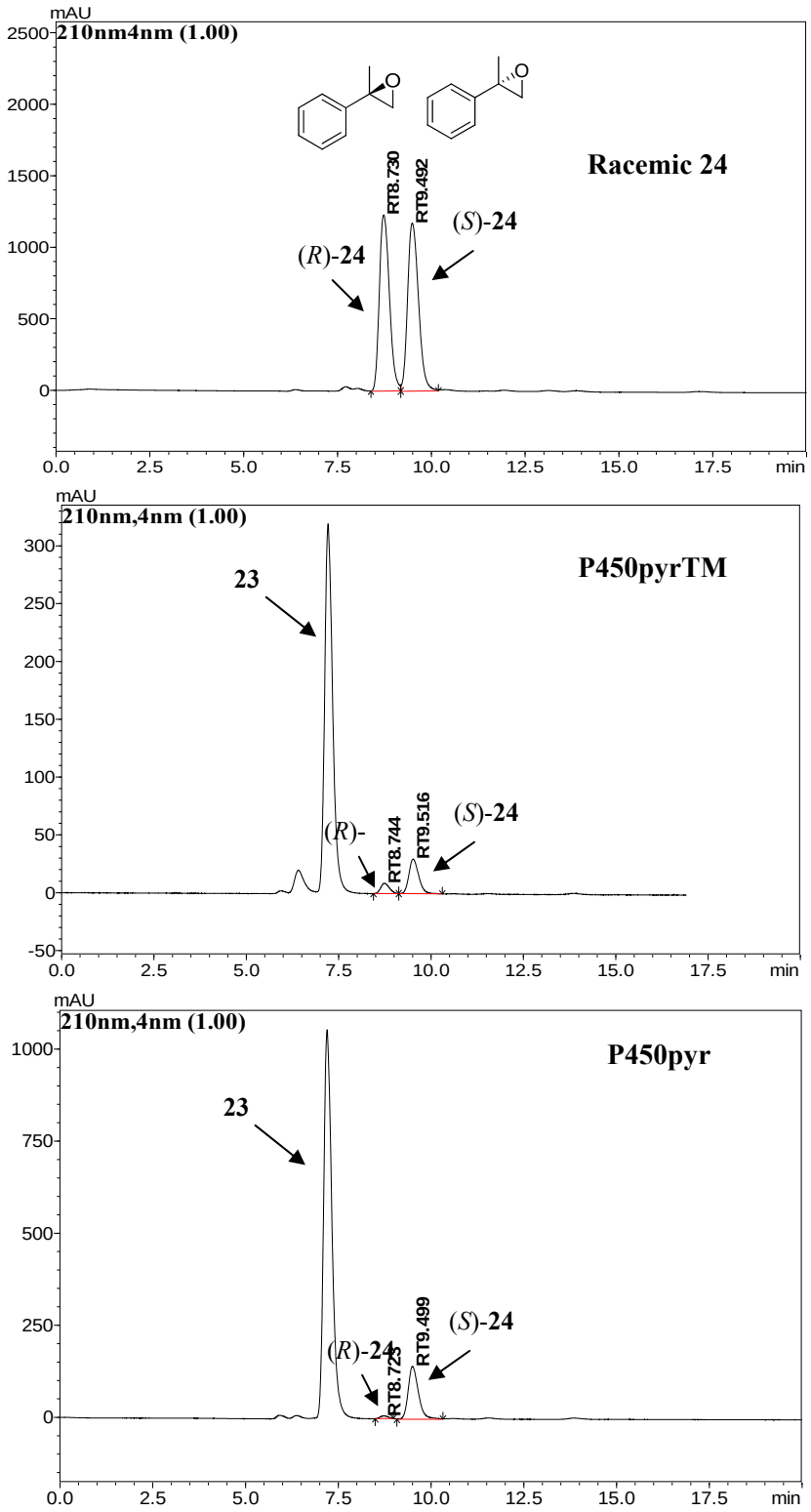
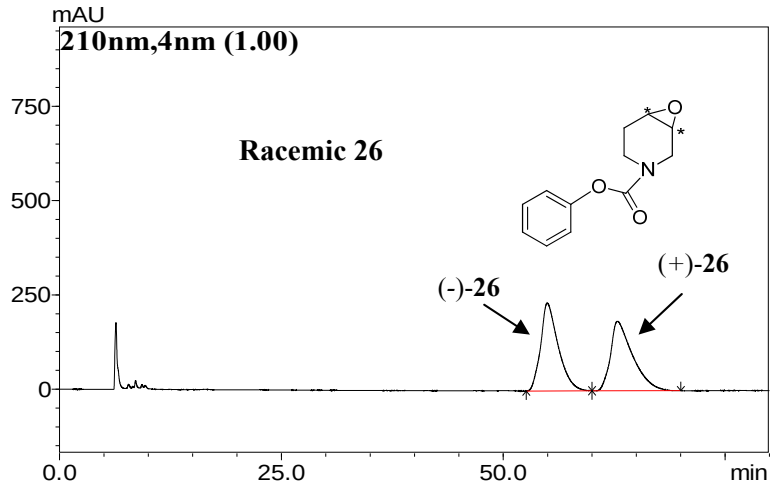
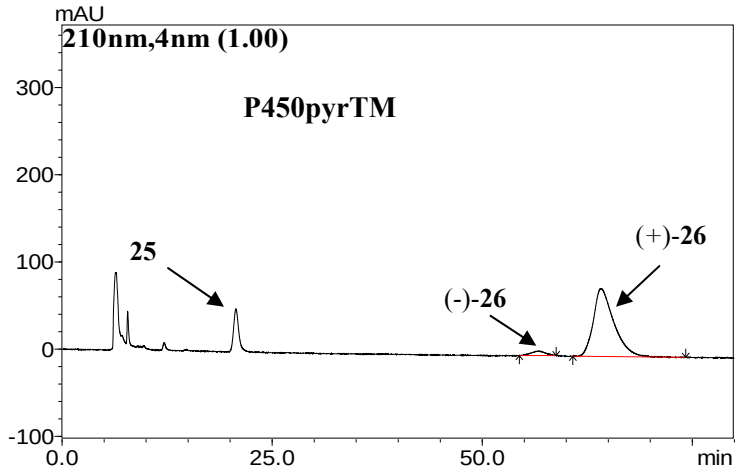


Figure S17. Chiral HPLC chromatograms for the analysis of 2-phenylpropylene oxide **24**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.





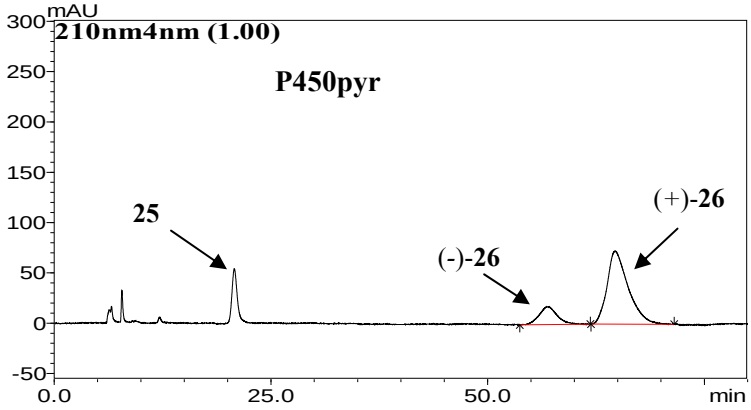
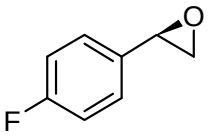


Figure S18. Chiral HPLC chromatograms for the analysis of *N*-phenoxycarbonyl-3, 4-epoxypiperidine **26**: Racemic, P450pyrTM-catalyzed epoxidation product, and P450pyr-catalyzed epoxidation product.

(*R*)-2-(4-fluorophenyl)oxirane (*R*)-**2:**



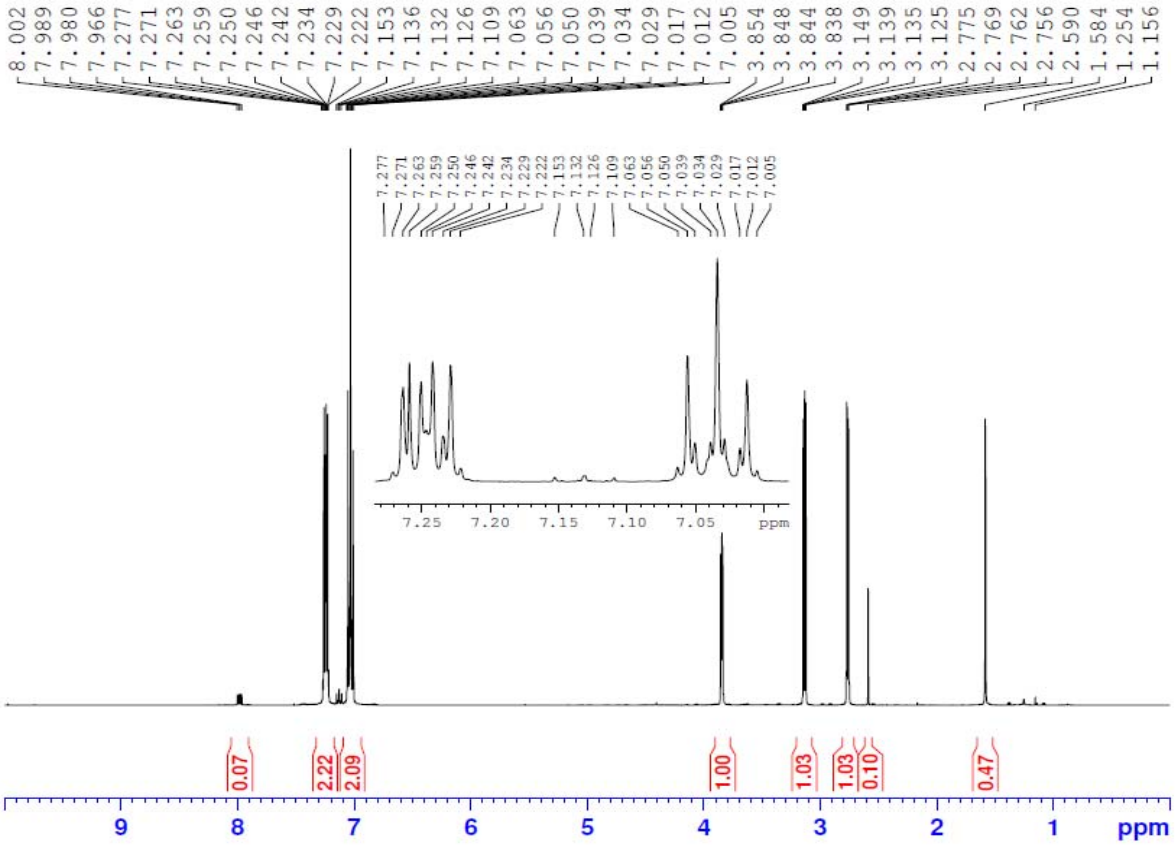
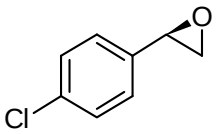


Figure S19. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **(***R***)-2-(4-fluorophenyl)oxirane 2** prepared by asymmetric epoxidation of 4-fluorostyrene **1** with the resting cells of *E. coli* (P450pyrTM).

(*R*)- 2-(4-chlorophenyl)oxirane (*R*)**-4:**



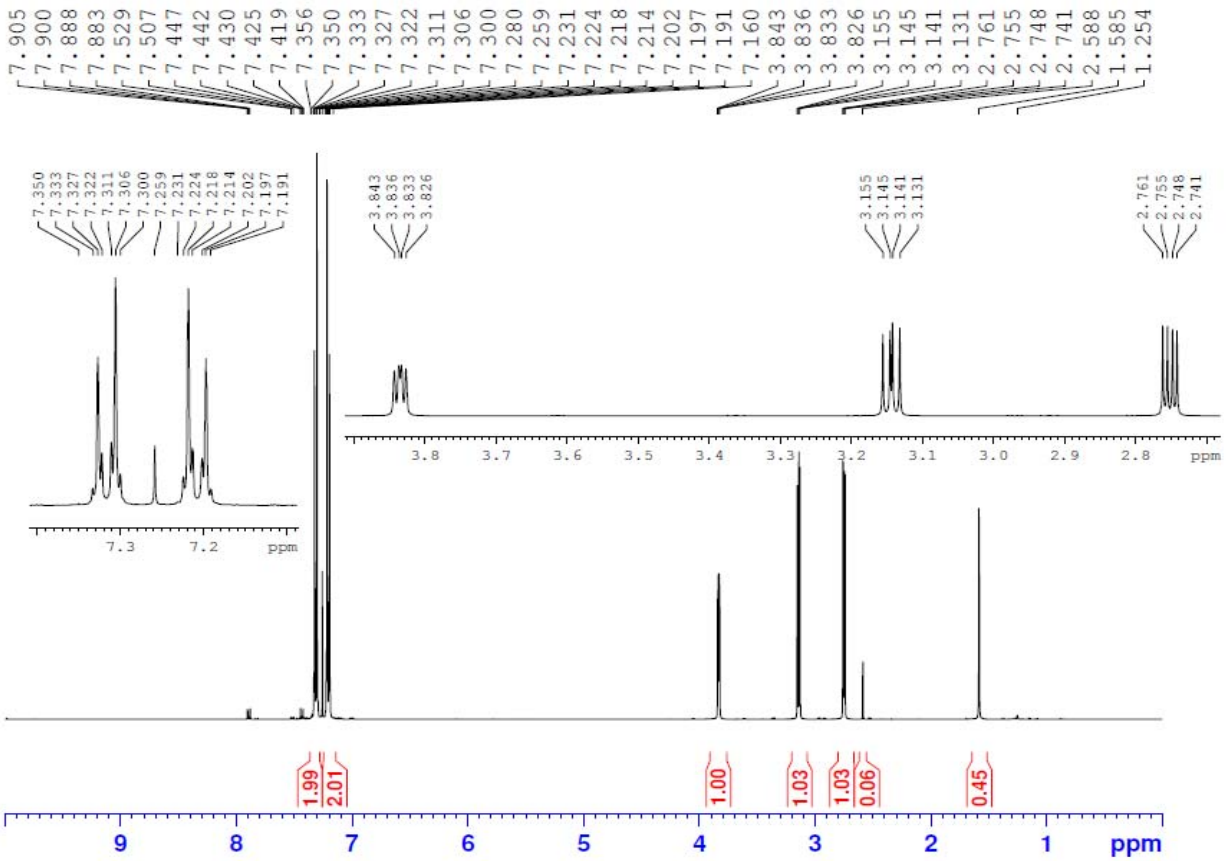
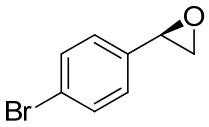


Figure S20. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **(***R***)**-2-(4-chlorophenyl)oxirane **4** prepared by asymmetric epoxidation of 4-chlorostyrene **3** with the resting cells of *E. coli* (P450pyrTM).

(*R*)- 2-(4-bromophenyl)oxirane (*R*)**-6:**



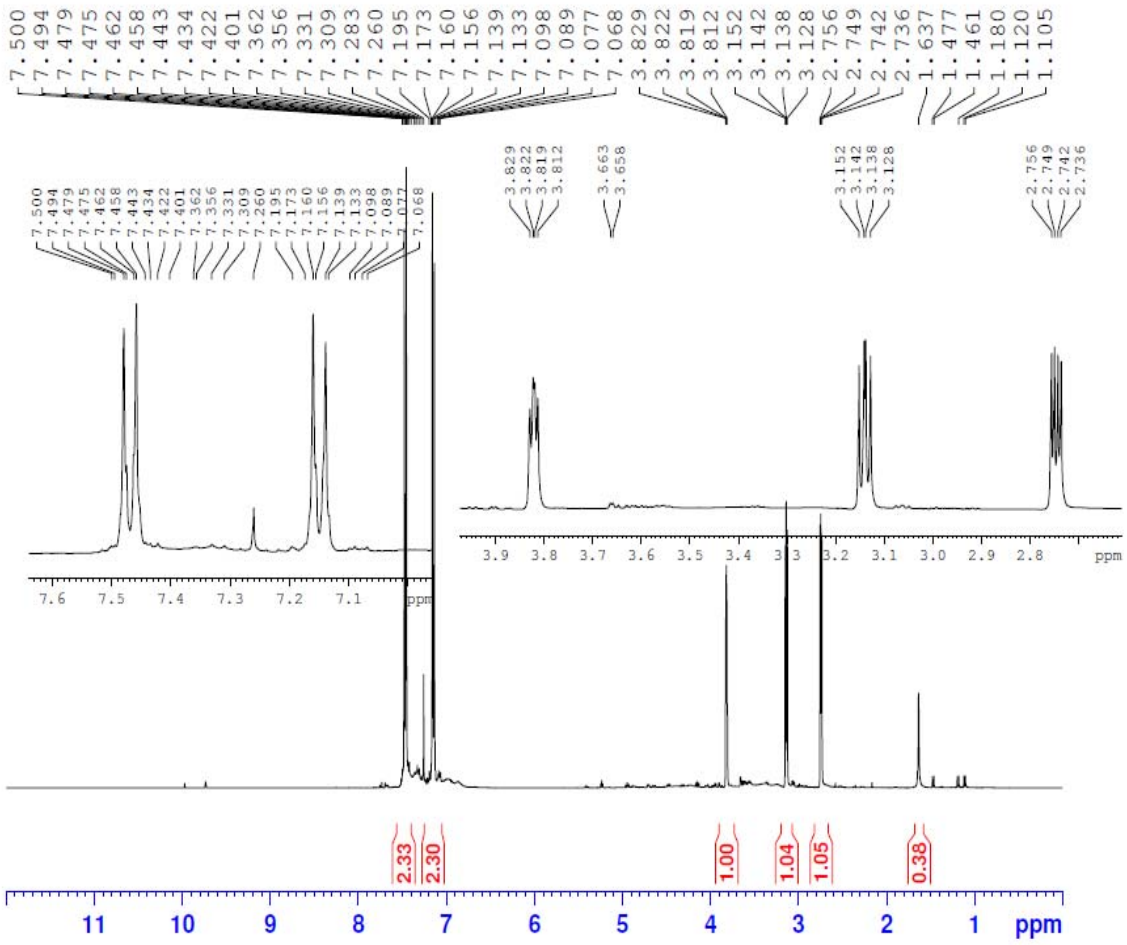
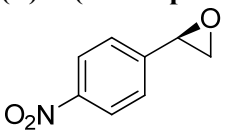


Figure S21. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **(***R***)**-2-(4-bromophenyl)oxirane **6** prepared by asymmetric epoxidation of 4-bromostyrene **5** with the resting cells of *E. coli* (P450pyrTM).

(*R*)-2-(4-nitrophenyl)oxirane (*R*)**-8:**



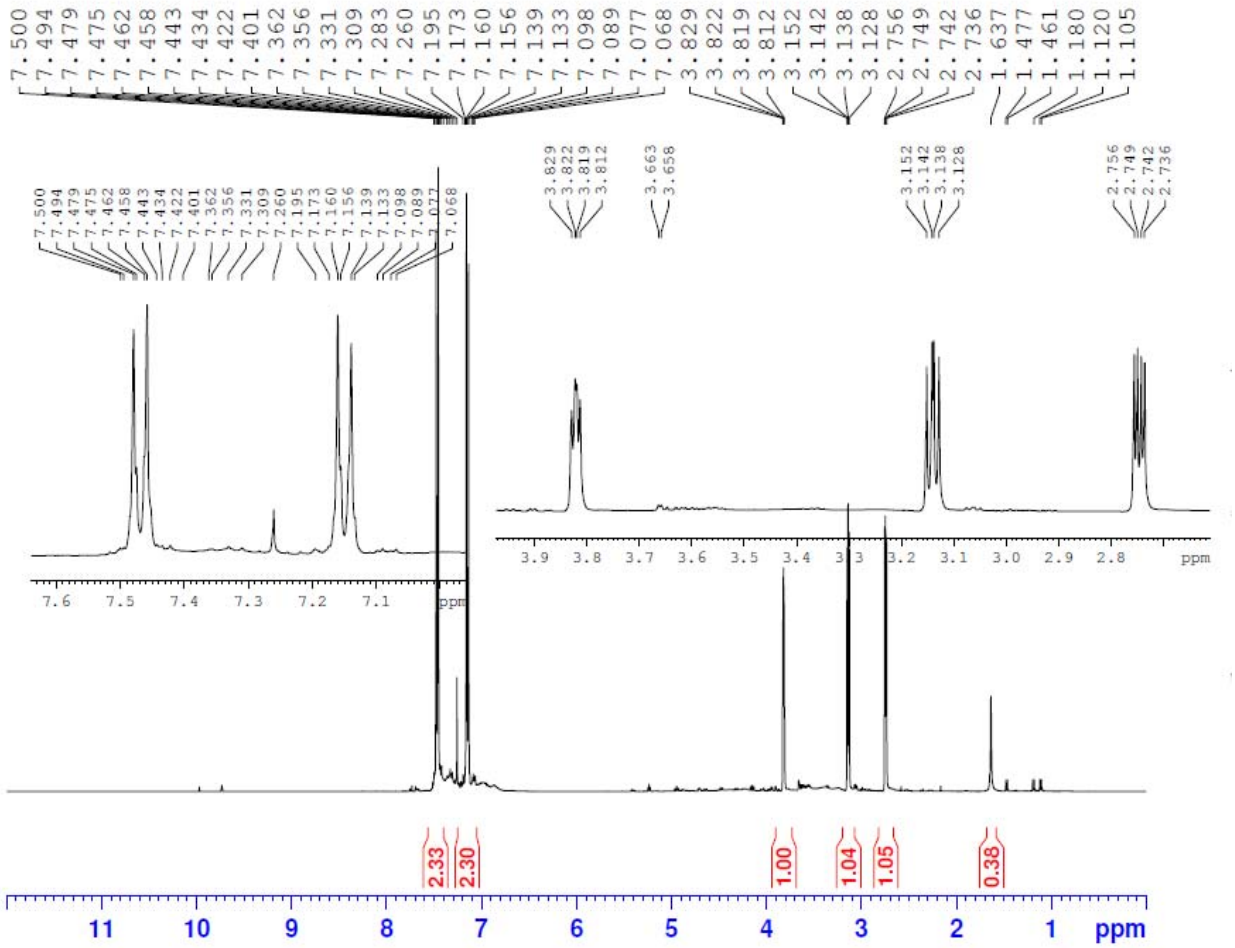
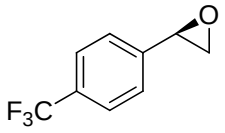


Figure S22. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **(***R*)-2-(4-nitrophenyl)oxirane **8** prepared by asymmetric epoxidation of 4-nitrostyrene **7** with the resting cells of *E. coli* (P450pyrTM).

(*R*)- 2-(4-trifluorophenyl)oxirane (*R*)**-10:**



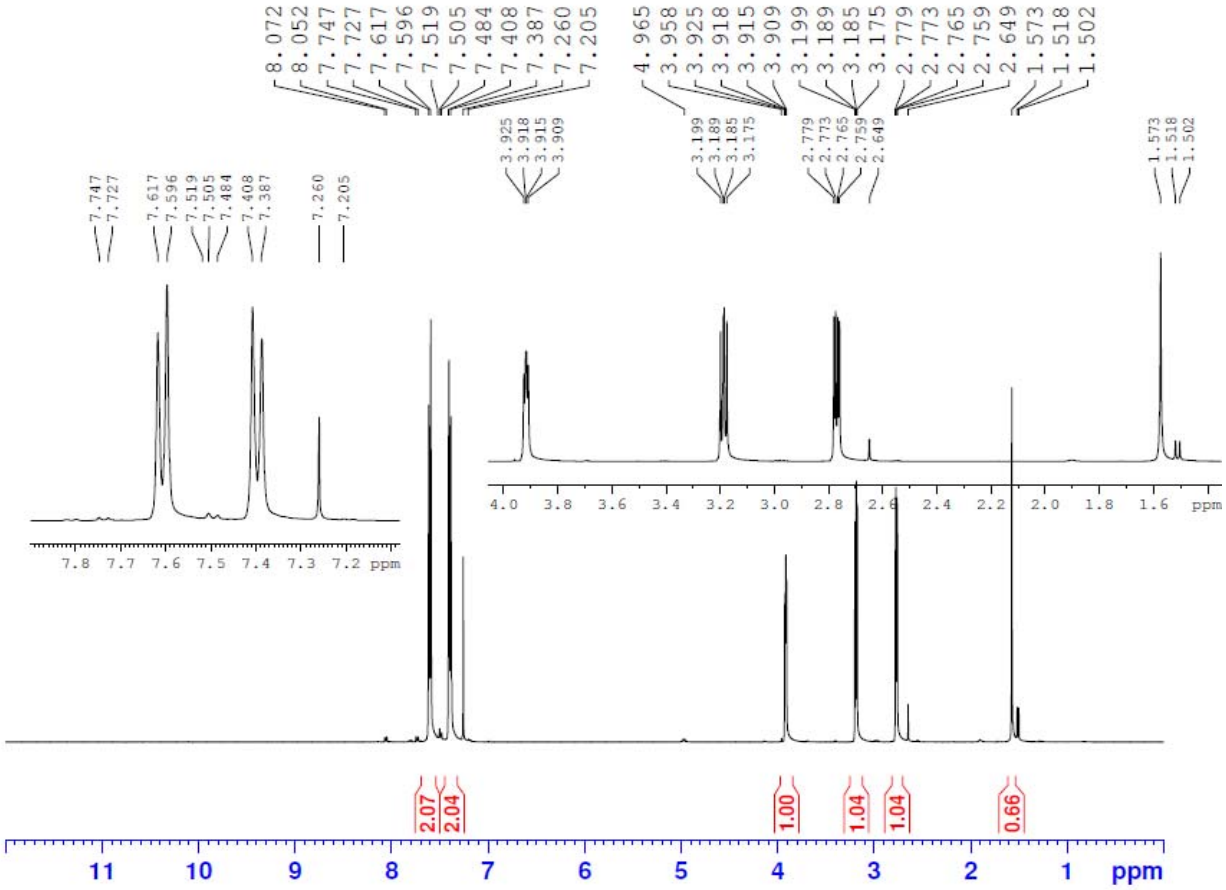
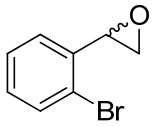


Figure S23. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **(***R***)**-2-(4-trifluorophenyl)oxirane **10** prepared by asymmetric epoxidation of 4-trifluorostyrene **9** with the resting cells of *E. coli* (P450pyrTM).

2-(2-bromophenyl)oxirane 16:



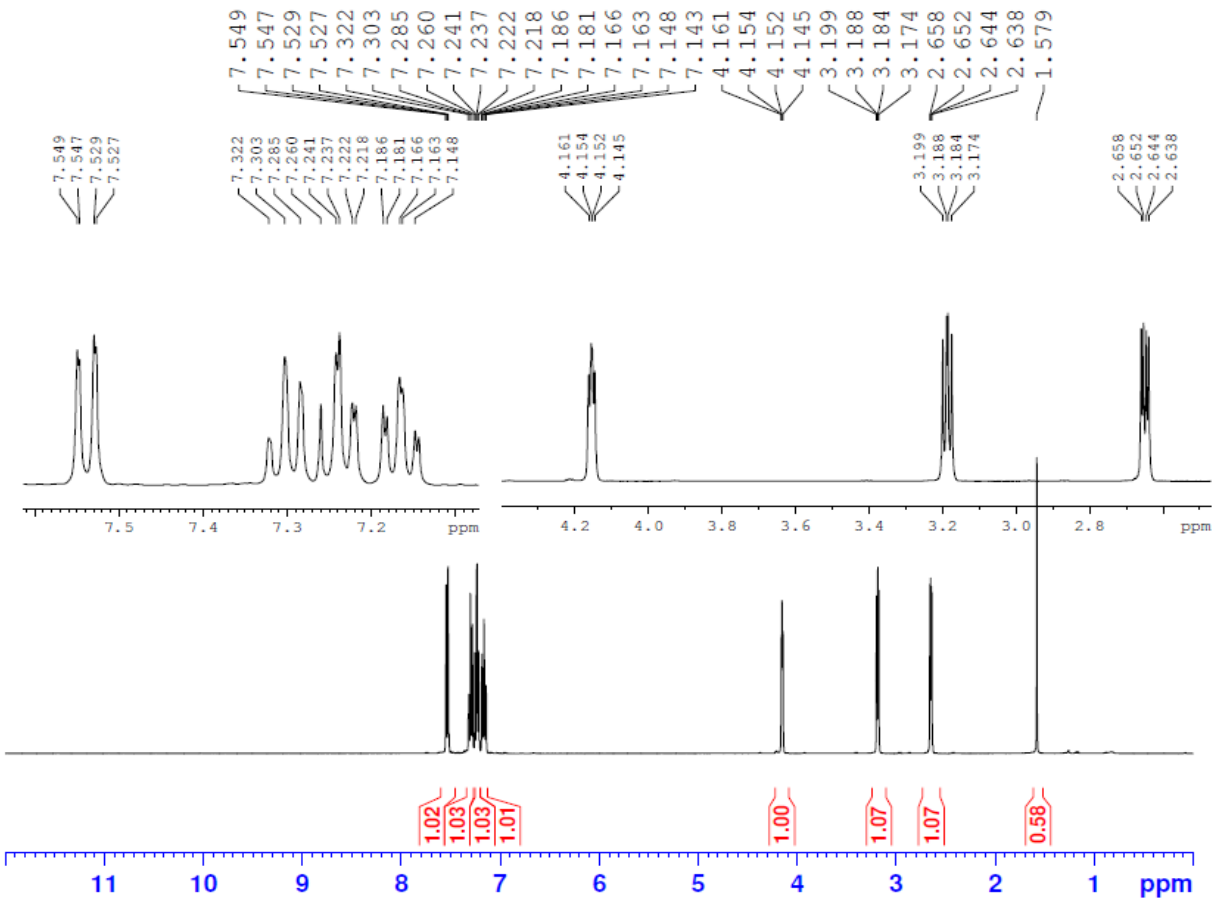
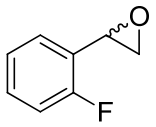


Figure S24. ^{1}H NMR (400 MHz, $CDCl_3$) chromatogram of racemic-2-(2-bromophenyl)oxirane **16** obtained from oxidation of 2-bromostyrene **15** with *m*-CPBA.

2-(2-fluorophenyl)oxirane 20:



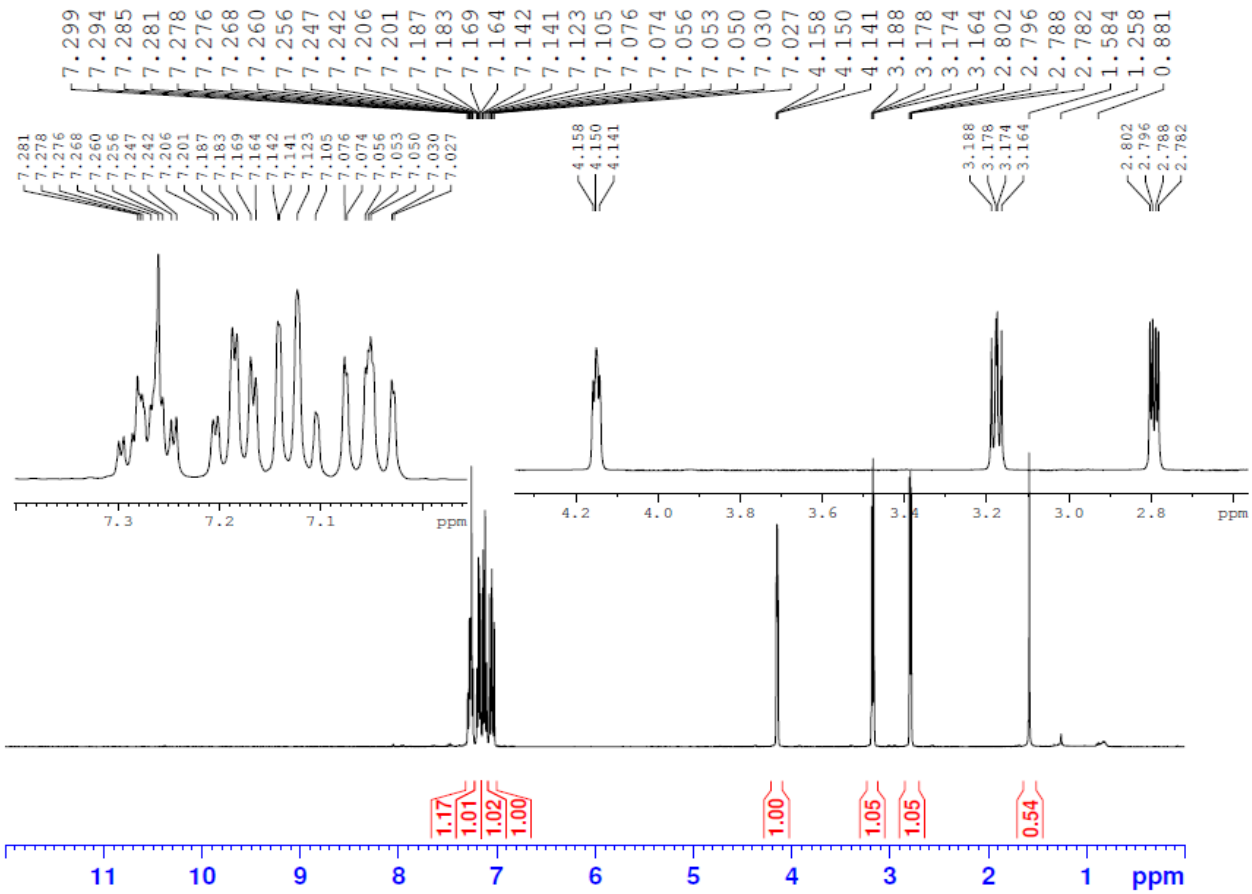
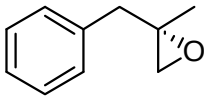


Figure S25. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of racemic-2-(2-fluorophenyl)oxirane **20** obtained from oxidation of 2-fluorostyrene **19** with *m*-CPBA.

(*S*)-2-methyl-3-phenylpropene epoxide (*S*)-22:



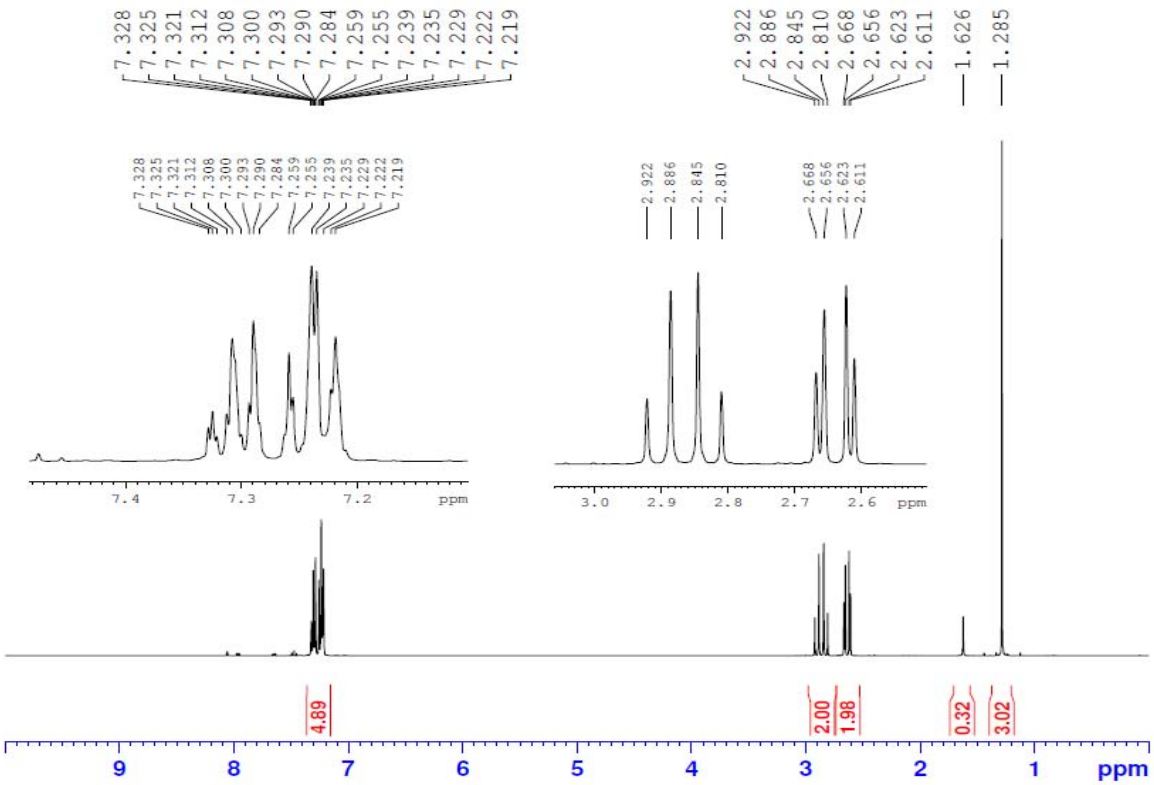
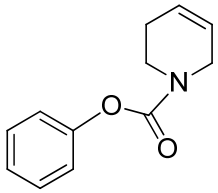


Figure S26. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of (*R*)-2-benzyl-2-methyloxirane **22** prepared by asymmetric epoxidation of 2-methyl-3-phenyl-1-propene **21** with the resting cells of *E. coli* (P450pyrTM).

***N*-phenoxycarbonyl-1, 2, 5, 6-tetrahydropyridine 25**:



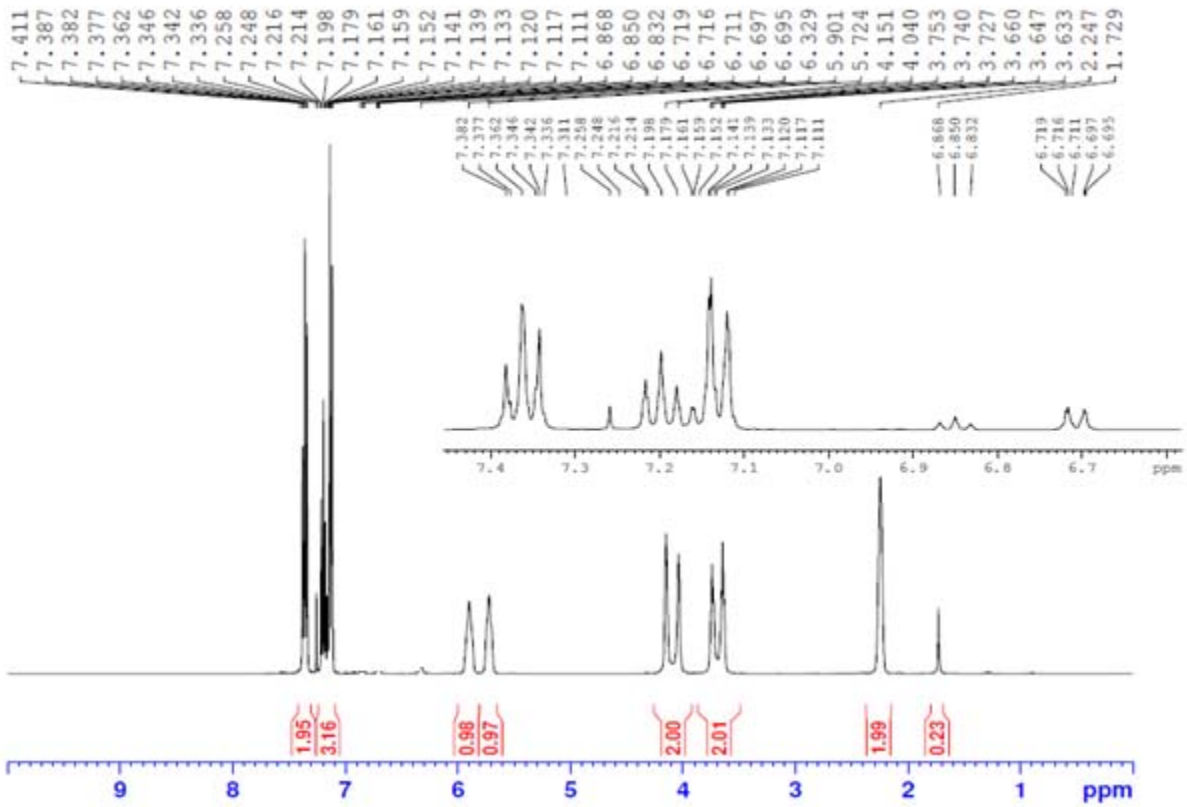


Figure S27. ^{1}H NMR (400 MHz, $CDCl_3$) chromatogram of substrate *N*-phenoxycarbonyl-1, 2, 5, 6-tetrahydropyridine **25** synthesized by chemical method.

***N*-phenoxycarbonyl-3, 4-epoxy-piperidine (+)-26:**

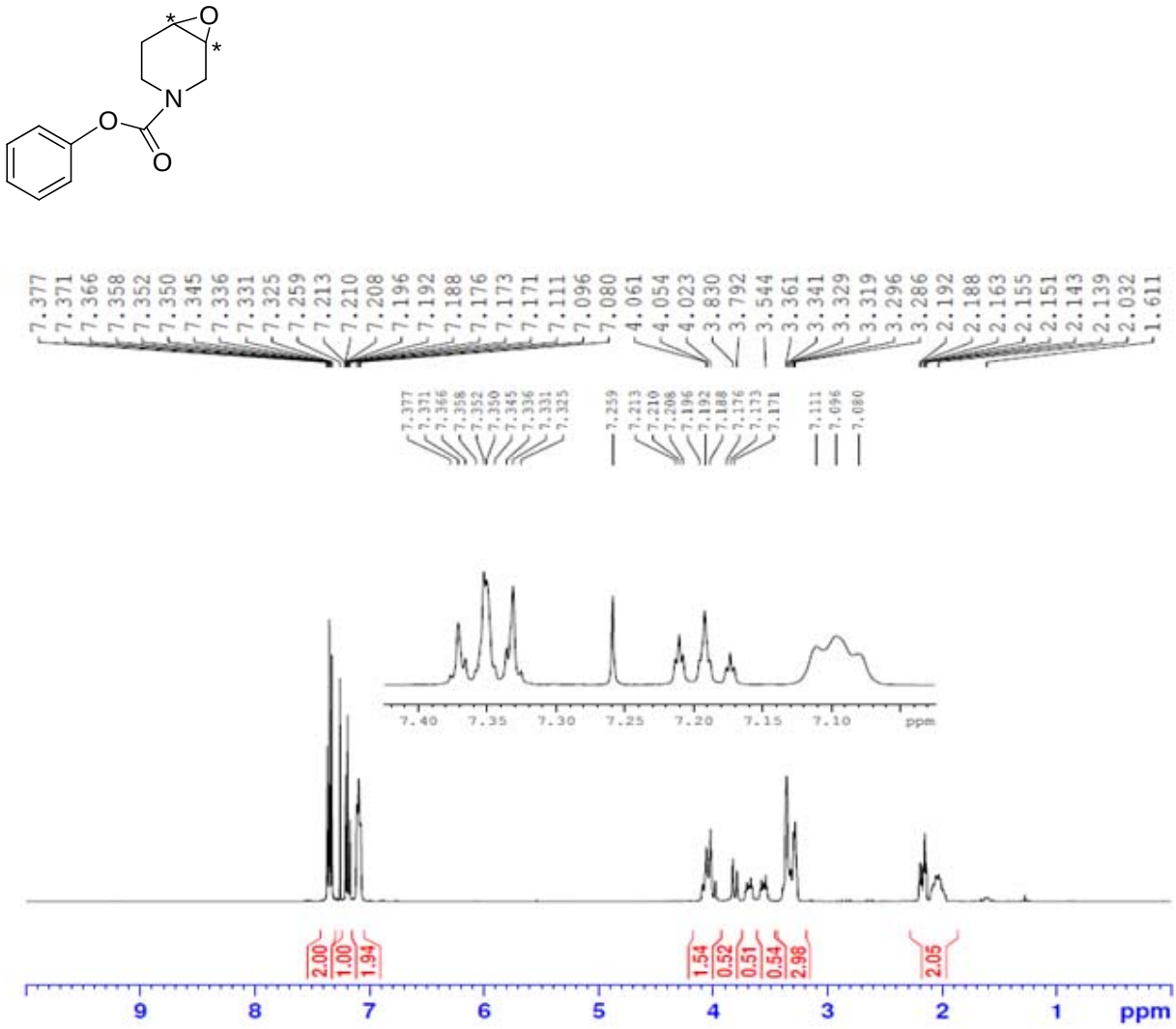


Figure S28. ^{1}H NMR (400 MHz, $CDCl_3$) chromatogram of (+)-*N*-phenoxycarbonyl-3, 4-epoxy-piperidine **26** prepared by asymmetric epoxidation of *N*-phenoxycarbonyl-1, 2, 5, 6-tetrahydropyridine **25** with the resting cells of *E. coli* (P450pyrTM).

9. Epoxidation of 4-bromostyrene 5 with different concentrations in single aqueous system

Different substrate concentrations ranging from 2 mM to 70 mM were employed for asymmetric epoxidation of 4-bromostyrene **5** in single aqueous system. As shown in Figure S29, incrases in substrate concentration resulted in increased product concentration for concentrations below 10 mM. The highest product concentration of 4.5 mM was obtained at 10 mM substrate. At higher substrate concentrations, severe inhibition was observed and the product concentration and conversion began to decrease significantly. The product obtained was only 1.93 mM with 2.7% conversion when substrate concentration was enhanced to 70 mM. Based on the highest product concentration, the substrate concentration of 10 mM was selected as the optimal condition for the epoxidation of 4-bromostyrene **5** in the single aqueous system.

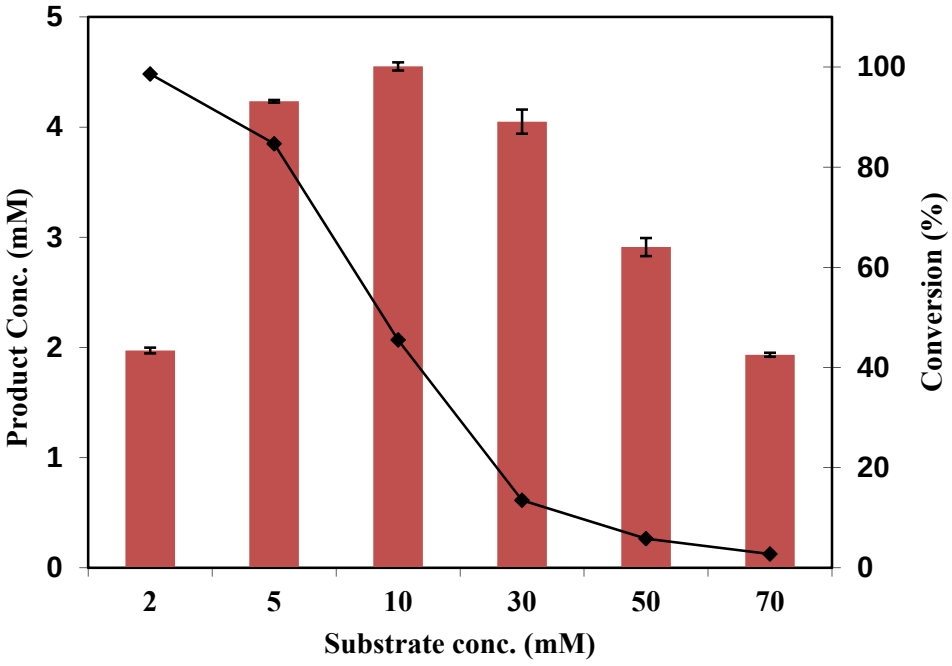


Figure S29. Effect of substrate concentration on the epoxidation of 4-bromostyrene **5** with resting cell of *E.coli* (P450pyrTM) in single aqueous system. Reaction conditions were conducted with 2-70 mM substrate in 5 mL cell suspension (10 g cdw/L) of *E.coli* (P450pyrTM) in 100 mM KP buffer (pH 8.0) containing 1wt% glucose at 30°C and 250 rpm for 12h. (Red bar) final product concentration; (◆) conversion. All the experiments were carried out in duplicate, and the mean values of the duplicate experiments were shown.

10. Epoxidation of 4-bromostyrene 5 in resin/water biphasic system

To overcome the severe substrate inhibition observed in single aqueous system,[S17] the resin/water biphasic system was attempted. In such a biphasic system, the hydrophobic resin acts as a substrate reservoir and prevents the cells in the aqueous phase from being damaged by high substrate concentration. Unlike most toxic organic solvets used in organic-aqueous biphasic systems, the resin is non-volatile, non-biodegradable, easily avaiable at low-cost and allows for simple recovery from the reaction mixture. The ratio of resin to substrate was firstly examined with cell density of 10 g cdw/L at 30 mM substrate. As shown in Figure S30, with a ratio of 2/3 (g/ mmole), the highest product concentration of 13.1 mM was achieved. Therefore, the ratio of resin to substrate of 2/3 (g/ m mole) was chosen for further experiments. Partition coefficients for substrate, 4-bromostyrene **5** and product, 2-(4-bromophenyl)oxirane **6**, were then determined with a 2/3 (g/m mole) ratio of resin to substrate in the resin/water system. The partition coefficients K(ratio of amount of solute in resin phase divided by the amount of the solute in the aqueous phase at equilibrium) for substrate **5** and product **6** were found to be 365 and 71, respectively, indicating that almost all of the substrate (> 99.0%) and most of the product (>98.0%) were partitioned in the resin phase.

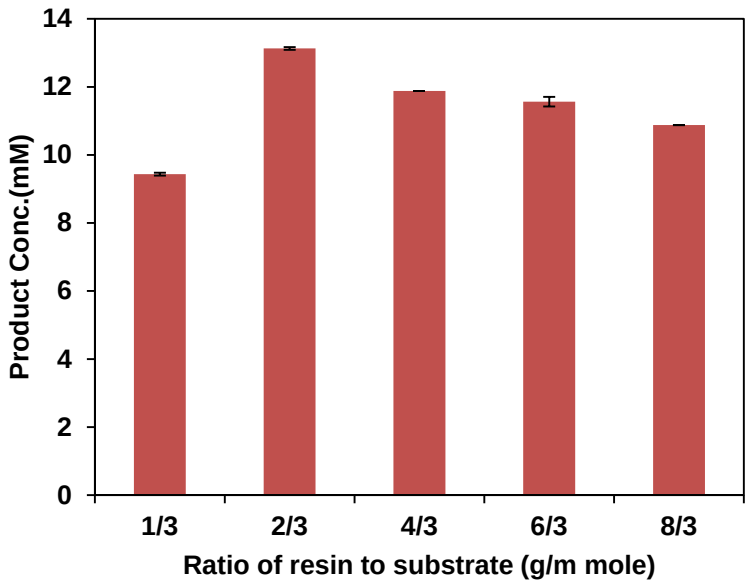


Figure S30. Effect of ratio of resin to substrate on the epoxidation of 4-bromostyrene **5** in resin/water biphasic system with resting cells of *E. coli* (P450pyrTM). Reactions were conducted with 30 mM substrate in 5 mL cell suspension (10 g cdw/L) of *E. coli* (P450pyrTM) in 100 mM KP buffer (pH 8.0) containing 1wt% glucose and different amount of resins (ratios of resin to substrate of 1/3-8/3, g /m mole) at 30°C and 250 rpm for 12h. All the experiments were carried out in duplicate, and the mean values of the duplicate experiments were shown.

Experiments with substrate concentrations ranging from 10 mM to 100 mM were then investigated for asymmetric epoxidation 4-bromostyrene **5** in resin/water biphasic system with the optimal ratio of resin to substrate obtained above (2/3, g/m mole). As shown in Figure S31, increases in substrate concentrations resulted in increased product concentration until 70 mM substrate, and then began to decrease . With 70 mM substrate concentration, the highest product concentration of 21.7 mM was achieved with 31.1% conversion, which is much better than the results obtained from the single aqueous system with the same substrate concentration (1.93 mM, 2.7% conversion). Therefore, based on the highest product concentration, the ratio of 2/3 (resin to substrate, g/m mole) and 70 mM substrate concentration were selected as the optimal reaction conditions for the epoxidation reaction of 4-bromostyrene **5** in resin/water biphasic system.

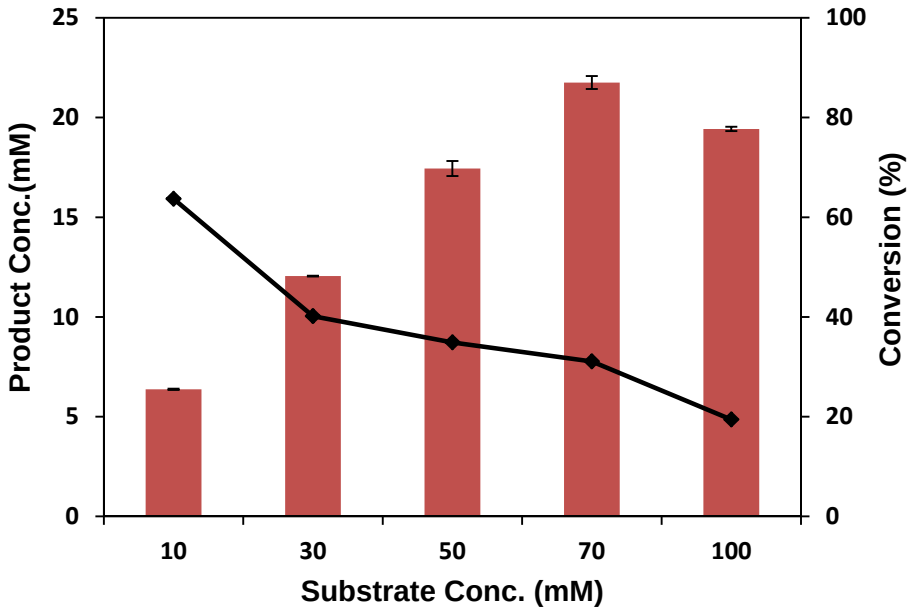


Figure S31. Effect of substrate concentration on the epoxidation of 4-bromostyrene **5** with resting cell of *E.coli* (P450pyrTM). Reaction conditions were conducted with 10-100 mM substrate in 5 mL cell suspension (10 g cdw/L) of *E.coli* (P450pyrTM) in 100 mM KP buffer (pH 8.0) containing 1wt% glucose and different amount of resins (ratios of resin to substrate were kept at 2/3, g/m mole) at 30°C and 250 rpm for 12h. (red bar) final product concentration in reaction mixture; (◆) conversion. All the experiments were carried out in duplicate, and the mean values of the duplicate experiments were shown.

The time course for P450pyrTM catalyzed epoxidation of 4-bromostyrene **5** in resin/water system under the optimal conditions was shown in Figure S32. The highest product concentration of 22 mM was achieved after 10 h reaction. Low product concentration maintained in the aqueous phase, it was only 0.4 mM at 24 h. The product *ee* obtained was very high, all *ee* values remained above 99%.

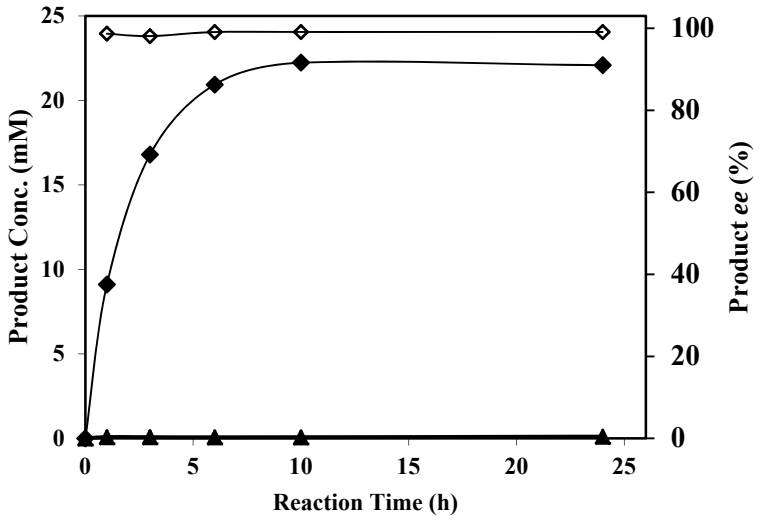


Figure S32. Time course for epoxidation of 4-bromostyrene **5** in resin/water system with resting cells of *E. coli* (P450pyrTM) under the optimal conditions. Reaction was conducted with 70 mM substrate in 5 mL cell suspension (10 g cdw/L) of *E. coli* (P450pyrTM) in 100 mM KP buffer (pH 8.0) containing 1wt% glucose (ratio of resin to substrate: 2/3, g/m mole) at 30°C and 250 rpm. (▲) product concentration in aqueous phase; (◆) total product concentration obtained in resin/water system; (◇) produce *ee* in resin/water system.

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