Electronic Supplementary Information

Asymmetric epoxidation of alkene and benzylic hydroxylation with P450tol monooxygenase from *Rhodococcus coprophilus* TC-2

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1. Chemicals

3-Bromostyrene **1** (98%), 3-chlorostyrene **3** (>98%), 2-(3-chlorophenyl)oxirane **4** (98%), 3-(trifluoromethyl)styrene **5** (99%), 2-(trifluoromethyl)styrene **9** (99%), 4-nitrostyrene **11** (>98%), 4-cyanostyrene **13** (98%), 4-phenyl-1-butene **15** (>98%), ethylbenzene **17** (>98%), 1-phenylethanol **18** (98%), 1-chloro-4-ethylbenzene **19** (97%), 1-(4-chlorophenyl)ethanol **20** (97%), 1-bromo-4-ethylbenzene **21** (97%), 1-(4-bromophenyl)ethanol **22** (97%), 4-ethylnitrobenzene **23** (98%), *n*-hexadecane (>99%), *δ*-aminolevulinic acid hydrochloride (ALA, >99%) and hydrophobic resin XAD 16 were purchased from Sigma-Aldrich, Singapore. 1-Bromo-2-ethylbenzene **25** (97%) and benzylacetone (>98%) were bought from Alfa Aesar, Singapore. 2-Nitrostyrene **7** (98%), 2-[3-(trifluoromethyl)phenyl]oxirane **6** (95%), 2-[2-(trifluoromethyl)phenyl]oxirane **10** (95%), 2-(4-cyanophenyl)oxirane **14** (97%), 1-(4-nitrophenyl)ethanol **24** (95%) and 1-(2-bromophenyl)ethanol **26** (95%) were obtained from Enamine (Kiev, Ukraine). The racemic epoxides 2-(3-bromophenyl)oxirane **2** (98%), 2-(2-nitrophenyl)oxirane **8** (98%), 2-(4-nitrophenyl)oxirane **12** (98%) and 2-(2-phenylethyl)oxirane **16** (98%) were synthesized according to the procedure reported previously. [S1]

2. Strains and Biochemicals

The toluene and ethylbenzene-degrading strains used for screening were from the strain collection in our lab. The T7 express (NEB) competent *Escherichia coli*, restriction enzymes and Quick DNA Ligase were purchased from NEB (New England Biolabs). Phusion DNA polymerase was obtained from Thermo Scientific, Singapore. The plasmids pRSFDuet1 and pETDuet1 were purchased from Novagen, USA. The genes of P450terp (terpC), terpredoxin reductase (terpA) and terpredoxin (terpB) from *Pseudomonas* sp.[S2] (Accession No. M91440.1) were purchased from GenScript, Singapore. Medium components Luria-Bertani (LB) premix, tryptone and yeast extract were purchased from Biomed Diagnostics, Singapore. Ampicillin sodium salt (>99%) and kanamycin disulfate salt (>99%) were obtained from

Sigma-Aldrich, Singapore. Isopropyl β-D-1-thiogalactopyranoside (ITPG, >99%) was obtained from Calbiochem, USA.

3. Analytic methods

3.1 Analysis of the conversion for enzyme catalyzed asymmetric epoxidation and hydroxylation

The concentrations of the alkenes and corresponding epoxides, ethylbenzene derivatives and corresponding benzylic alcohols were analyzed by using a Shimadzu Prominence HPLC (Japan) system (reverse phase) on an Agilent Poroshell 120 EC-C18 column (150 mm × 4.6 mm, Agilent) at 25^{o}C with UV detection at 210 nm. The analytical conditions (flow rate and eluent) and the retention time for each compound were shown in Table S1. The calibration curves of the substrates and products were established by analyzing samples containing both 2 mM internal standard (benzyl alcohol for alkenes and epoxides; benzylacetone for ethylbenzenes and benzylic alcohols) and individual substrate or product at different concentration (2 mM, 4 mM, 6 mM, and 8 mM) in acetonitrile. Based on the calibration curves, the concentrations of the substrates and products in the analytic samples were quantified.

Table S1. HPLC analysis for alkenes and their corresponding epoxides products as well as ethylbenzene derivatives and their corresponding benzylic alcohols 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**	16.5	**2**	8.6
60:40	0.5	**3**	18.4	**4**	9.3
60:40	0.5	**5**	17.0	**6**	9.1
60:40	0.5	**7**	8.8	**8**	6.7
60:40	0.5	**9**	16.8	**10**	10.2
60:40	0.5	**11**	9.2	**12**	6.1
60:40	0.5	**13**	7.7	**14**	5.4
60:40	0.5	**15**	21.3	**16**	8.1
60:40	0.5	**17**	15.5	**18**	5.2
60:40	0.5	**19**	22.5	**20**	5.7
60:40	0.5	**21**	23.7	**22**	5.8
60:40	0.5	**23**	12.5	**24**	5.1
60:40	0.5	**25**	24.0	**26**	6.0

3.2 Analysis of the *ee* of the epoxides and benzylic alcohols from biotransformations

The product *ee* from the biotransformation was determined by using a Shimadzu Prominence HPLC (Japan) at 25°C with UV detection 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 (all chiral columns were bought from Daicel Corporation, Japan).

Table S2. HPLC analysis of the *ee* of chiral epoxides and benzylic alcohols.

Products	Chiral column	Eluent (hexane: IPA, *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	12.4	13.0
4 [a]	AS-H	90:10	0.5	11.1	11.8
6 [c]	AS-H	90:10	0.5	9.1	9.7
8 [b]	AS-H	90:10	0.5	13.5	14.8
10 [a]	IA-3	100:0	0.5	13.2	14.1
12 [a]	AS-H	93:7	1.0	15.2	21.7
14 [a]	AD-H	90:10	1.0	8.3	8.6
16 [d]	OD-H	98:2	1.0	5.9	7.0
18 [e]	OB-H	98:2	0.8	24.0	14.2
20 [e]	OB-H	98:2	0.8	18.1	15.9
22 [e]	OB-H	98:2	0.8	19.8	17.1
24 [e]	OB-H	98:2	0.8	57.0	52.7
26 [e]	OB-H	98:2	0.8	17.3	12.3

[a] The absolute configuration was established in our previous study. [S1, S3]

[b] The absolute configuration was deduced from the elution order on the chiral HPLC (Chiralpak AS-H column). *R* enantiomer was eluted before the *S* enantiomer. [S4]

[c] The absolute configuration was verified by chiral GC analysis (Lipodex E column) according to the elution order. For product **6**, *S* enantiomer was eluted before the *R* enantiomer. [S5]

[d] The absolute configuration was deduced from the elution order on the chiral HPLC (Chiralpak OD-H column). *R* enantiomer was eluted before the *S* enantiomer. [S6]

[e] The absolute configuration was deduced from the elution order on the chiral HPLC (Chiralpak OB-H column). *S* enantiomer was eluted before the *R* enantiomer. [S7]

4. Applications of enantiopure epoxides and benzylic alcohols

Table S3. Applications of enantiopure epoxides as synthetic intermediates

Entry	Epoxides	Final product	Applications of final product	Ref.
1	(*R*)-**2**	Enantio-riched 1-aryl-2-(1H-1,2,4-triazol-1-yl)ethanols	Antifungal activity for the treatment of topical or inner mycoses	[S8]
2	(*R*)-**4**	β3 Adrenergic receptor agonists	Anti-stress effect for the treatment of diabetes and obesity	[S9]
3	(*R*)-**6**	β3 Adrenergic receptor agonists	Anti-stress effect for the treatment of diabetes and obesity	[S9]
4	(*R*)-**8**	Benzisoxazolones	Antibacterial and antileukemic activity for treatment of psoriasis and proliferative skin disorders	[S10]
5	(*R*)-**10**	Neurotherapeutic azole compounds	For the treatment of anxiety, depression, obesity, sleep disorder, neuropathic pain, *etc*.	[S11]
6	(*S*)-**12**	Enantiomerically enriched benzoyl piperidine compounds	For the treatment of psychoses and cognition disorders	[S12]
7	(*S*)-**14**	Sphingsine-1-phosaphate receptor agonists	For treatment of autoimmune and vascular diseases	[S13]
8	(*R*)-**16**	1) (*R*)-Yashabushiketol; 2) β-Lactone-fused tetrahydrofurans	1) Natural product showing chiral aldol functionality; 2)"Activated aldol products" as versatile intermediates in organic synthesis	[S14] [S6]

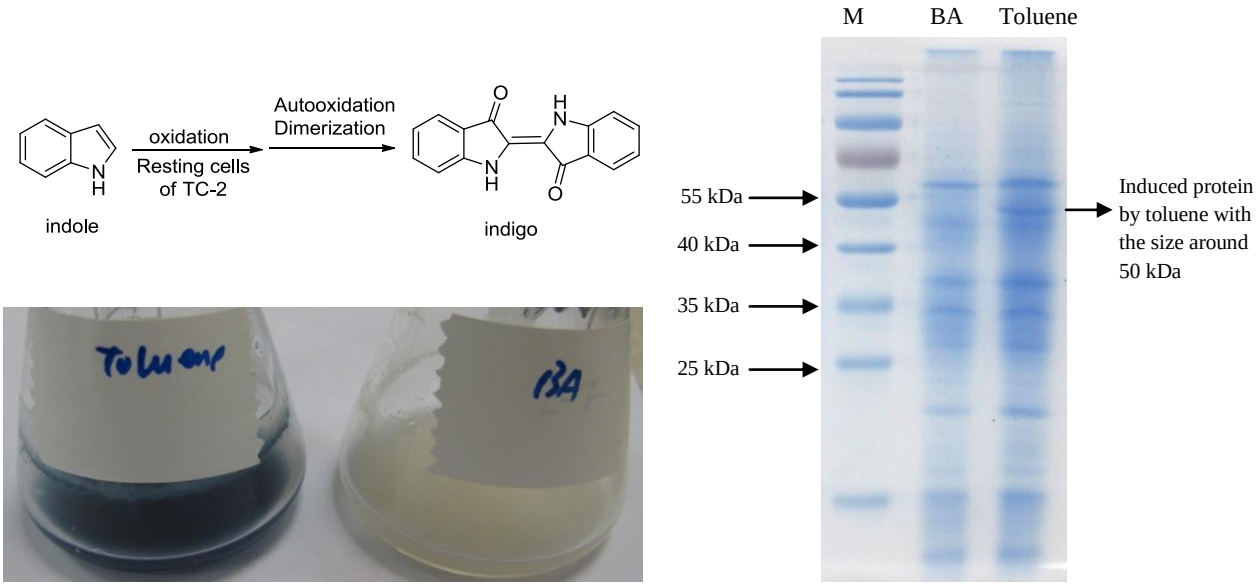
Table S4. Applications of enantiopure benzylic alcohols as synthetic intermediates

Entry	Alcohols	Final product	Applications of final product	Ref.
1	(*R*)-**18**	Fendiline derivatives	For cancer treatment	[S15]
2	(*R*)-**20**	Selective histamine H1 antagonist : Clemastine	Anticholinergic and sedative effects for treatment of gastrointestinal disorders and respiratory disorders	[S16]
3	(*R*)-**22**	Benzotriazole derivatives (mGluR2 receptor)	For treatment or prevention of neurological and psychiatric disorders	[S17]
4	(*R*)-**24**	Antisepsis agent	For the treatment of sepsis and septic shock	[S18]
5	(*S*)-**26**	Fractalkine receptor (CX3CR1) antagonists	For the treatment of multiple sclerosis	[S19]

5. Screening of microorganisms for (*R*)-enantioselective epoxidation of 3-bromostyrene

Each isolate of 100 toluene- or ethylbenzene-degrading strains was inoculated from M9 medium[S20] agar plate into 5 mL LB broth medium and incubated at 30^{o}C and 250 rpm for 12 h. 5 mL LB seed culture were transferred into 100 mL M9 medium in a 250-mL conical shaking flask containing a tube with 1 mL toluene, or ethylbenzene. The culture was shaken at 250 rpm and 30^{o}C for 24-30 h, and the cell growth was monitored by measuring OD at 600 nm. Cells were harvested at early stationary phase by centrifugation at 8,000 g for 20 min, washed twice with 100 mM potassium phosphate (KP) buffer (pH 8.0), and re-suspended to 6 g CDW/L in 1 mL KP buffer containing 1 wt% glucose and 5 mM 3-bromostyrene in a 2-mL culture tube. The mixture was shaken at 1000 rpm and 30^{o}C for 5 h. 0.5 mL sample was taken, mixed with 0.5 mL acetonitrile containing 2 mM benzyl alcohol as internal standard, and the supernatant was separated by centrifugation at 21, 000 g for 10 min and then subjected to reversed HPLC analysis. For the determination of product *ee*, 0.5 mL sample was taken and mixed with 0.5 mL *n*-hexane, and the organic phase was separated by centrifugation at 21, 000 g for 5 min and used for chiral HPLC analysis.

6. Effect of toluene and benzyl alcohol as carbon sources on enzyme activity



a) **b)**

Figure S1. **a)** Biotransformation of indole to indigo with the resting cells of *Rhodococcus coprophilus* TC-2 grown on toluene (left) or benzyl alcohol (right) as carbon source. The blue color in the left bottle indicates the happening of the oxidation of indole. **b)** SDS-PAGE analysis of the cell-free extracts (CFE) of *Rhodococcus coprophilus* TC-2 grown on toluene or benzyl alcohol as carbon source. Lane M: protein marker; Lane BA: CFE of cells grown on benzyl alcohol as carbon source; Lane Toluene: CFE of cells grown on toluene as carbon source.

Table S5. Epoxidation of 3-bromostyrene with resting cells of *Rhodococcus coprophilus* TC-2 grown on toluene or benzyl alcohol as carbon source

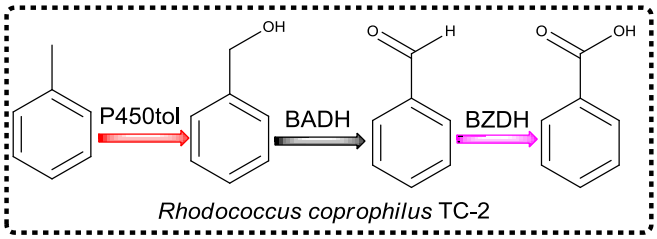
Carbon source	**Cell density (g CDW/L)** [a]	**Specific activity (U/g CDW)** [b]	***ee* (%)**[c]
Toluene	1.0	8.8	97.6 (*R*)
Benzyl alcohol	1.0	-	-

[a] Cell density at 24 h growth. CDW, cell dry weight. [b] Activity was tested with 2 mM substrate in 4 mL cell suspension (6 g CDW/L) of *Rhodococcus coprophilus* TC-2 in KP buffer (100 mM, pH 8.0) containing 1 wt% glucose at 30°C and 250 rpm for 20 min. [c] Determined by chiral HPLC analysis.

7. The gene clusters in the genome of *Rhodococcus coprophilus* TC-2 for the toluene degradation

The genome of *Rhodococcus coprophilus* TC-2 was sequenced and analyzed by BGI-HONG KONG CO., LIMITED. Two gene clusters containing a group of genes responsible for the oxidations of toluene to benzyl alcohol, benzaldehyde, and then benzoic acid were identified. One of them was shown in Figure S2 (b), the P450tol monooxygenase in this gene cluster was identified as the responsible enzyme in TC-2 strain for the epoxidation.

a)



b)

Figure S2. **a)** Pathway of *Rhodococcus coprophilus* TC-2-catalyzed biotransformation of toluene to benzyl alcohol, benzaldehyde and benzoic acid. P450tol monooxygenase consisting of ferredoxin reductase (FdR), ferredoxin (FdX) and the hydroxylase (P450tol) catalyzes the initial oxidation of toluene to benzyl alcohol. Benzyl alcohol dehydrogenase (BADH) catalyzes the formation of benzaldehyde, followed by benzaldehyde dehydrogenase (BZDH)-catalyzed oxidation to benzoic acid. **b)** Gene organization in the gene cluster from the genome of *Rhodococcus coprophilus* TC-2. *badh*: gene of benzyl alcohol dehydrogenase; *bzdh*: gene of benzaldehyde dehydrogenase; N.A.: unknown gene; *p450tol*: gene of P450tol hydroxylase; *fdx*: gene of ferredoxin; *CoA-ligase*: gene of CoA-ligase; *fdr*: gene of ferredoxin reductase.

8. Primers used for cloning in this study

Table S6. Primers used for cloning in this study.

P450tol (P)	Forward: C*GAGCTC*GATGACGACCGTCGAATCGAACACAA (*sac* I) Reverse: CCC*AAGCTT*TCAGCTGAAGGTGAGGTGTACCGGC (*Hind* III)
Ferredoxin reductase 967 (FdR_967)	Forward: GGA*AGATCT*CATGAACTCGCTAACGGAACCGGCCG (*Bgl* II) Reverse: CGG*GGTACC* TCACGATGCAGCGGGTGAACGTTCTG (*Kpn* I)
Ferredoxin 965 (FdX_965)	Forward: GGA*AGATCT*CATGCCTTACGTCGTTTACCATCTCCCCG (*Bgl* II) Reverse: CGG*GGTACC*CTACCTACTGTCGACCACACGGACGTG (*Kpn* I)
Ferredoxin reductase 934 (FdR_934)	Forward: GGA*AGATCT*CATGCCCACGCCACATCCTGCACCCAC (*Bgl* II) Reverse: CGG*GGTACC*CTACAGAACCGCGTCCCGCAACGATGTGG (*Kpn* I)
Ferredoxin 936 (FdX_936)	Forward: GGA*AGATCT*CATGCCCACCGTCGTGTACCAGCTCC (*Bgl* II) Reverse: CGG*GGTACC*TCAGTCGGTCGAGGACGGCACAGTC (*Kpn* I)
Ferredoxin reductase 1500 (FdR_1500) (from *Mycobacterium* sp.HXN-1500)	Forward: GGGAATTC*CATATG*ATCCACACCGGCGTGACCGAAG (*Nde* I) Reverse: CGG*GGTACC*TTAGAGGGAGGTTGGGGACGTTGCGC (*Kpn* I)
Ferredoxin reductase 200 (FdR_200) (from *Sphingomonas* sp. HXN-200)	Forward: GGGAATTC*CATATG*ATCCACACCGGCGTGACCGAAG (*Nde* I) Reverse: CGG*GGTACC*CTATACTGGCAGCAATTCTTTCAGGGGAAGG (*Kpn* I)
Ferredoxin 200 (FdX_200) (from *Sphingomonas* sp. HXN-200)	Forward: GAATTC*CATATG*CCAACAGTGACCTATGTTG (*Nde* I) Reverse: CGG*GGTACC*TCAATGCTGCGCGAGAGGAAG (*Kpn* I)
Glucose dehydrogenase (GDH)	Forward: GGATTC*CCATGG*TTTATCCGGATTTAAAAGGAAAAGTC(*Nco* I) Reverse: GGATTC*CTGCAG*TTAACCGCGGCCTGCCTG (*Pst* I)
P450terp (terpC)	Forward: CGCGGATCCG ATGGACGCT AGAGCCACCA TTCCGG (BamH) Reverse: CGAGCTCCTAAGCCTTGGTGAAGCGAATCGGC (SacI)
Terpredoxin reductase (terpA)	Forward: GGAAGATCTCATGGGGGAACGTCGAGACACTACG (*Bgl* II) Reverse: CCGCTCGAGCTATGCGAAATGAAGGGCGTCTTTC (*Xho* I)
Terpredoxin (terpB)	Forward: GGAATTCCATATGCCACGTGTCGTGTTTATCG (*Nde* I) Reverse: CGGGGTACCCTAAGCAGGTAGTGGAACCCTGACG (*Kpn* I)

9. Engineering of recombinant *E. coli* (P450tol-1) co-expressing P450tol hydroxylase with the native ferredoxin and ferredoxin reductase

The genomic DNA from *Rhodococcus coprophilus* TC-2 was used as the PCR template to amplify the P450tol gene (P), ferredoxin gene (FdX_965) and ferredoxin reductase gene (FdR_967), respectively. Primers with corresponding restriction sites for the genes amplification were shown in the Table S6. PCR amplified gene products were digested with their restrictive enzymes. The digested P fragment was ligated into the multiple cloning site (MCS) 1 of the pETDuet1 digested with *sac* I and *Hind* III restriction enzymes to give the plasmid pETDuet-P450tol. The digested FdR_967 fragment was then ligated into the MCS 2 of plasmid pETDuet-P450tol digested with *Bgl* II and *Kpn* I to give the plasmid pETDuet-P450tol-FdR_967. The digested FdX_965 fragment was ligated into the MCS 2 of pRSFDuet1 digested with *Bgl* II and *Kpn* I, resulting in the plasmid pRSFDuet-FdX_965. The two plasmids were then co-transformed into T7 express (NEB) competent *E. coli* cells which were then plated on LB agar plates containing 50 mg mL^{-1} kanamycin (for pRSFDuet1) and 100 mg mL^{-1} ampicillin (for pETDuet1). The resulting recombinant *E. coli* co-expressing P450tol and the native ferredoxin and ferredoxin reductase was named as *E. coli* (P450tol-1).

The *E. coli* (P450tol-1) was inoculated to 3 mL LB medium containing 50 mg mL^{-1} kanamycin and 100 mg mL^{-1} ampicillin and grown at 37 °C for 10-12 h. The pre-culture (2 mL) was transferred into 50 mL terrific broth (TB) medium containing kanamycin (50 mg L^{-1}) and ampicillin (100 mg L^{-1}). Cells were grown at 37°C and 250 rpm to OD_{600} about of 0.6~0.8, and then induced by addition of IPTG to a concentration of 0.25 mM (ALA was added to 0.5 mM). The cells continued to grow for another 10−12 h at 22°C. The cells were then harvested by centrifugation at 5000 g for 10 min and used for SDS-PAGE analysis according to a protocol reported previously. [S21]

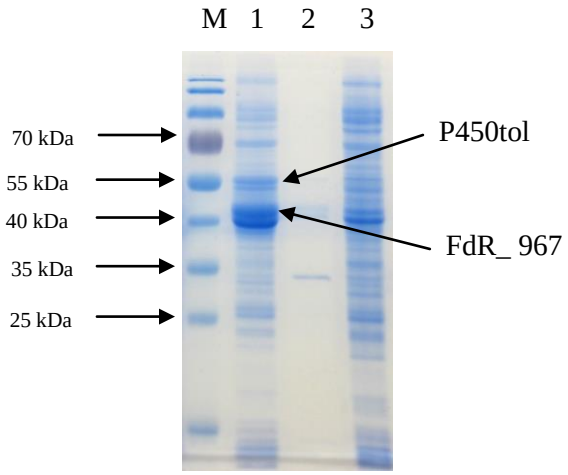


Figure S3. SDS-PAGE analysis of the polypeptides produced in recombinant *E. coli* (P450tol-1). Lane M: protein marker; Lane 1: supernatant of cell lysate; Lane 2: precipitate of cell lysate; Lane 3: cell lysate of *E. coli* cells without IPTG addition.

10. Engineering of recombinant *E. coli* co-expressing P450tol with different pairs of ferredoxins (FdX) and ferredoxin reductases (FdR)

The P450tol hydroxylase was co-expressed with different pairs of ferredoxins and ferredoxin reductases (Table S7). The genomes of *Rhodococcus coprophilus* TC-2 and *Sphingomonas* sp. HXN-200 were used as the templates to amplify ferredoxin reductases (FdR_967, FdR_934 and FdR_200) and ferredoxins (FdX_965, FdX_934 and FdX_200). Meanwhile, plasmid pRSFDuet1-GDH-Fdx [S22] was used for the amplification of ferredoxin reductase FdR_1500 gene. The recombinant *E. coli* cells were engineered with the same plasmids configurations described above (section 9), which resulted in four different recombinants (Table S7). Among the four recombinants, the recombinant *E. coli* (P450tol-4) co-expressing P450tol with the FdR_200 and FdX_200 from *Sphingomonas* sp. HXN-200 showed the highest activity for epoxidation of 3-bromostyrene.

Table S7. Effect of co-expression of different pairs of ferredoxins and ferredoxin reductases in recombinant *E. coli* on epoxidation of 3-bromostyrene.

Recombinants	Ferredoxin Reductase	Ferredoxin	Sources of reductase	Specific activity (U/g CDW)[a]	Conv. (%)[b]
E. coli (P450tol-1)	FdR_967	FdX_965	*Rhodococcus coprophilus* TC-2	0.52	31
E. coli (P450tol-2)	FdR_934	FdX_936	*Rhodococcus coprophilus* TC-2	1.7	43
E. coli (P450tol-3)	FdR_1500	FdX_200	*Mycobacterium* sp.HXN-1500/ *Sphingomonas* sp. HXN-200	2.5	77
E. coli (P450tol-4)	FdR_200	FdX_200	*Sphingomonas* sp. HXN-200	3.0	90
E. coli (P450tol-GDH)	FdR_200	FdX_200	*Sphingomonas* sp. HXN-200	5.8	>99

[a] Activity was tested with 2 mM substrate in 4 mL cell suspension (4 g CDW/L) of recombinant *E. coli* cells in KP buffer (100 mM, pH 8.0) containing 1 wt% glucose at 30°C and 250 rpm for 20 min. [b] Determined by HPLC analysis.

11. Engineering of recombinant *E. coli* (P450tol-GDH) co-expressing P450tol, FdR_200, FdX_200 and GDH

The glucose dehydrogenase (GDH) gene was amplified using the genome of *Bacillus subtilis* as the template. The recombinant *E. coli* (P450tol-GDH) co-expressing P450tol, FdR_200, FdX_200 and GDH was then constructed based on the dual plasmid configurations (Figure 1a in main text). The activity of the *E. coli* (P450tol-GDH) was then measured. As shown in Table S7, compared with *E. coli* (P450tol-4), nearly two-fold improvement in epoxidation activity was achieved by *E. coli* (P450tol-GDH).

12. Sequence alignment of P450tol with several known P450 monooxygenaes

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P450tol      ---MTTVESNTTAAIPDEIARQIVLPEGHKD----NVPLFEAYRWLRENQPLGQARVEGY 53
P450terp     --------MDARATIPEHIARTVILPQGYAD----DEVIYPAFKWLRDEQPLAMAHIEGY 48
P450pyr      ---MEHTGQSAAATMPLDSIDVSIPELFYND----SVGEY--FKRLRKDDPVHYCADSAF 51
P450cam      MTTETIQSNANLAPLPPHVPEHLVFDFDMYNPSNLSAGVQEAWAVLQESNVPDLVWTRCN 60
                          *.:* .     :      :    .      :  *:..:

P450tol      DPLWLITKYADLMEVERQPQIFAAGGGEDKGSNN--PILANQAGDEFTRQLLGGNLRILD 111
P450terp     DPMWIATKHADVMQIGKQPGLFSNAEGSE--------ILYDQNNEAFMRSISGGCPHVID 100
P450pyr      GPYWSITKYNDIMHVDTNHDIFSSDAGYGGIIIDDG------------IQKGGDGGLDLP 99
P450cam      GGHWIATRGQLIREAYEDYRHFSSECPFIP-------------------REAGEAYDFIP 101
             .  *  *:   : .   :   *:                            *     :

P450tol      ALPYLDQPEHSVVKDVAFDWFRPANLKKWEDRIRETARASIDRLLAGGPDLDAVQEFAVF 171
P450terp     SLTSMDPPTHTAYRGLTLNWFQPASIRKLEENIRRIAQASVQRLLDFDGECDFMTDCALY 160
P450pyr      SFIAMDRPRHDEQRKAVSPIVAPANLAALEGTIRERVSKTLDGLPVG-EEFDWVDRVSIE 158
P450cam      --TSMDPPEQRQFRALANQVVGMPVVDKLENRIQELACSLIESLRPQ-GQCNFTEDYAEP 158
                 :* * :   :  .   .  . :   *  *:. .   :: *     : :     :

P450tol      FPLRVIMSLFGVPEEDEPRMMALTQDFFGVADPDAQRDDIEALSPDAAAQQWAATIADFY 231
P450terp     YPLHVVMTALGVPEDDEPLMLKLTQDFFGVHEPDEQAVAAPRQSADEAARRFHETIATFY 220
P450pyr      ITTQMLATLFDFPFEERRKLTRWS----------DVTTAAPGGGVVESWDQRKTELLECA 208
P450cam      FPIRIFMLLAGLPEEDIPHLKYLT----------------DQMTRPDGSMTFAEAKEALY 202
              . ::.    ..* ::   :   :                      .

P450tol      AYFDVLVESRRA-EPRDDLATLIAVAKDENGEYFPKTFAYGWFVAIATAGHDTTASTLAG 290
P450terp     DYFNGFTVDRRS-CPKDDVMSLLANSK-LDGNYIDDKYINAYYVAIATAGHDTTSSSSGG 278
P450pyr      AYFQVLWNERVNKDPGNDLISMLAHSP-ATRNMTPEEYLG-NVLLLIVGGNDTTRNSMTG 266
P450cam      DYLIPIIEQRRQ-KPGTDAISIVANGQ-VNGRPITSDEAKRMCGLLLVGGLDTVVNFLSF 260
              *:  :  .*    *  *  :::* .     .   .         : ..* **. .

P450tol      CLQSLAAHPEVLDRVKGDPDLIPDLVNESLRIVSPVKHFTRVALQDYEMRGQKIKAGDRL 350
P450terp     AIIGLSRNPEQLALAKSDPALIPRLVDEAVRWTAPVKSFMRTALADTEVRGQNIKRGDRI 338
P450pyr      GVLALHKNPDQFAKLKANPALVETMVPEIIRWQTPLAHMRRTAIADSELGGKTIRKGDKV 326
P450cam      SMEFLAKSPEHRQELIERPERIPAACEELLRRFSLVADG-RILTSDYEFHGVQLKKGDQI 319
              :  *   *:        *  :     * :*  : :    *    * *. *  :: **::

P450tol      MLLFQSGNRDAEVFDRPDDFDIDR-RPNKHIAFGYGPHMCIGQHLAKLELKVMLQELLPH 409
P450terp     MLSYPSANRDEEVFSNPDEFDITR-FPNRHLGFGWGAHMCLGQHLAKLEMKIFFEELLPK 397
P450pyr      VMWYYSGNRDDEVIDRPEEFIIDRPRPRQHLSFGFGIHRCVGNRLAEMQLRILWEEILTR 386
P450cam      LLPQMLSGLDERENACPMHVDFSR-QKVSHTTFGHGSHLCLGQHLARREIIVTLKEWLTR 378
             ::    .. * .    * .. : *     *  ** * * *:*::**. :: :  :* *.:

P450tol      LERVEVS-GEPKLIQTNFVGGLRKLPVHLTFS----- 440
P450terp     LKSVELS-GPPRLVATNFVGGPKNVPIRFTKA----- 428
P450pyr      FSRIEVM-AEPERVRSNIVRGYAKMMVRVHA------ 416
P450cam      IPDFSIAPGAQIQHKSGIVSGVQALPLVWDPATTKAV 415
             :  ..:  .      :.:* *   : :
```

Figure S4. Sequence alignment of P450tol with several known cytochrome P450 monooxygenases (the multiple alignment by ClustalW2). P450tol, P450 from *Rhodococcus coprophilus* TC-2 (this study); P450terp, P450 from *Pseudomonas aeruginosa* (UniProt: U919J5); P450pyr, P450 from *Sphingomonas* sp. HXN-200 (UniProt: Q5F4D9); P450cam, P450 from *Pseudomonas putida* (UniProt: M5b4l7). "*": the identical amino acids; ".": similar amino acids; ":": highly similar amino acids.

Based on the amino acid sequence, P450tol shows 47% similarity with P450terp, 26% similarity with P450pyr and 24% similarity with P450cam.

13. Cell growth, epoxidation/hydroxylation activity, and SDS–PAGE analysis of recombinant *E. coli* (P450tol-GDH)

E. coli (P450tol-GDH) was grown in 25 mL LB medium containing 50 mg mL^{-1} kanamycin and 100 mg mL^{-1} ampicillin in 125-mL flask at 37 °C for 10-12 h, the pre-culture (2 mL) was then inoculated into 50 mL TB medium containing kanamycin (50 mg L^{-1}) and ampicillin (100 mg L^{-1}) in multiple 250-mL flasks. Cells were grown at 37°C and 250 rpm to OD_{600} about of 0.6~0.8 and then induced by adding IPTG to final concentration of 0.25 mM (ALA was added to 0.5 mM). At different time points, samples were taken for cell density measurement at 600 nm. To determine the cell activity, each time a whole flask containing 50 mL cell culture was taken, cells were harvested by centrifugation at 5000 g for 10 min and re-suspended in KP buffer (100 mM, pH 8.0) containing 1wt% glucose for activity test.

Activity test: freshly prepared *E. coli* (P450tol-GDH) cells were diluted by KP buffer (100 mM, pH 8.0) containing 1 wt% glucose to 4 mL with a cell density of 4 g CDW/L. And then 16 μL 0.5 M 3-bromostyrene (or 1-bromo-4-ethylbenzene) in ethanol was added to the cell suspension in the flask to a concentration of 2 mM. The reaction was carried out at 30°C, 250 rpm for 20 min. After reaction, 4 mL acetonitrile containing 2 mM benzyl alcohol (2 mM benzylacetone for hydroxylation) as internal standard was added to the flask and mixed with the sample. 1 mL mixture was taken and centrifuged at 21, 000 g for 10 min, 0.5 mL supernatant was separated and subjected to reverse HPLC analysis.

E. coli (P450tol-GDH) cells cultivated for 14 h were harvested by centrifugation at 5000 g for 10 min and used for SDS–PAGE analysis. The result was shown in Figure 1b in main text.

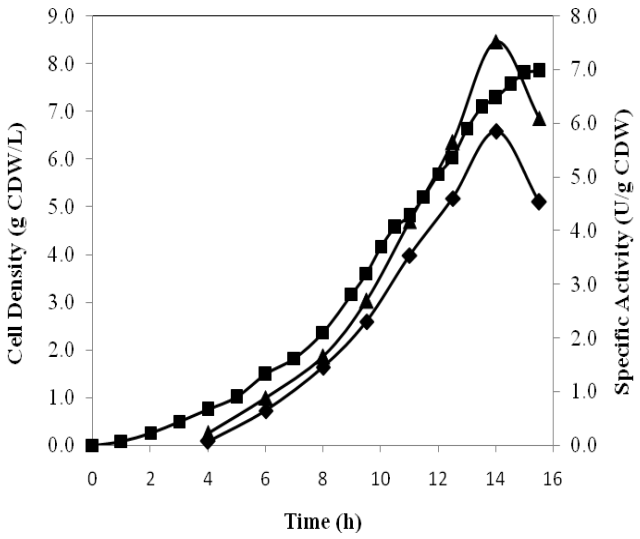


Figure S5. Cell growth and activity curves of *E. coli* (P450tol-GDH). (■) Cell density; (◆) activity for epoxidation of 3-bromostyrene **1**; (▲) activity for hydroxylation of 1-bromo-4-ethylbenzene **21**.

14. Engineering of recombinant *E. coli* (P450terp-GDH) co-expressing P450terp monooxygenase and GDH

The synthetic genes from the company were used as templates. The genes of P450terp (terpC), terpredoxin reductase (terpA) and terpredoxin (terpB) were re-amplified using the corresponding primers listed in Table S6. The fragments were digested with restriction enzymes and inserted into the plasmids with the same configurations for expression of P450tol monooxygenase (Figure 1a in main text). The resulted plasmids pETDuet-P450terp-terpA and pRSFDuet-GDH-terpB were co-transformed into T7 express (NEB) competent *E. coli* cells, and the recombinant was selected on LB agar plates containing 50 mg mL^{-1} kanamycin and 100 mg mL^{-1} ampicillin. This gave the recombinant *E. coli* (P450terp-GDH).

The *E. coli* (P450tol-GDH) was inoculated to 3 mL LB medium containing 50 mg mL^{-1} kanamycin and 100 mg mL^{-1} ampicillin and grown at 37 °C for 10-12 h. The pre-culture (2 mL) was transferred into 50 mL TB medium containing kanamycin (50 mg L^{-1}) and ampicillin (100 mg L^{-1}). Cells were grown at 37°C and 250 rpm to OD_{600} about of 0.6~0.8, and then induced by addition of IPTG to a concentration of

0.25 mM (ALA was added to 0.5 mM). The cells continued to grow for another 10−12 h at 22°C. The cells were harvested by centrifugation at 5000 g for 10 min and used for SDS-PAGE analysis.

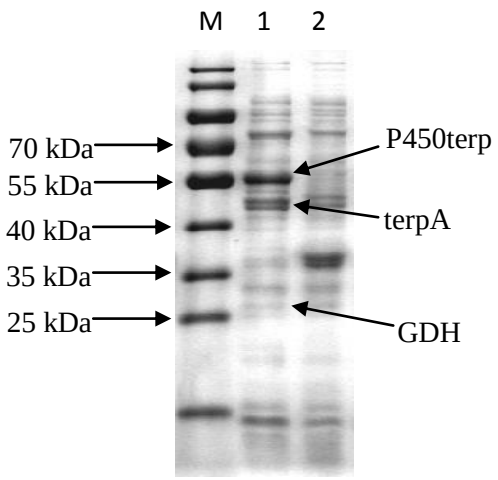


Figure S6. SDS-PAGE analysis of cell-free extracts of *E. coli* recombinants. Lane M: protein marker; Lane 1: *E. coli* (P450terp-GDH); Lane 2: *E. coli* containing dual plasmids pETDuet/pRSFDuet as a negative control.

15. General procedure for the asymmetric epoxidation of alkenes 1, 3, 5, 7, 9, 11, 13 and 15 with resting cells of *E. coli* (P450tol-GDH) or *E. coli* (P450terp-GDH)

To 4 mL cell suspension (10 g CDW/L) of *E. coli* (P450tol-GDH) [or *E. coli* (P450terp-GDH)] in KP buffer (100 mM, pH 8.0) containing 1wt% glucose was added 16 μL 0.5 M alkene substrate (**1**, **3**, **5**, **7**, **9**, **11**, **13** and **15**) in ethanol to give a 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 5 h. After reaction, 200 μL sample was taken, mixed with 200 μL *n*-hexane, the supernatant was separated by centrifugation 21, 000 g for 10 min and then subjected to chiral HPLC analysis for the determination of product *ee*. Acetonitrile 3.8 mL containing 2 mM benzyl alcohol as internal standard was added to the flask and mixed with the remained 3.8 mL sample. 1 mL mixture was taken, and centrifuged at 21, 000 g for 10 min, 0.5 mL supernatant was then subjected to reverse HPLC analysis.

16. General procedure for the asymmetric hydroxylation of ethylbenzene derivatives 17, 19, 21, 23 and 25 with resting cells of *E. coli* (P450tol-GDH)

To 4 mL cell suspension (10 g CDW/L) of *E. coli* (P450tol-GDH) in KP buffer (100 mM, pH 8.0) containing 1wt% glucose was added 0.5 M ethylbenzenes substrate (40 μL for **17**, 24 μL for **19** and **21**, 16 μL for **23** and **25**) in ethanol to give a final concentration of 5 mM for **17**, 3 mM for **19** and **21**, 2 mM for **23** and **25**, respectively. The reaction was conducted in a 100-mL flask with screw cap at 30^{o}C and 250 rpm for appropriate time (8 h for **17**, 5 h for **19**, **21** and **23**, 10 h for **25**). After reaction, 200 μL sample was taken, mixed with 200 μL ethyl acetate, and centrifuged at 21, 000 g for 10 min, the organic phase was separated and then completely evaporated. The residue was dissolved in 200 μL *n*-hexane and the sample was then subjected to chiral HPLC analysis. Acetonitrile 3.8 mL containing 2 mM benzylacetone as internal standard was added to the reaction flask and mixed with the remained 3.8 mL sample. 1 mL mixture was taken and centrifuged at 21, 000 g for 10 min, and 0.5 mL supernatant was subjected to reverse HPLC analysis.

17. General procedure for the asymmetric epoxidation of alkenes 1, 3, 5, 7 and 13 in *n*-hexadecane /water biphasic system

To 2 mL cell suspension (10 g CDW/L) of *E. coli* (P450tol-GDH) in KP buffer (100 mM, pH 8.0) containing 1wt% glucose was added 1 mL *n*-hexadecane containing 40 mM alkene substrate (**1**, **3**, **5**, **7** and **13**) (based on the volume of aqueous phase) as a second phase. The reaction was carried out in a 100-mL flask with screw cap at 30°C and 250 rpm for 12 h. After reaction, 100 μL aliquot was taken, and the organic phase was separated by centrifuged at 21, 000 g for 5 min. Acetonitrile 40 μL was added to 10 μL organic phase and mixed with 50 μL acetonitrile containing 2 mM benzyl alcohol as internal standard, the mixture was then subjected to reversed HPLC analysis. For the determination of product *ee*, 20 μL organic phase was mixed with 80 μL *n*-hexane, and the mixture was used for chiral HPLC analysis.

18. Asymmetric hydroxylation of 1-bromo-4-ethylbenzene 21 in resin/water biphatic system

To 5 mL cell suspension (10 g CDW/L) of the *E. coli* (P450tol-GDH) in KP buffer (100 mM, pH 8.0) containing 1wt% glucose were added 0.21 g resin XAD16 and 70 mM substrate (based on the volume of aqueous phase). The reaction was conducted in 100-mL flask with screw cap at 30^{o}C and 250 rpm for 12 h. After reaction, for determination of product concentration in aqueous phase, 0.5 mL acetonitrile containing 2 mM benzylacetone as internal standard was mixed with 0.5 mL reaction mixture, followed by centrifugation at 21, 000 g for 10 min, and 0.5 mL supernatant was subjected to reversed HPLC analysis. For the determination of the amount of product absorbed by the resin, the resin was separated 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, 0.5 mL acetonitrile containing 2 mM benzyl alcohol as internal standard was mixed with 0.5 mL methanol extract, the mixture was centrifuged at 21, 000 g for 10 min and 0.5 mL supernatant was then subjected to reversed HPLC analysis. To determine the product *ee*, 0.5 mL sample from aqueous phase was taken, mixed with 0.5 mL ethyl acetate, and centrifuged at 21, 000 g for 10 min, the organic phase was separated and then completely evaporated. The residue was dissolved in 200 μL *n*-hexane and the sample was subjected to chiral HPLC analysis.

19. Chiral HPLC chromatograms

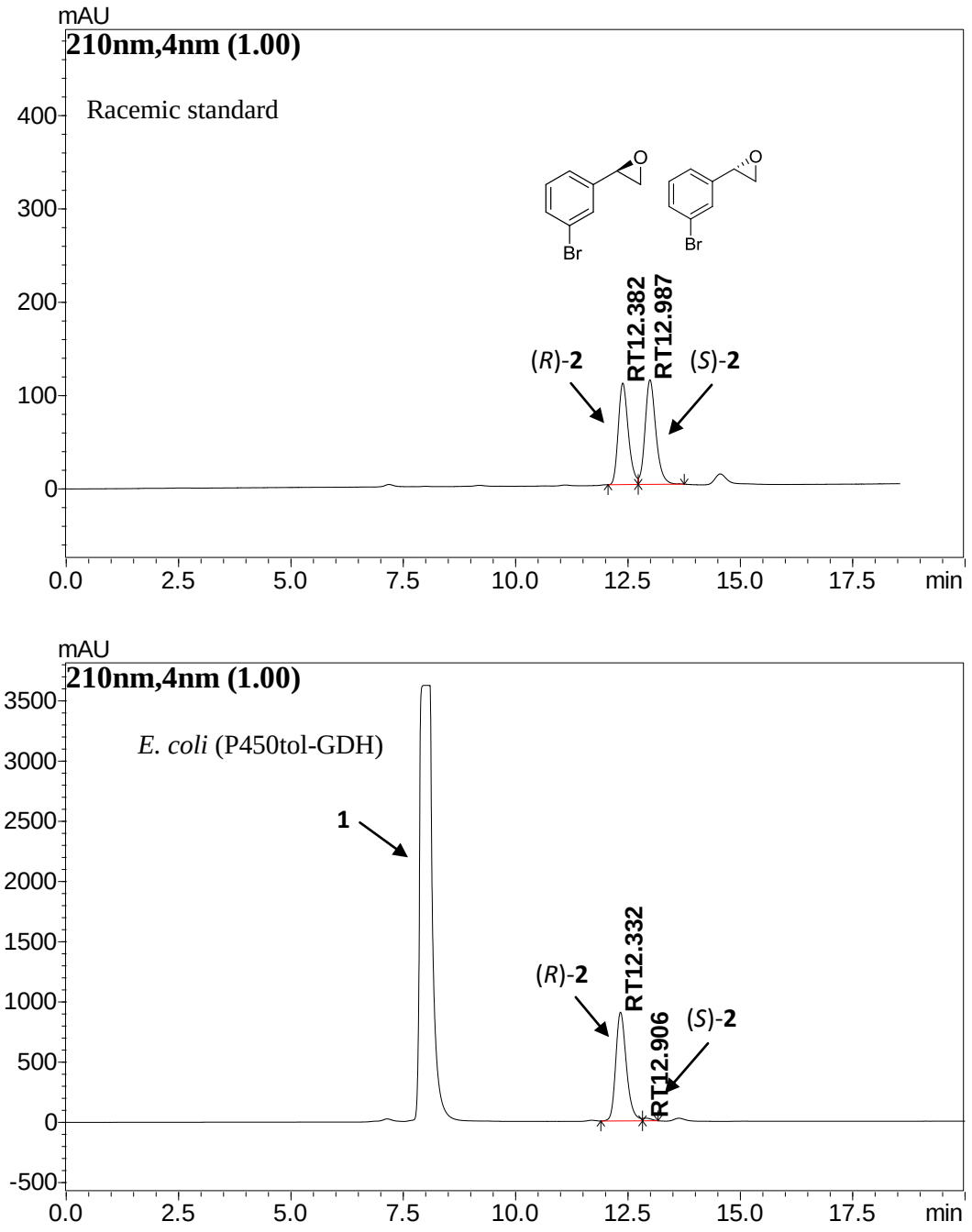
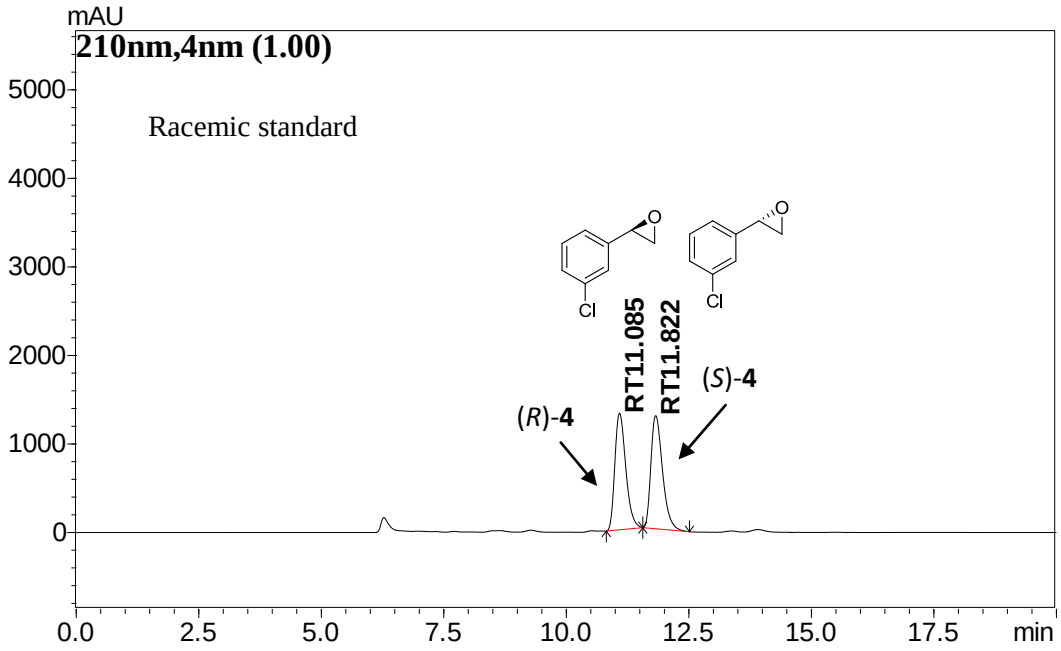


Figure S7. Chiral HPLC chromatograms for the analysis of 2-(3-bromophenyl)oxirane **2**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).



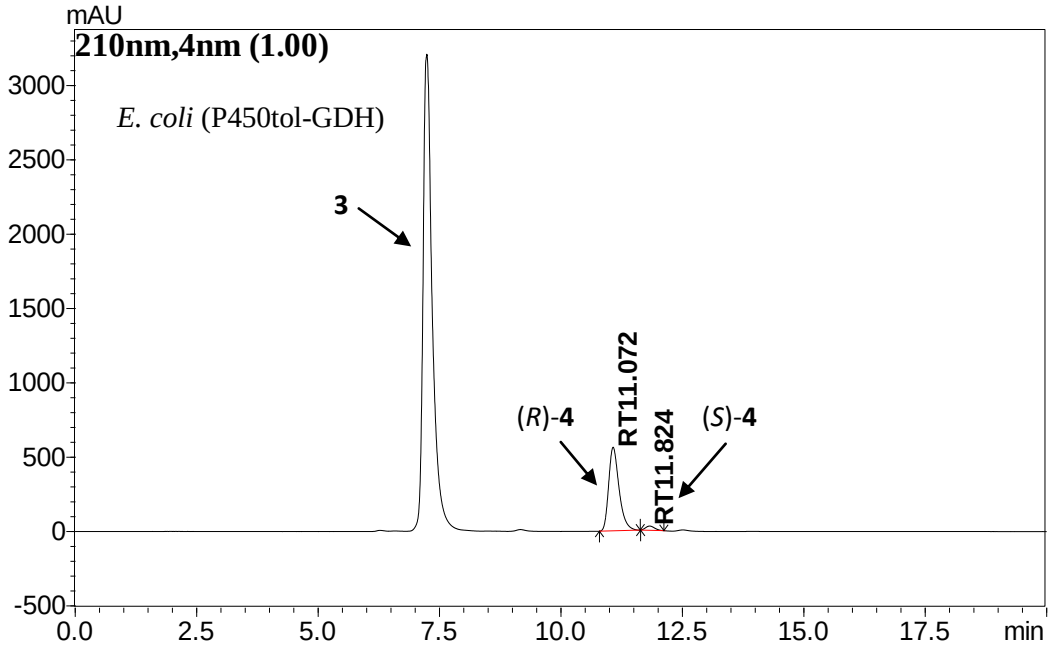


Figure S8. Chiral HPLC chromatograms for the analysis of 2-(3-chlorophenyl)oxirane **4**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).

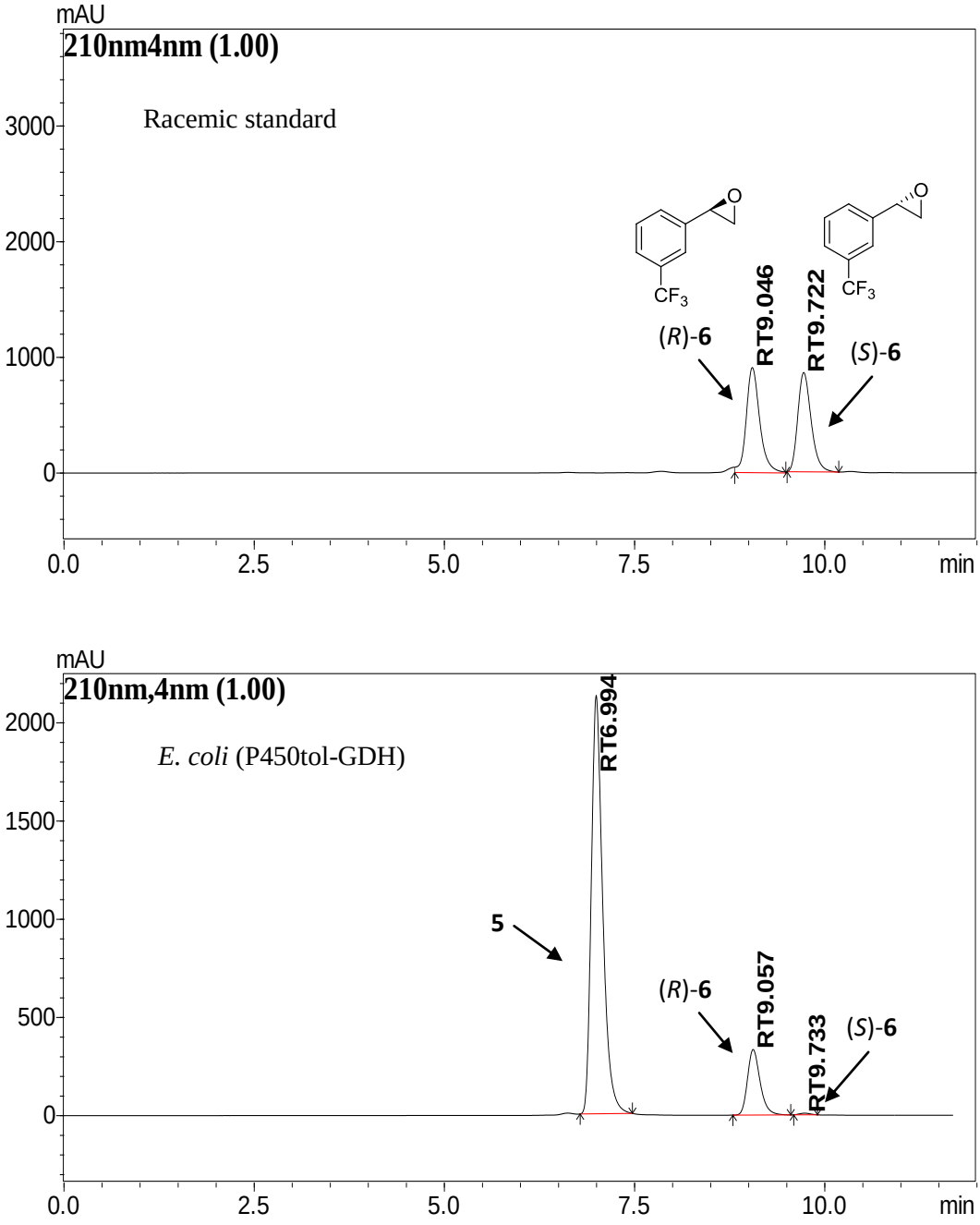


Figure S9. Chiral HPLC chromatograms for the analysis of 2-[3-(trifluoromethyl)phenyl]oxirane **6**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).

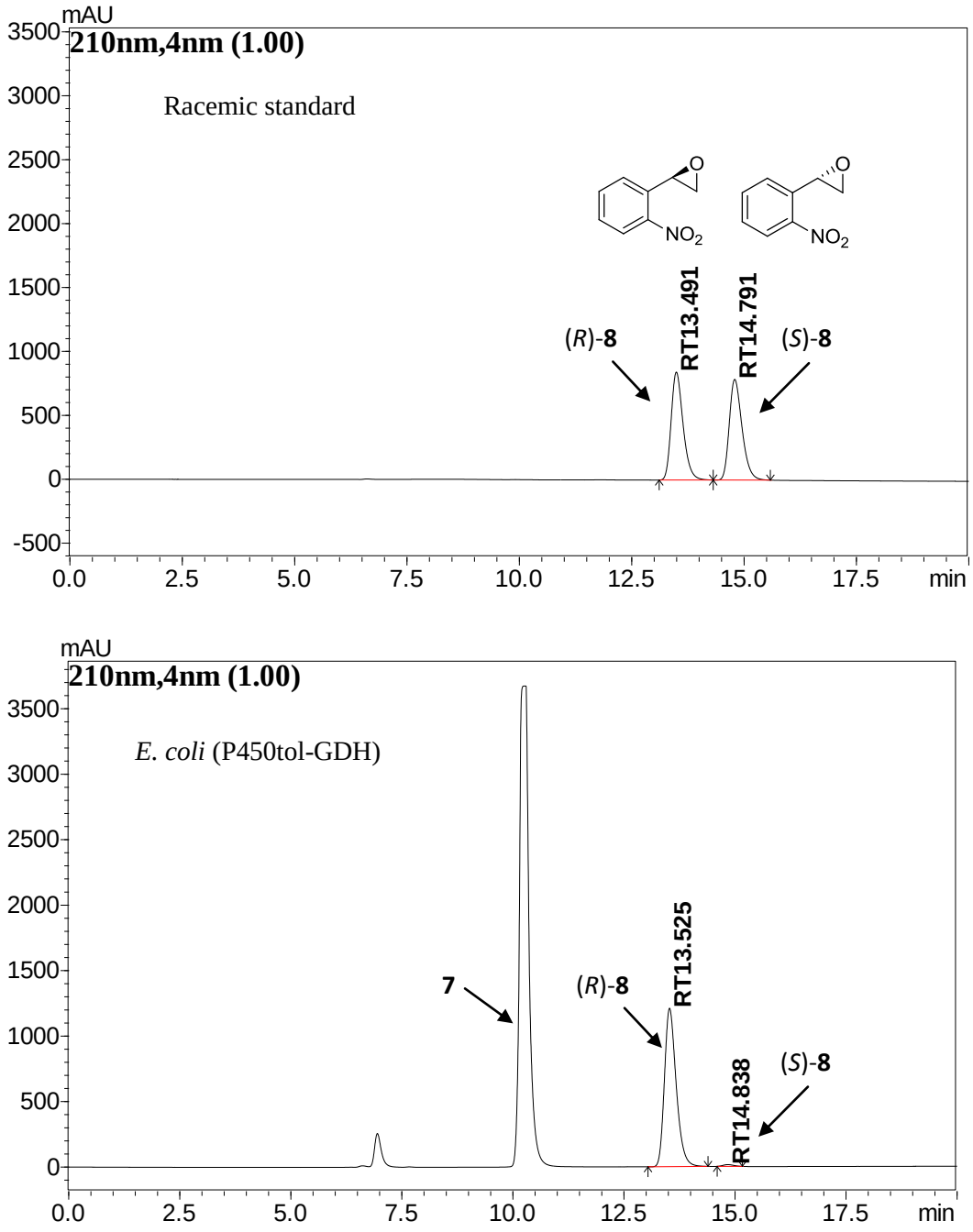
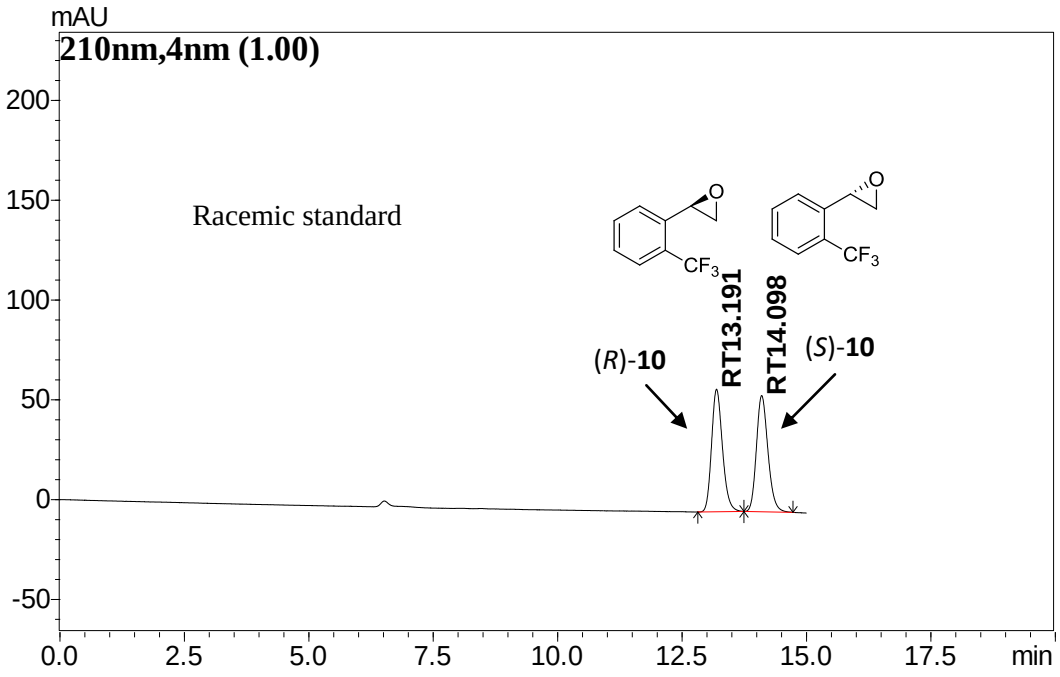


Figure S10. Chiral HPLC chromatograms for the analysis of 2-(2-nitrophenyl)oxirane **8**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).



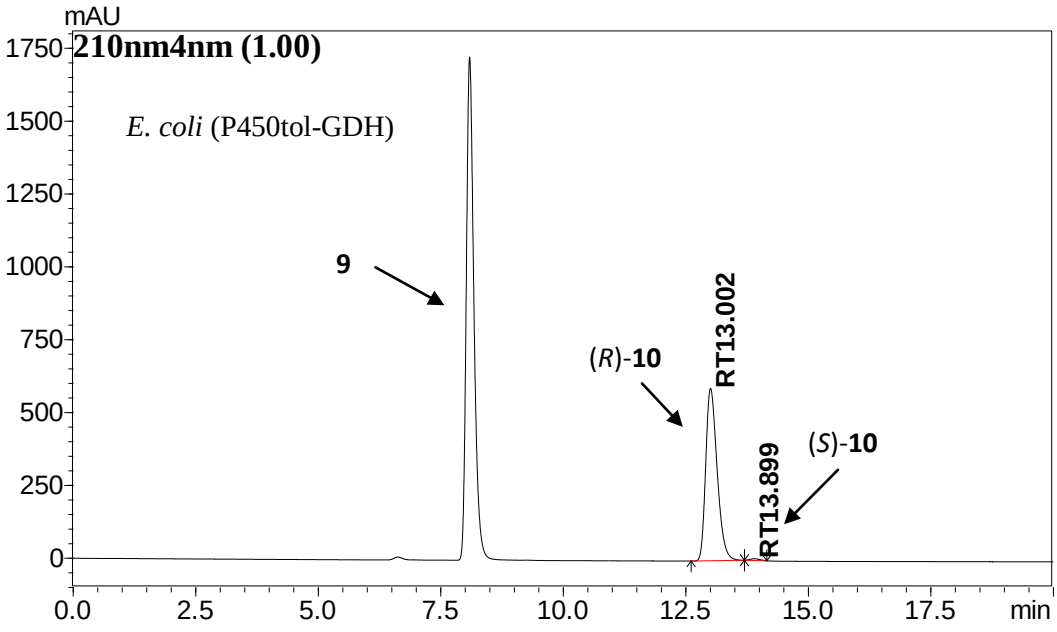
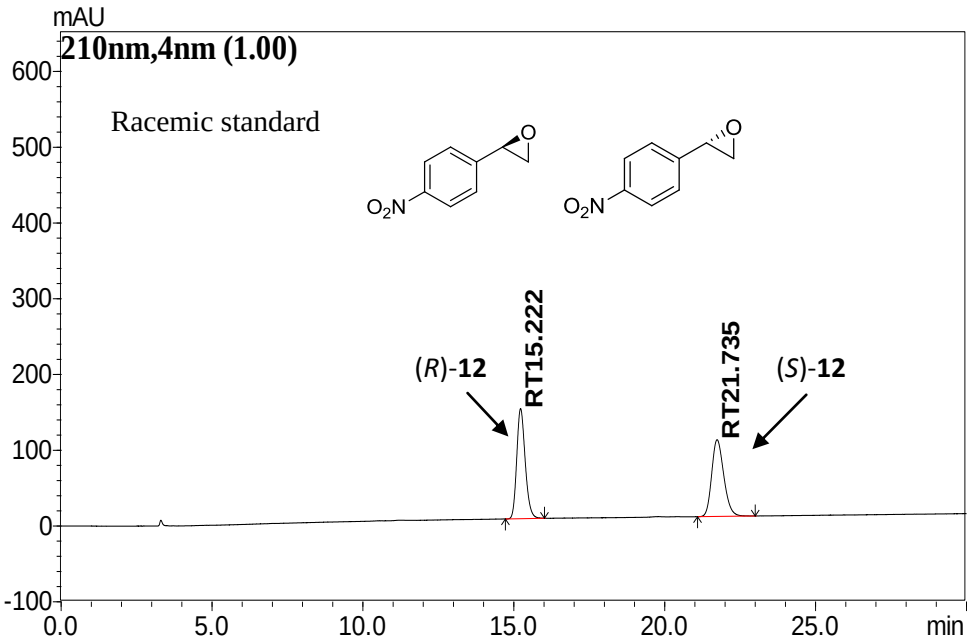


Figure S11. Chiral HPLC chromatograms for the analysis of 2-[2-(trifluoromethyl)phenyl]oxirane **10**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).



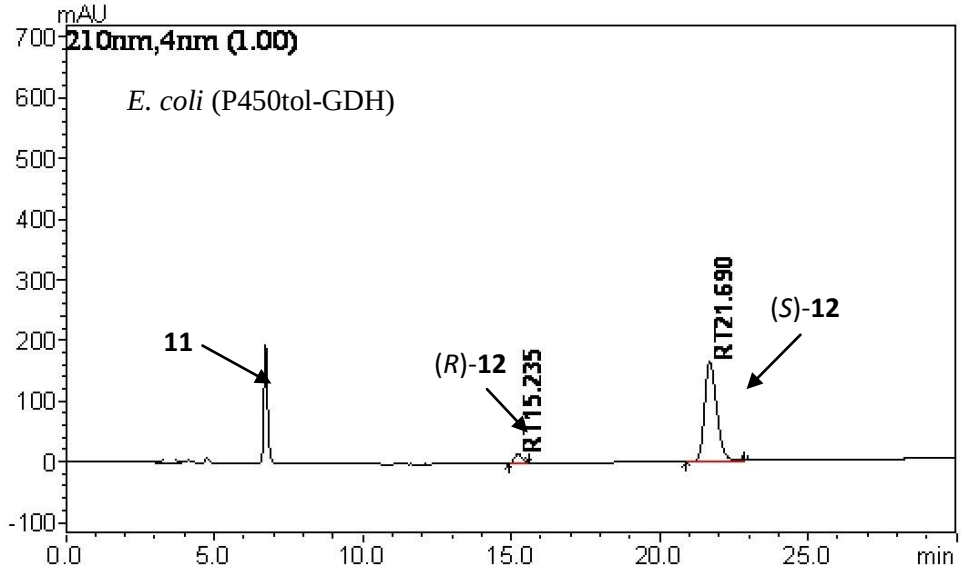
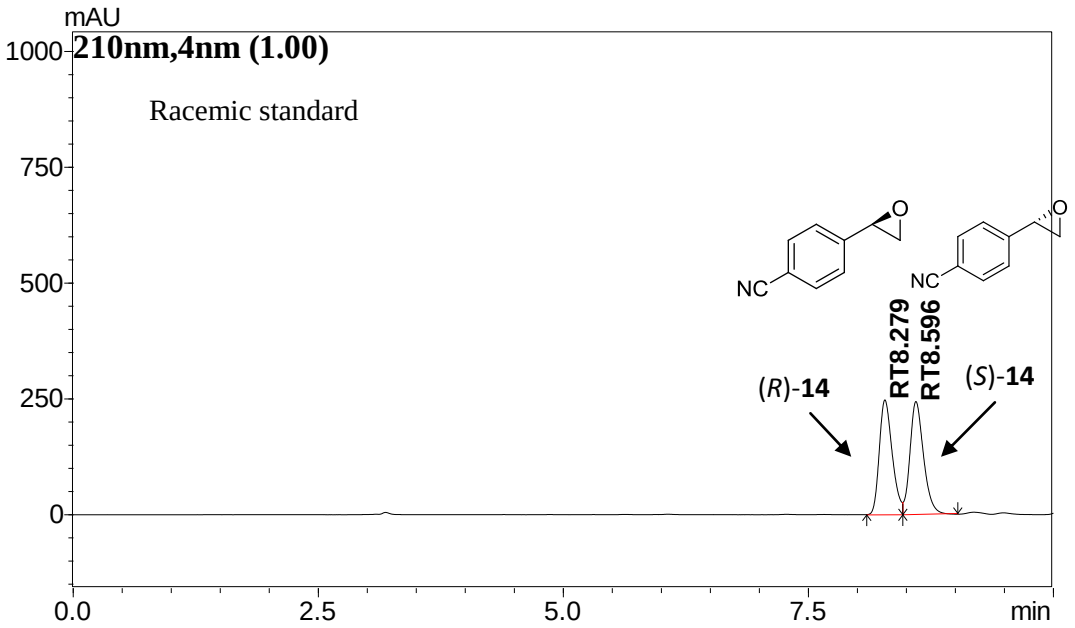


Figure S12. Chiral HPLC chromatograms for the analysis of 2-(4-nitrophenyl)oxirane **12**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).



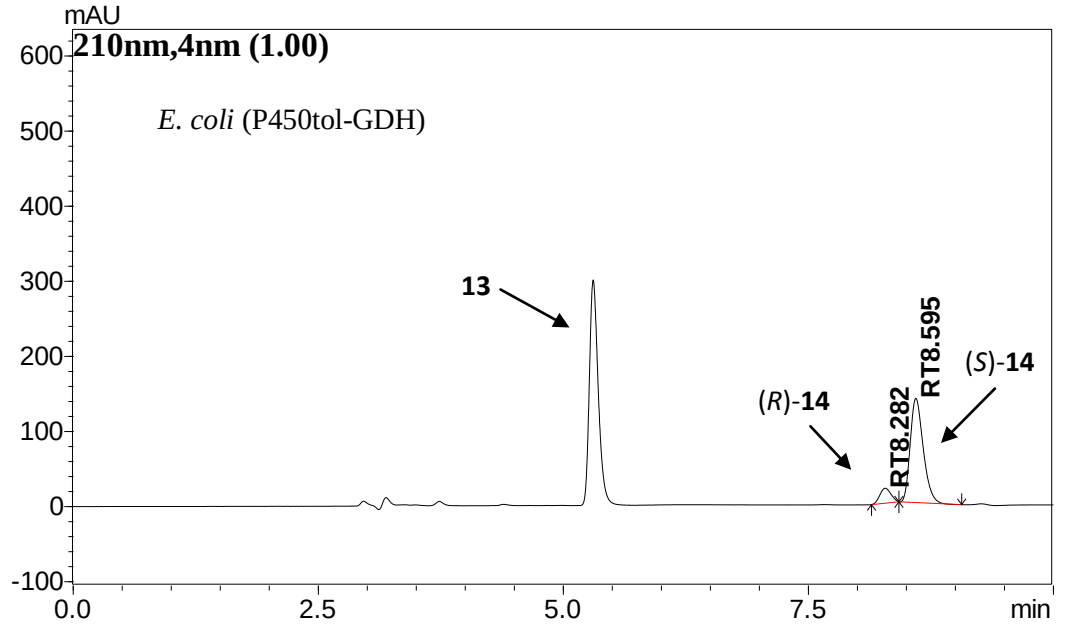
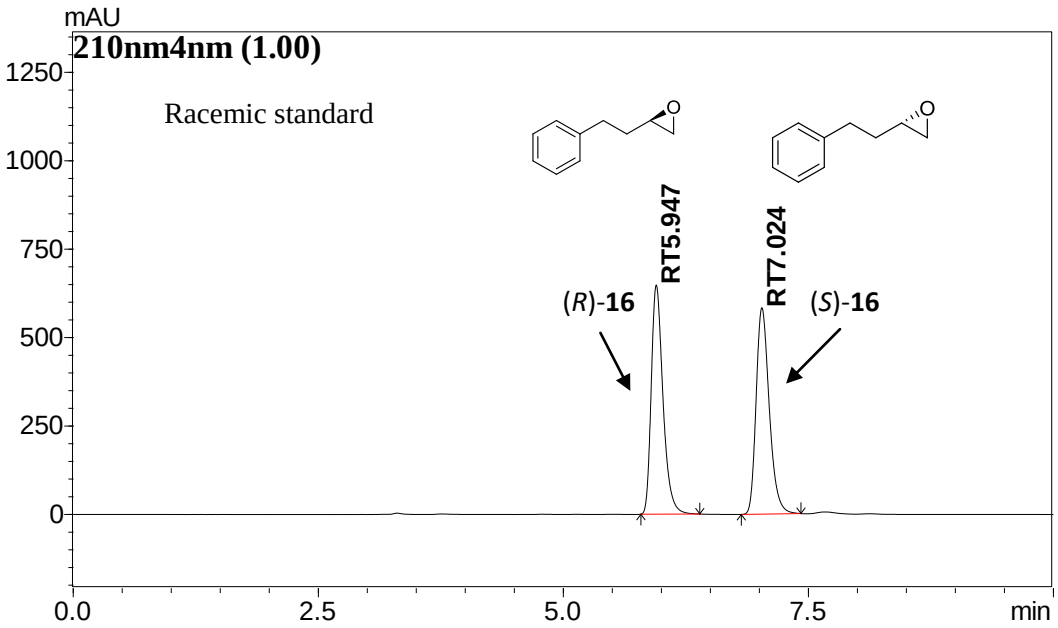


Figure S13. Chiral HPLC chromatograms for the analysis of 2-(4-cyanophenyl)oxirane **14**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).



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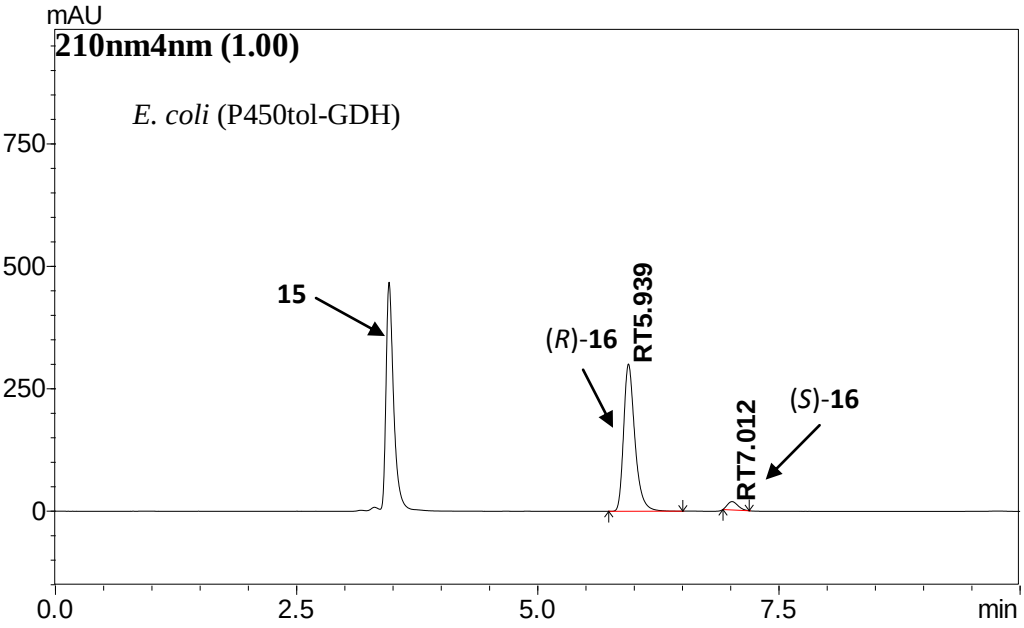

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3

4 **Figure S14**. Chiral HPLC chromatograms for the analysis of 2-(2-phenylethyl)oxirane **16**: racemic
5 standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed epoxidation (lower).

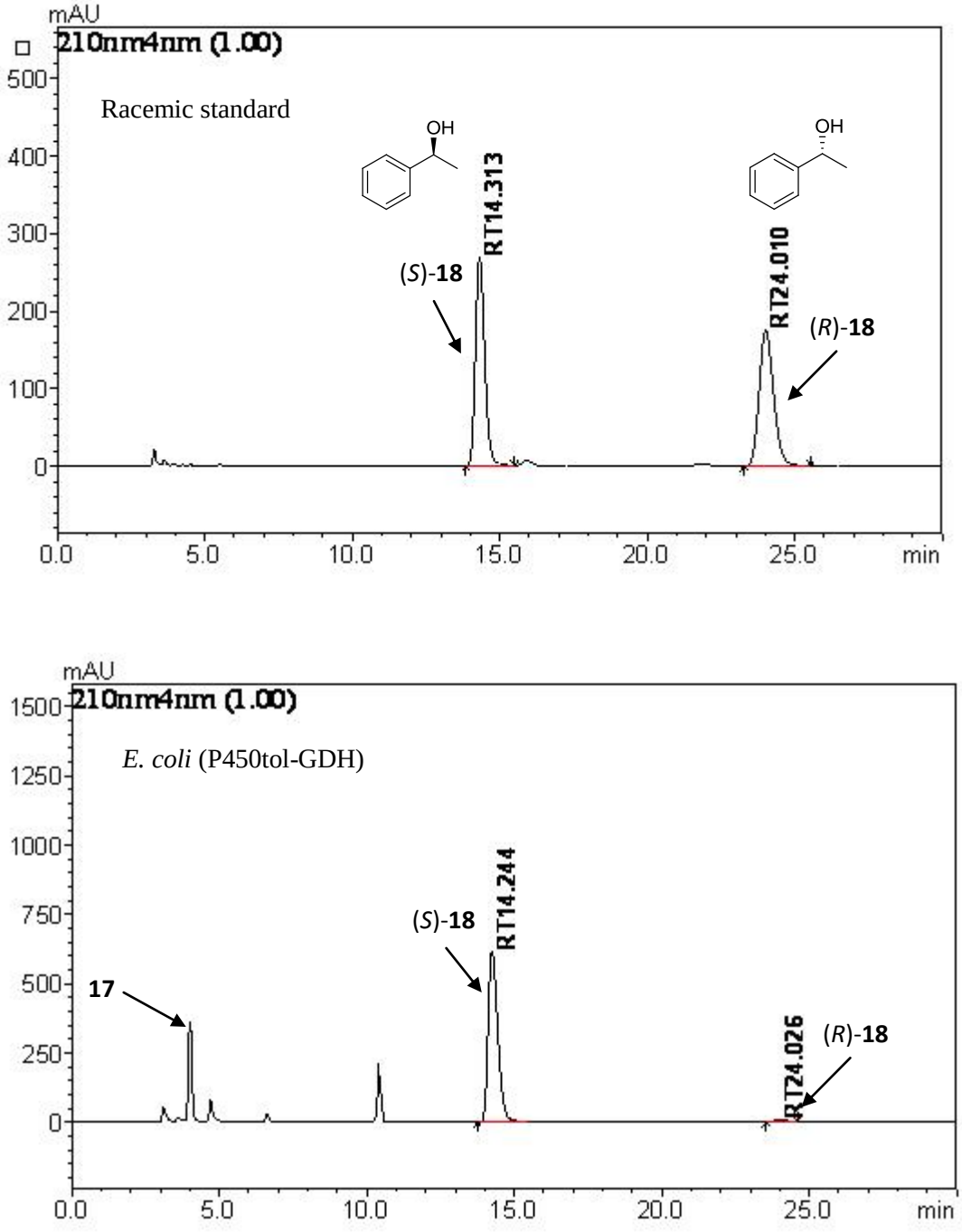

Figure S15. Chiral HPLC chromatograms for the analysis of 1-phenylethanol **18**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed hydroxylation (lower).

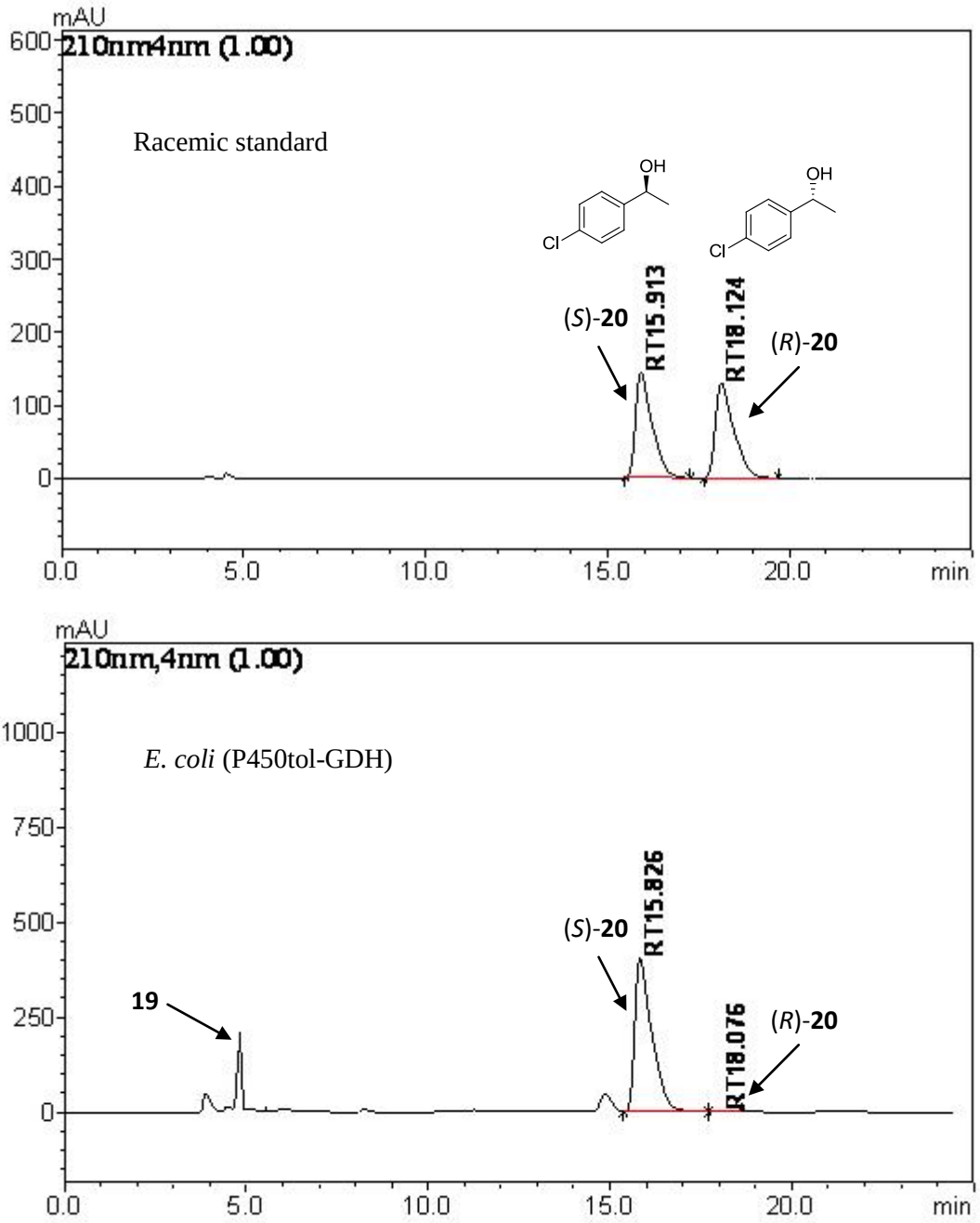

Figure S16. Chiral HPLC chromatograms for the analysis of 1-(4-chlorophenyl)ethanol **20**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed hydroxylation (lower).

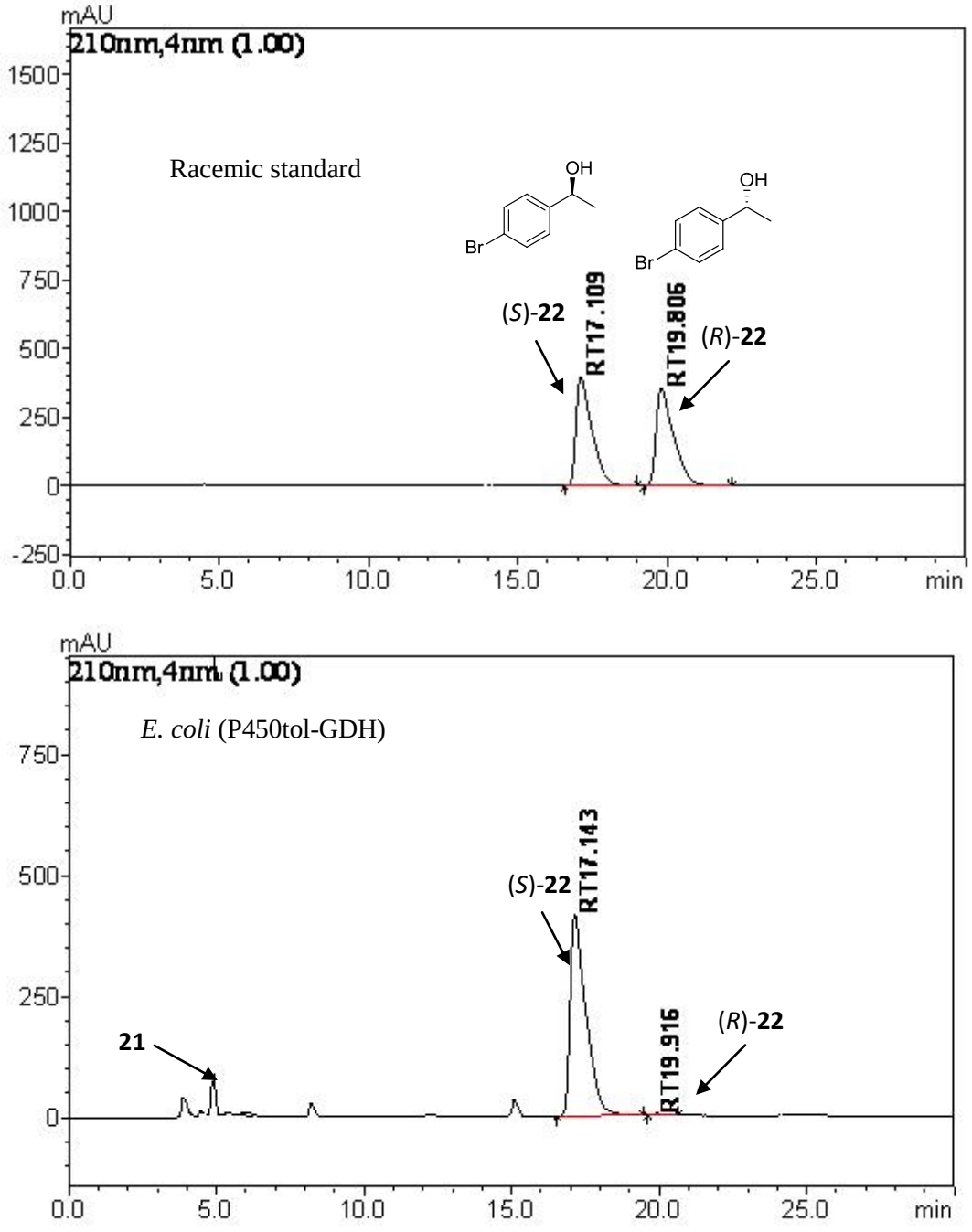

Figure S17. Chiral HPLC chromatograms for the analysis of 1-(4-bromophenyl)ethanol **22**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed hydroxylation (lower).

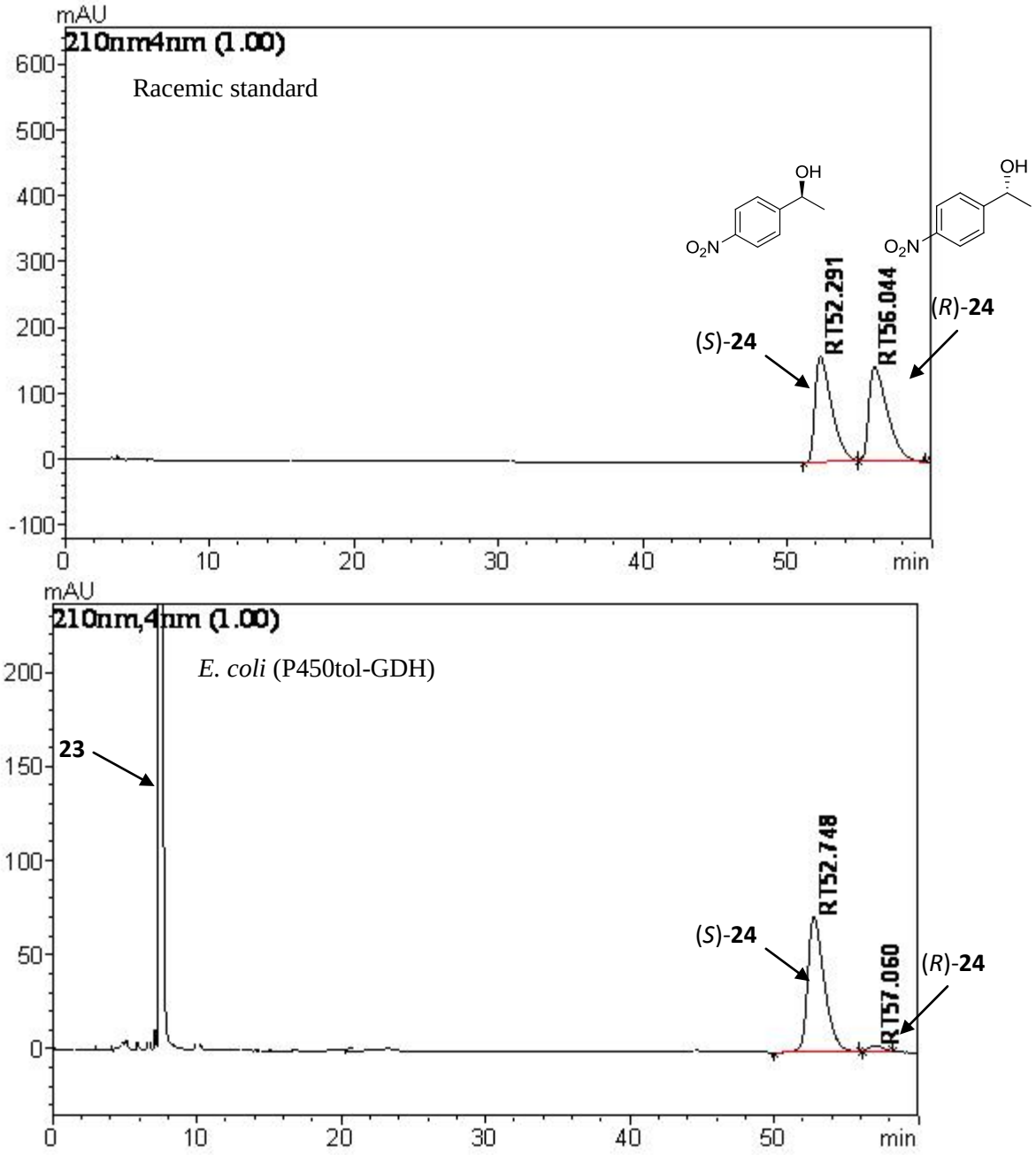

Figure S18. Chiral HPLC chromatograms for the analysis of 1-(4-nitrophenyl)ethanol **24**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed hydroxylation (lower).

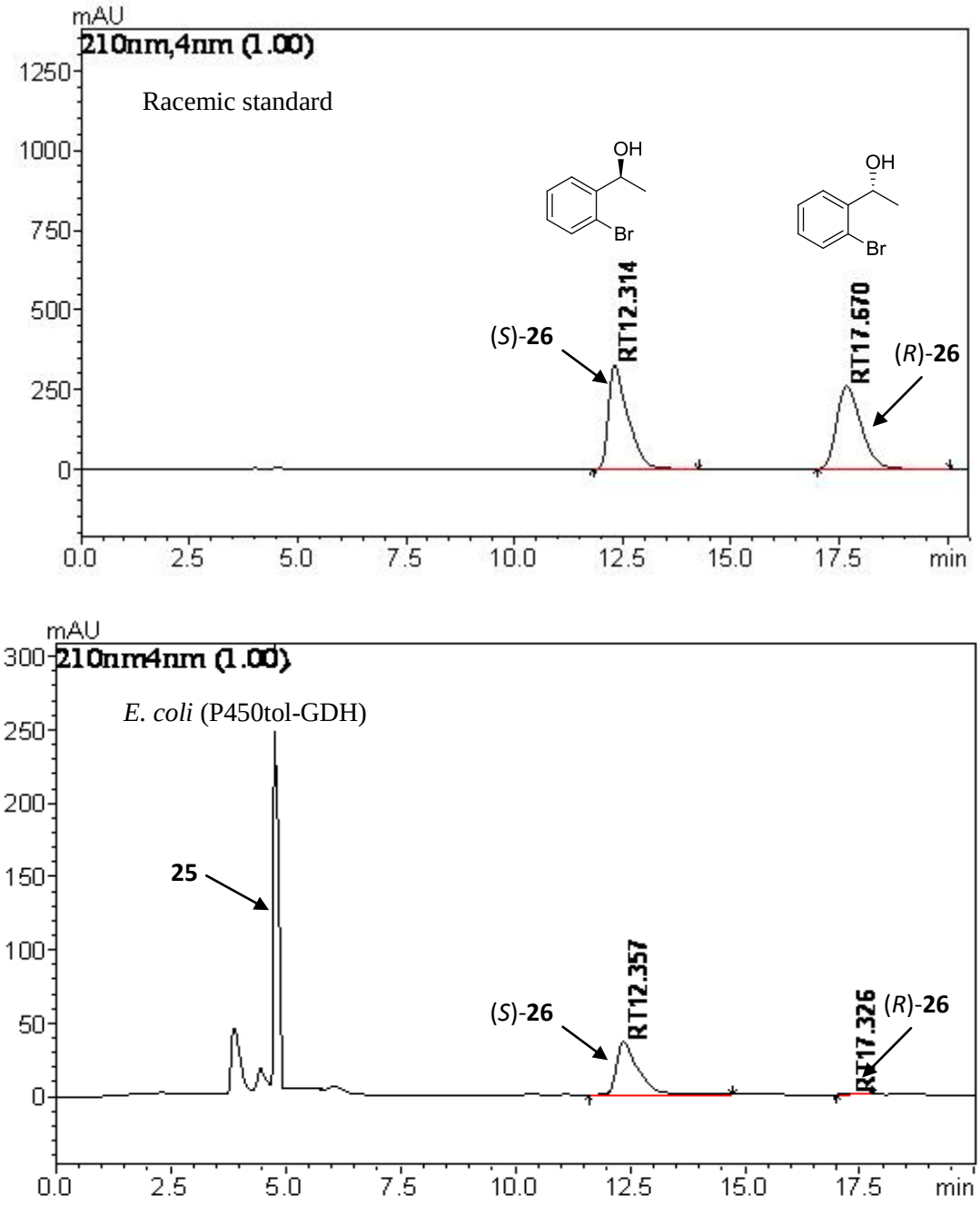

Figure S19. Chiral HPLC chromatograms for the analysis of 1-(2-bromophenyl)ethanol **26**: racemic standard (upper) and the product from *E. coli* (P450tol-GDH)-catalyzed hydroxylation (lower).

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