Supplementary Information

Asymmetric Ring Opening of Racemic Epoxides for Enantioselective Synthesis of (*S*)-β-Amino Alcohols by A Cofactor Self-sufficient Cascade Biocatalysis System

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1 Chemicals

Tryptone and yeast extract were obtained from Oxoid (Shanghai, China), T4 DNA ligase and restriction enzymes were purchased from New England Biolabs (Beijing, China). Taq plus DNA polymerase and IPTG (inducer, >99%) were purchased from Sangon Biotech (Shanghai, China). Plasmid isolation kit was from Tiangen (Shanghai, China). Antibiotics ampicillin and kanamycin were from Sigma Aldrich. (±)-1,2-Epoxybutane, (±)-1,2-epoxy-3-methylbutane, (±)-Styrene oxide (±)-4-Fluorostyrene oxide, (±)-4-Chlorostyrene oxide, (±)-4-Bromostyrene oxide, (±)-2-amino-1-butanol, (±)-valinol, (±)-2-amino-2-phenylethanol, 1-hydroxy-2-butanone, 1-hydroxy-3-methyl-2-butanone, 1-phenyl-2-hydroxyethanone and pyridoxal-5'-phosphate (PLP) were from Energy Chemical (Shanghai, China). (R)-2-Amino-2-(4-fluorophenyl)ethanol, (S)-2-Amino-2-(4-fluorophenyl)ethanol, (R)-2-Amino-2-(4-chlorophenyl)ethanol, (S)-2-Amino-2-(4-chlorophenyl)ethanol, (R)-2-Amino-2-(4-bromophenyl)ethanol, (S)-2-Amino-2-(4-bromophenyl)ethanol, (±)-2-Amino-2-(4-fluorophenyl)ethanol, (±)-2-Amino-2-(4-chlorophenyl)ethanol and (±)-2-Amino-2-(4-bromophenyl)ethanol were from Amatek Scientific (Suzhou, China). All other chemicals were of analytical grade and were commercially available.

2 Bacterial strains, vectors and culture conditions

E. coli DH5α and *E. coli* BL21 (DE3) supercompetent cells were obtained from Tiangen (Shanghai, China). They were routinely grown in Luria-Bertani (LB) medium at 37°C unless stated otherwise. Ampicillin (100 μg/mL) and kanamycin (50 μg/mL) were used for the selection of recombinant strains in *E. coli*. The plasmids pET28a (+), pETduet-1 and pRSFduet-1 for the heterogeneous expression studies were obtained from Novagen (Shanghai, China). Recombinant plasmids pETduet-BDHA,

pET28-GoSCR[1] and pET28-MVTA[2] were constructed as our previously described, and preserved in our lab.

3 Engineering of recombinant strains of *E. coli* (BDHA), *E. coli* (GoSCR), *E. coli* (SpEH), *E. coli* (BDHA-GoSCR), *E. coli* (SpEH-MVTA) and *E. coli* (SpEH-BDHA-GoSCR-MVTA)

E. coli (BDHA), *E. coli* (GoSCR)[1] and *E. coli* (MVTA)[2] were constructed as our previously described.

For *E. coli* (SpEH), epoxide hydrolase (SpEH) gene from *Sphingomonas* sp. HXN-200 was synthesized by General Biosystems (Anhui, China). The PCR amplifications were performed with Taq plus DNA polymerase (Sangon Biotech, Shanghai, China), with initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 65°C for 40 s, extension at 72°C for 1.5 min and followed by a final extension at 72°C for 10 min. The PCR product of epoxide hydrolase genes were isolated and digested with corresponding restriction endonucleases (*BamH*I, *Hind*III), cloned into the pRSFduet vector. The sequence of the insert DNA was subsequently confirmed by sequencing.

For *E. coli* (BDHA-GoSCR), GoSCR gene and recombinant plasmid pETduet-BDHA were digested with the same restriction endonucleases of *BamH*I and *Hind*III, respectively. After ligation and transform into *E. coli* BL21 (DE3), the recombinant cells were plated on LB plate containing ampicillin (100 μg/mL). The transformed strain co-expressing BDHA and GoSCR was abbreviated as *E. coli* (BDHA-GoSCR). The co-expression system was confirmed by SDS-PAGE and testing the activity of alcohol dehydrogenase for oxidation of vicinal diol.

For *E. coli* (SpEH-MVTA), MVTA gene and plasmid pRSFduet-SpEH were digested with the

same restriction endonucleases of *Nde*I and *Xho*I, respectively. After ligation and transform into *E. coli* BL21 (DE3), the recombinant cells were plated on LB plate containing kanamycin (50 μg/mL). The transformed strain co-expressing MVTA and SpEH was abbreviated as *E. coli* (SpEH-MVTA). The co-expression system was confirmed by SDS-PAGE and testing the activity of the enzyme.

For *E. coli* (SpEH-BDHA-GoSCR-MVTA), the constructed plasmids pETduet-BDHA-GoSCR and pRSFduet-SpEH-MVTA with the same origin of replication and different antibiotic selection were transformed into *E. coli* BL21 competent cells simultaneously and plated on LB plates containing ampicillin (100 μg/mL) and kanamycin (50 μg/mL). The transformed strain co-expressing SpEH, BDHA, GoSCR and MVTA was abbreviated as *E. coli* (SpEH-BDHA-GoSCR-MVTA).

4 Protein expression and activity of SpEH, BDHA, GoSCR and MVTA

The recombinant *E. coli* strains were grown overnight at 37°C in LB medium (3 mL in 20 mL tube) containing appropriate antibiotics (100 μg/mL ampicillin or 50 μg/mL kanamycin, or both) as indicated above. One milliliter of seed culture was transferred to 50 mL of terrific broth (TB) medium containing appropriate antibiotics in a 250 mL flask. The cells were grown for 2 to 3 h to reach an OD600 of 0.6−0.8 and then induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) (0.5 mM). Cell growth was continued at 20°C and 200 rpm for 12−18 h. Cells were harvested by centrifugation at 8,000 × g and 4°C for 10 min, washed twice with sodium phosphate buffer (100 mM, pH 7.5), and resuspended in the same buffer. The cell suspension was put on ice and sonicated for 90 times at 400 W for 4 s with 4 s of interval to yield cell-free extracts, after centrifugation (12,000 × *g* and 4°C), the supernatant of cell lysate was stored at −20°C for further use. SDS-PAGE was carried out employing a 12% polyacrylamide gels, stained with Coomassie Brilliant Blue R250.

The activity of epoxide hydrolase was tested as described previously[3].

The oxidation activity of alcohol dehydrogenase was measured by monitoring the NADH concentration increase using UV absorbance at 340 nm. The assay mixture contained 1 mL sodium phosphate buffer (pH 7.5, 100 mM), including 0.2 mM NAD^+, 10 mM substrate **2** and 10 μL enzyme solution (3 mg/mL). Reactions were started by addition of the enzyme solution and measured over a period of 1 min. The reaction mixture without NAD^+ or enzyme was used as a negative control. One unit of the enzyme activity was defined as the amount of enzyme catalyzing 1.0 μmol of NADH increase per minute under standard conditions (25°C, pH 7.5). The protein concentration of the enzyme solution was determined by the Bradford method[4].

The activity of MVTA was assayed by 2,3,5-triphenyltetrazolium chloride (TTC) method as previously described[5]. Briefly, the reaction mixture (200 μL) contained 100 mM sodium phosphate buffer (pH 8.0), 5 mM β-amino alcohols substrates, 5 mM pyurvate, 0.1 mM pyridoxal 5'-phosphate (PLP) and an appropriate amount of enzyme. After the enzyme solution was added, the reaction mixture were immediately incubated at 30°C in 96-well microplate for 5 min, 40 μL TTC-solution was quickly added, then, the color formation commenced at room temperature within 10 min due to the reaction with the α-hydroxy ketones. The activity measurements were performed on a Multiskan Spectrum Microplate Reader (Thermo Scientific Fisher Inc.) at 510 nm. All activity measurements were performed in triplicate. One unit of enzyme is defined as the amount of enzyme that catalyses the production of 1 μmol of α-hydroxy ketone in 1 min.

5 General procedure for conversion of vicinal diols 2a-f to (S)-β-amino alcohols 4a-f with the mixtures of lyophilized cell-free extract of *E. coli* (BDHA), *E. coli* (GoSCR) and *E. coli* (MVTA)

The freshly prepared cells of *E. coli* (BDHA), *E. coli* (GoSCR) and *E. coli* (MVTA) were resuspended in deionized (DI) water to a cell density of 20 g cell dry weight (CDW)/L, respectively. The cell suspension was put on ice and sonicated for 90 times at 400 W for 4 s with 4 s of interval to break the cells, and the mixture was centrifuged at 12,000 ×g at 4°C for 30 min to remove the cell debris. The protein concentration of the cell-free extracts was determined by Bradford method. Then, the cell-free extracts were frozen at -80^{o}C overnight, followed by lyophilization for 48 h to get the lyophilized enzyme powders. The lyophilized powders of the cell free-extracts of *E. coli* (MVTA), *E. coli* (BDHA) and *E. coli* (GoSCR) were mixed at different protein concentration. The standard reaction mixture was sodium phosphate buffer (100 mM, pH 7.5) containing 10-50 mM **2**a-f, 0.002-0.5 mM NAD^{+}, 20 mg/mL BDHA, 20 mg /mL GoSCR, 10 mg /mL MVTA, 0.1 mM PLP, (*R*)-MBA (15-55 mM) was also added as the amino donor. The reactions were carried out in 5 mL sodium phosphate buffer at 25°C and 200 rpm in capped 50 mL conical flask. To quantitate the formation of **4**a-f, 300 μL aliquots were taken out at different time points, basified by adding NaOH (0.1 mL, 10 N), saturated with NaCl and extracted with 0.5 mL ethyl acetate (EtOAc) containing 20 mM of *n*-dodecane as an internal standard. The organic phase were dried over anhydrous sodium sulfate and subjected to GC analysis. All experiments were performed in duplicate.

6 General procedure for conversion of racemic epoxide 1a-f to (S)-β-amino alcohols 4a-f with the mixtures of lyophilized cell-free extracts of *E. coli* (SpEH), *E. coli* (BDHA), *E. coli* (GoSCR) and *E. coli* (MVTA)

The freshly prepared cells of *E. coli* (SpEH), *E. coli* (BDHA), *E. coli* (GoSCR) and *E. coli* (MVTA) were resuspended in deionized (DI) water to a cell density of 20 g cdw/L, respectively. The cell

suspension was put on ice and sonicated for 90 times at 400 W for 4 s with 4 s of interval to break the cells, and the mixture was centrifuged at 12,000 ×*g* at 4°C for 30 min to remove the cell debris. The protein concentration of the cell-free extracts was determined by Bradford method. Then, the cell-free extracts were lyophilized and used for conversion of **1**a-f to **4**a-f or frozen at -80°C for further use. The standard reaction mixture was sodium phosphate buffer (100 mM, pH 7.5) containing 10-20 mM **1a-f**, 0.5 mM NAD^+, 10 mg/mL SpEH, 20 mg/mL BDHA, 20 mg/mL, 10 mg/mL MVTA, 0.1 mM, PLP, 10% DMSO, 15-25 mM (*R*)-MBA was also added as the amino donor. The reactions were carried out in 5 mL sodium phosphate buffer (100 mM, pH 7.5) at 25°C and 200 rpm in capped 50 mL conical flask. To quantitate the formation of **4**a-f, 300 μL aliquots were taken out at different time points, basified by adding NaOH (0.1 mL, 10 N), saturated with NaCl and extracted with 0.5 mL ethyl acetate (EtOAc) containing 20 mM of *n*-dodecane as an internal standard. The organic phase were dried over anhydrous sodium sulfate and subjected to GC analysis. All experiments were performed in duplicate.

7 General procedure for conversion of epoxides 1a-f to (*S*)-β-amino alcohols 4a-f with the mixture of resting cells of *E. coli* (BDHA-GoSCR) and *E. coli* (SpEH-MVTA)

The freshly prepared cells of *E. coli* (SpEH-MVTA) and *E. coli* (BDHA-GoSCR) were mixed in 5 mL 100 mM sodium phosphate buffer (pH 7.5) to a cell density of 10 g cdw/L and 15 g cdw/L, respectively. The standard reaction mixture was containing 10-20 mM epoxides **1**a-f, 0.1 mM PLP, 10% DMSO, 15-25 mM (*R*)-MBA, and the mixtures were shaken at 200 rpm at 30°C. To quantitate the formation of β-amino alcohols **4**a-f, 300 μL aliquots were taken out at different time points. Analytic samples were basified by adding NaOH (0.1 mL, 10 N), saturated with NaCl and extracted with 0.5 mL EtOAc containing 20 mM of *n*-dodecane as an internal standard. The organic phase were dried over

anhydrous sodium sulfate and subjected to GC analysis. All experiments were performed in duplicate.

8 General procedure for conversion of epoxides 1a-f to (*S*)-β-amino alcohols 4a-f with the resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA)

The freshly prepared cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) were resuspended in 5 mL 100 mM sodium phosphate buffer (pH 7.5) to a cell density of 10-20 g cdw/L, respectively. The standard reaction mixture was containing 10-50 mM epoxides **1**a-f, 0.1 mM PLP, 10% DMSO, 15-20 mM (*R*)-MBA, and the mixtures were shaken at 200 rpm at 30°C. For two-liquid phase system reaction, an equal volume of hexadecane was added. Samples were taken out at different time points to prepare analytic samples for the determination of the product concentration and ee, as described above. All experiments were performed in duplicate.

9 Preparation of (*S*)-4c from racemic 1c by cascade biocatalysis with the resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA)

The freshly prepared cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) were resuspended in 50 mL 100 mM sodium phosphate buffer (pH 7.5) to a cell density of 15 g cdw/L. 50 mL hexadecane containing racemic **1c** (50 mM, 300 mg) was added. The mixtures were shaken at 250 rpm and 30°C for 24 h. The reaction mixture was centrifuged to remove the cells. Aqueous phase was separated, acidified (pH<2, 1 M HCl) by adding 1 M HCl and extracted using ethyl acetate (3×50 ml) to remove the ketone and diol. Then the aqueous phase was basified (pH>12) by adding NaOH (10 N), saturated with NaCl, and extracted with ethyl acetate for three times (3 × 50 mL). All the organic phases were combined and then dried over Na_2SO_4. After filtration, the organic solvent was removed by evaporation at reduced

pressure. The crude products were purified by flash chromatography on a silica gel column to give (*S*)-**4c** as a white solid in 80.0% yield (240 mg) and >99% *ee*. ^{1}H NMR (400 MHz, 298K, $CDCl_3$) δ_H 7.30−7.18 (5H, m, Ph), 3.97 (1H, dd, $^3J_{HH}$ = 4.0 Hz, 8.5Hz, CH), 3.66 (^{1}H, dd, $^2J_{HH}$ = 11.0Hz, $^3J_{HH}$ = 4.0 Hz, CH_2), 3.48 (1H, dd, $^2J_{HH}$ = 11.0Hz, $^3J_{HH}$ = 8.5 Hz, CH_2), 2.05 (2H, s, NH_2).

10 Assay method

The concentrations of β-amino alcohol were measured by gas chromatograph. Gas chromatography analysis was carried out with a GC-14C gas chromatography (Shimadzu, Japan) equipped with a flame ionization detector (FID) and an HP-5 column (30 m × 0.320 mm × 0.25 mm; Agilent Technologies, lnc.). The analytical conditions were as follows: for **4**a, **4**b, **4**c and **4**d, column temperature, 120°C; for **4**e, column temperature, 140°C; for **4**f, column temperature, 150°C; injection temperature, 250°C; detector temperature, 250°C.

The enantiomeric excesss of β-amino alcohols was determined by GC using a method described by Mutti[6]. Briefly, the samples were analyzed with a chiral column (CP-Chirasil-Dex CB, 25 m x 0.32 mm x 0.25 μm; Agilent Technologies, lnc.) after derivatization with 4-dimethylamino pyridine (DMAP) and acetic anhydride. The analytical conditions were as follows: For **4**a, column temperature, 120°C for 0 min, 2°C min^{-1} to 160°C and hold 10 min; For **4**b, column temperature, 100°C for 0 min, 2°C min^{-1} to 120°C and hold 3 min, then 2°C min^{-1} to 160°C and hold 5 min; For **4**c, column temperature, 120°C for 0 min, 2°C min^{-1} to 160°C and hold 20 min; For **4**d, column temperature, 100°C for 0 min, 2°C min^{-1} to 120°C, then 5°C min^{-1} to 160°C and hold 22 min; For **4**e, column temperature, 140°C for 0 min, 2°C min^{-1} to 180°C and hold 20 min; For **4**f, column temperature, 140°C for 0 min, 2°C min^{-1} to 180°C and hold 30 min; injection temperature, 250°C; detector temperature, 250°C. The absolute configuration of β-amino alcohols was determined as described by Mutti et al[6] and Pressnitz et al[7].

Table S1. Substrate specific and enantioselectivity of MVTA for reduction amination of α-hydroxy ketones[a]

Substrate	Time (h)	Conversion [%][b]	Product	ee [%][c]
3a	2	>99	(*S*)-**4**a	97
3b	3	>99	(*S*)-**4**b	98
3c	2	>99	(*S*)-**4**c	>99
3d	2	>99	(*S*)-**4**d	>99
3e	5	>99	(*S*)-**4**e	>99
3f	5	>99	(*S*)-**4**f	>99

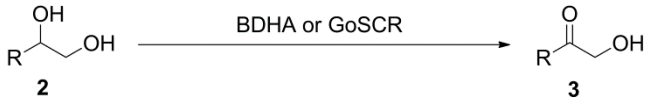
[a] Standard conditions: 1 mL sodium phosphate buffer (pH 8.0, 100 mM), including 20 mM substrate, 20 mM (*R*)-MBA, 10% (V/V) DMSO, 0.1 mM PLP, 5 mg/mL MVTA, 30°C, 200 rpm. [b] Conversion was determined by GC; [c] ee value was determined by chiral GC.

Table S2. Selection of alcohol dehydrogenases[a]

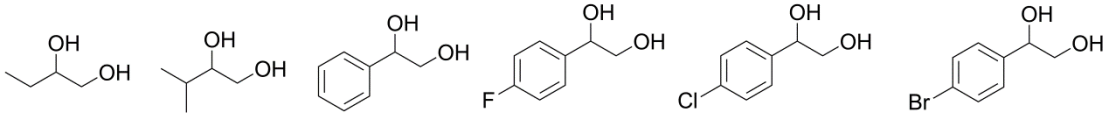
Enzyme	Specific activity		
	(*R*)-**2**c [U/mg protein]	(*S*)-**2**c [U/mg protein]	Acetophenone [U/mg protein]
Go0716[b]	-	-	1.206
Go2108[b]			0.724
Go2378[b]	0.036	0.044	0.119
Go1538[b]			0.503
Go0313[b]	0.12	0.07	0.238
GoSCR[b]	-	0.12	1.257
YucD[c]	-	-	1.272
SCD[c]	-	-	0.905
GutB[c]	0.22	-	0.392
YxnA[c]	-	-	0.415
BDHA[c]	0.80	-	0.428
IOlG[c]	-	-	0.286
YugJ[c]	-	-	0.252
YutJ[c]			0.318
RDR[d]	-	-	2.204

[a] Standard conditions: 1 mL sodium phosphate buffer (pH 8.0, 100 mM), including 10 mM substrate, 0.5 mM NAD^+, 10 µl alcohol dehydrogenase (3 mg/mL), 25°C. [b] enzymes from *Gluconobacter oxydans*; [c] enzymes from *Bacillus subtilis*; [d] enzyme from *Devosia riboflavina*

Table S3. Substrate specific of BDHA and GoSCR for oxidation of diol **2**.



2 **3**

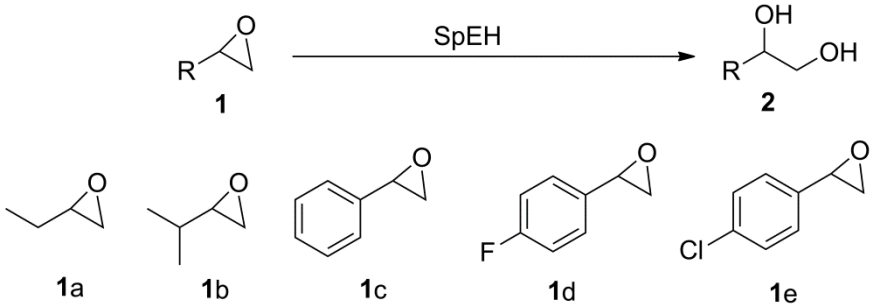


2a **2b** **2c** **2d** **2e** **2f**

Entry	Substrate	BDHA [U/mg protein][a]	GoSCR [U/mg protein] [a]
1	(*R*)-**2**a	0.181	-
2	(*R*)-**2**b	0.488	-
3	(*R*)-**2**c	0.791	-
4	(*R*)-**2**d	0.133	-
5	(*R*)-**2**e	0.168	-
6	(*R*)-**2**f	0.488	-
7	(*S*)-**2**a	-	0.087
8	(*S*)-**2**b	-	0.068
9	(*S*)-**2**c	-	0.119
10	(*S*)-**2**d	-	0.068
11	(*S*)-**2**e	-	0.071
12	(*S*)-**2**f	-	0.067

[a] Standard conditions: 1 mL sodium phosphate buffer (pH 8.0, 100 mM), including 10 mM substrate, 0.5 mM NAD^+, 10 µl alcohol dehydrogenase (3 mg/mL), 25°C.

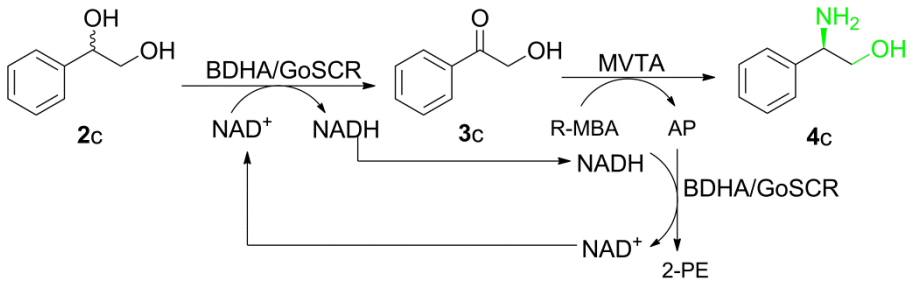
Table S4. Substrate specific and enantioselectivity of SpEH for hydrolysis of epoxides **1**[a]



Entry	Substrate	Time [h]	Conversion [%][b]	Product	ee [%][c]
1	**1**a	2	99	**2**a (*S*)	26.0
2	**1**b	2	99	**2**b (*S*)	3.0
3	**1**c	3	99	**2**c (*S*)	25.8
4	**1**d	3	99	**2**d (*S*)	34.6
5	**1**e	3	99	**2**e (*S*)	32.8
6	**1**f	3	99	**2**f (*S*)	39.9

[a] Standard conditions: 1 mL sodium phosphate buffer (pH 8.0, 100 mM), including 20 mM substrate, 10% DMSO, 5 mg/mL cell free extract of *E. coli* (SpEH), 30°C, 200 rpm. [b] Conversion was determined by GC, error limit: <2% of the state values; [c] ee value was determined by chiral GC.

Table S5. Effects of different pH on the synthesis of (*S*)-phenylglycinol **4**c with the mixture of BDHA, GoSCR and MVTA[a]



Entry	Sub.	BDHA [mg mL^{-1}]	GoSCR [mg mL^{-1}]	MVTA [mg mL^{-1}]	pH	Conv. to **4**c [%][b]	(*S*)-**4**c ee [%][c]
1	(*R*)-**2**c	20		10	7.0	53.0	>99
2	(*R*)-**2**c	20		10	7.4	59.1	>99
3	(*R*)-**2**c	20		10	8.0	49.2	>99
4	(*S*)-**2**c		20	10	7.0	58.0	>99
5	(*S*)-**2**c		20	10	7.4	62.2	>99
6	(*S*)-**2**c		20	10	8.0	47.2	>99

[a] Standard conditions: 5 mL sodium phosphate buffer (100 mM, pH7.0-8.0), including 50 mM **2**c, 0.2 mM NAD$^+$, 55 mM R-MBA, 20 mg/mL BDHA, 20 mg/mL GoSCR, 10 mg/mL MVTA, 0.1 mM PLP, 30^{o}C, 200 rpm, 7 h. [b] Conversion was determined by GC, error limit: <2% of the state values; [c] ee value was determined by chiral GC.

Table S6. Effects of different temperature on synthesis of (*S*)-phenylglycinol **4**c with the mixture of BDHA, GoSCR and MVTA[a]

Entry	Sub.	BDHA [mg mL^{-1}]	GoSCR [mg mL^{-1}]	MVTA [mg mL^{-1}]	Temp. [^{o}C]	Conv. to **4**c [%][b]	(*S*)-**4**c ee [%][c]
1	(*R*)-**2**c	20		10	25	67.1	>99
2	(*R*)-**2**c	20		10	30	75.7	>99
3	(*R*)-**2**c	20		10	35	49.8	>99
4	(*S*)-**2**c		20	10	25	73.8	>99
5	(*S*)-**2**c		20	10	30	80.2	>99
6	(*S*)-**2**c		20	10	35	69.0	>99

[a] Standard conditions: 5 mL sodium phosphate buffer (100 mM, pH 7.5), including 50 mM **2**c, 0.2 mM NAD^{+}, 55 mM R-MBA, 20 mg/mL BDHA, 20 mg/mL GoSCR, 10 mg/mL MVTA, 0.1 mM PLP, 25-35^{o}C, 200 rpm, 7 h. [b] Conversion was determined by GC, error limit: <2% of the state values; [c] ee value was determined by chiral GC.

Table S7. Effects of different concentration of cofactor on synthesis of (*S*)-Phenylglycinol **4**c with the mixture of BDHA, GoSCR and MVTA[a]

Entry	Sub.	NAD^+ [mM]	BDHA [mg mL^{-1}]	GoSCR [mg mL^{-1}]	MVTA [mg mL^{-1}]	Conv. to **4**c [%][b]	(*S*)-**4**c ee [%][c]
1	(*R*)-**2**c	0.5	20		10	97.7	>99
2	(*R*)-**2**c	0.2	20		10	67.8	>99
3	(*R*)-**2**c	0.1	20		10	65.2	>99
4	(*R*)-**2**c	0.05	20		10	57.0	>99
5	(*S*)-**2**c	0.5		20	10	92.9	>99
6	(*S*)-**2**c	0.2		20	10	85.0	>99
7	(*S*)-**2**c	0.1		20	10	70.9	>99
8	(*S*)-**2**c	0.05		20	10	66.4	>99

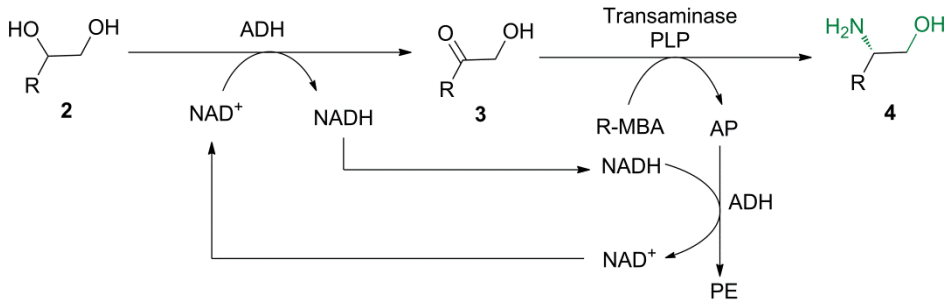
[a] Standard conditions: 5 mL sodium phosphate buffer (100 mM, pH 7.5), including 50 mM **2**c, 0.05-0.5 mM NAD^+, 55 mM R-MBA, 20 mg/mL BDHA, 20 mg/mL GoSCR, 10 mg/mL MVTA, 0.1 mM PLP, 30^{o}C, 200 rpm, 7 h. [b] Conversion was determined by GC, error limit: <2% of the state values; [c] ee value was determined by chiral GC.

Table S8. Effect of different ratios of two enzymes on synthesis of (*S*)-phenylglycinol **4**c with the mixture of BDHA, GoSCR and MVTA[a]

Entry	Sub.	Sub. conc. [mM]	BDHA [mg mL^{-1}]	GoSCR [mg mL^{-1}]	MVTA [mg mL^{-1}]	Conv. to **4**c [%][b]	(*S*)-**4**c ee [%][c]
1	(*R*)-**2**c	50	20		10	93.1	>99
2	(*R*)-**2**c	50	15		15	85.8	>99
3	(*R*)-**2**c	50	10		20	70.2	>99
4	(*S*)-**2**c	50		20	10	97.1	>99
5	(*S*)-**2**c	50		15	15	89.4	>99
6	(*S*)-**2**c	50		10	20	64.9	>99
7	Rac-**2**c	50	20	20	10	99.0	>99

[a] Standard conditions: 5 mL sodium phosphate buffer (100 mM, pH 7.5), including 50 mM **2**c, 0.5 mM NAD^+, 55 mM R-MBA, 10-20 mg/mL BDHA, 10-20 mg/mL GoSCR, 10-20 mg/mL MVTA, 0.1 mM PLP, 30°C, 200 rpm, 7 h. [b] Conversion was determined by GC, error limit: <2% of the state values; [c] ee value was determined by chiral GC.

Table S9. Enantioselective conversion of racemic vicinal diols **2** to chiral β-amino alcohol **4** with the mixture of BDHA, GoSCR and MVTA[a]



Entry	Sub.	Sub. concn [mM]	R-MBA [mM]	BDHA [mg mL^{-1}]	GoSCR [mg mL^{-1}]	MVTA [mg mL^{-1}]	T [h]	Conv. to **4** [%][b]	(*S*)-**4**c ee [%][c]
1	**2**a	20	25	20	20	10	6	99.0	97
2	**2**b	20	25	20	20	10	24	80.6	98
3	**2**c	50	55	20	20	10	7	99.0	>99
4	**2**c	100	110	40	40	20	12	90.0	>99
5	**2**d	20	25	20	20	10	12	93.1	>99
6	**2**e	20	25	20	20	10	12	55.6	>99
7	**2**f	20	25	20	20	10	12	31.6	>99

[a] Standard conditions: 5 mL sodium phosphate buffer (100 mM, pH 7.5), including 20-100 mM substrate **2**, 0.5 mM NAD^+, 25-110 mM R-MBA, 20-40 mg/mL BDHA, 20-40 mg/mL GoSCR, 10 mg/mL MVTA, 0.1 mM PLP, 30°C, 200 rpm, 7 h. [b] Conversion was determined by GC, error limit: <2% of the state values; [c] ee value was determined by chiral GC.

Table S10 Asymmetric ring opening of racemic epoxides **1** to (S)-β-amino alcohols **4** with the mixtures of resting cells of *E. coli* (SpEH-MVTA) (Catalyst 1) and *E. coli* (BDHA-GoSCR) (Catalyst 2)

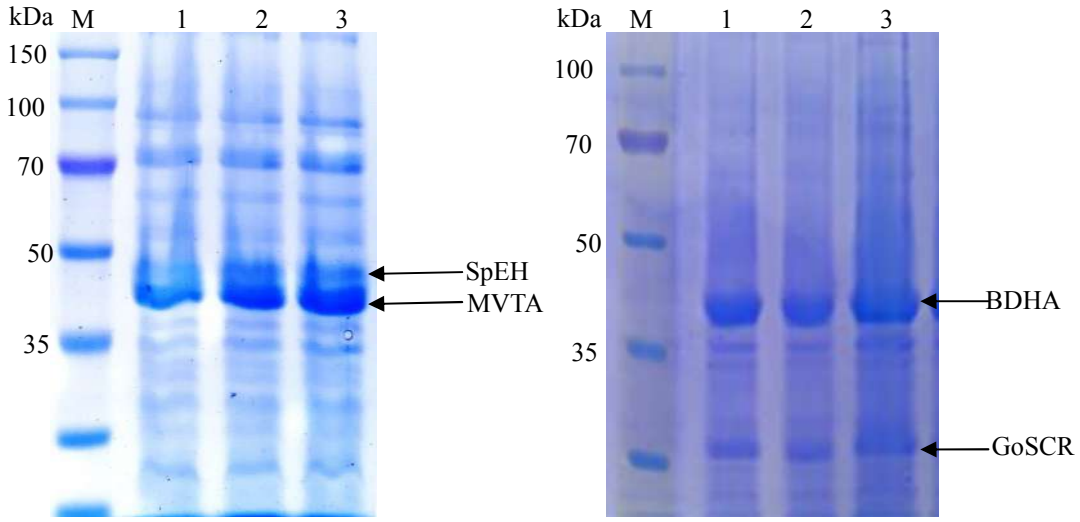
Entry	Sub.	Sub. conc. [mM]	Catalyst 1 [g cdw L^{-1}]	Catalyst 2 [g cdw L^{-1}]	Time [h]	Conv. to **4** [%][a]	(S)-**4** ee [%][b]
1	**1**a	10	10	16	12	42.5	97
2	**1**b	10	10	16	12	49.9	98
3	**1**c	20	10	16	12	68.1	>99
4	**1**d	20	14	22	24	77.7	>99
5	**1**e	20	14	22	24	66.2	>99
6	**1**f	20	14	22	24	61.8	>99

The reactions were conducted using 15-25 mM of the amine donor (R-MBA) in 100 mM sodium phosphate buffer (pH 7.5) containing 0.1 mM PLP, 10-20 mM substrates, 10% DMSO at 30°C. [a] Conversion was determined by GC, error limit: <2% of the state values. [b] ee was determined by chiral GC.

Table S11 Asymmetric ring opening of racemic epoxides **1** to (S)-β-amino alcohol **4** with the resting cell of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (Catalyst 3)

Entry	Sub.	Sub. conc. (mM)	R(+)-MBA [mM]	Catalyst 3 [g cdw L-1]	Time [h]	Conv. to **4** [%][a]	(S)-**4** ee [%][b]
1	**1**a	10	15	10	20	71.0	97
2	**1**b	10	15	10	20	72.0	98
3	**1**c	10	15	10	12	99.0	>99
4	**1**c	20	25	20	24	99.0	>99
5	**1**c	50	55	20	24	5.6	>99
6	**1**d	10	15	12	20	91.3	>99
7	**1**d	20	25	25	24	63.9	>99
8	**1**e	10	15	12	20	61.7	>99
9	**1**f	10	15	12	28	48.4	>99

The reactions were conducted using 15-55 mM of the amine donor (R-MBA) in 100 mM sodium phosphate buffer (pH 7.5) containing 0.1 mM PLP, 10-50 mM substrates, 10% DMSO at 30^{o}C. [a] Conversion was determined by GC, error limit: <2% of the state values. [b] ee was determined by chiral GC.



(a) (b)

Figure S1. SDS-PAGE of the cell-free extracts of *E. coli* (SpEH-MVTA) (a) and *E. coli* (BDHA-GoSCR) (b) after induction for 4-12 h. a: Lane M: protein marker, lane 1: *E. coli* (SpEH-MVTA) after induction for 4 h, lane 2: *E. coli* (SpEH-MVTA) after induction for 8 h,, lane 3: *E. coli* (SpEH-MVTA) after induction for 12 h. b: Lane M: protein marker, lane 1: *E. coli* (BDHA-GoSCR) after induction for 4 h,, lane 2: *E. coli* (DHA-GoSCR) after induction for 8 h, lane 3: *E. coli* (BDHA-GoSCR) after induction for 12 h.

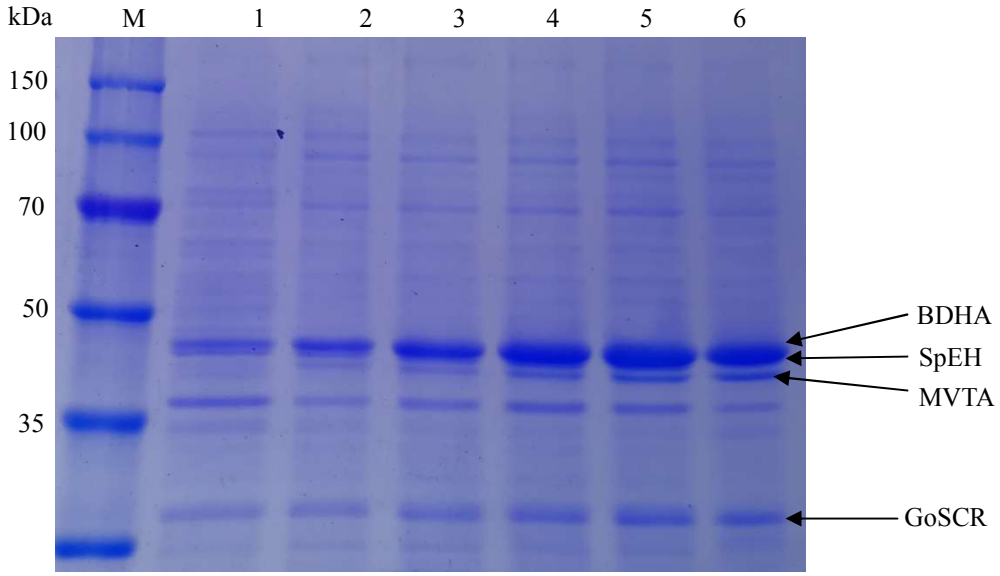


Figure S2. SDS-PAGE of the cell-free extracts of *E. coli* (SpEH-BDHA-GoSCR-MVTA) strains after inducton for 2-16 h. Lane M: protein marker, lane 1: *E. coli* (SpEH-BDHA-GoSCR-MVTA) after induction for 2 h, lane 2: *E. coli* (SpEH-BDHA-GoSCR-MVTA) after induction for 4 h,, lane 3: *E. coli* (SpEH-BDHA-GoSCR-MVTA) after induction for 8 h,, lane 4: *E. coli* (SpEH-BDHA-GoSCR-MVTA) after induction for 12 h,, lane 5: *E. coli* (SpEH-BDHA-GoSCR-MVTA) after induction for 14 h, lane 6: *E. coli* (SpEH-BDHA-GoSCR-MVTA) after induction for 16 h.

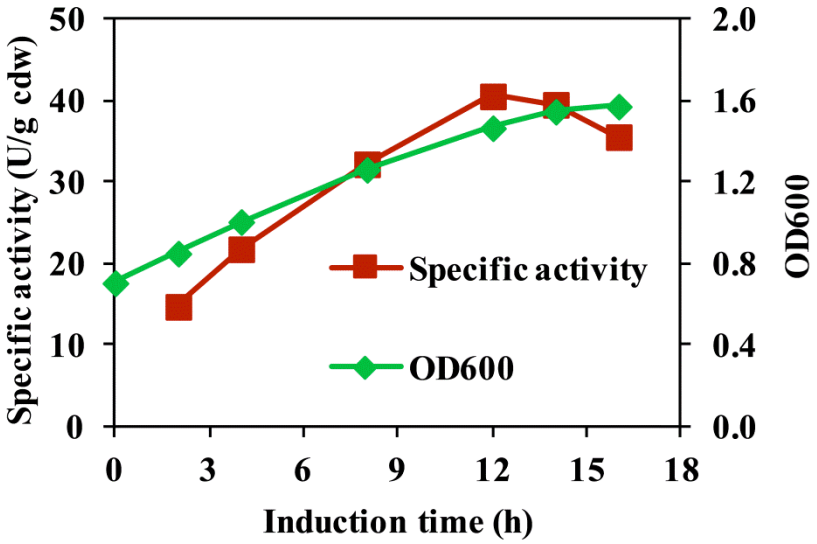


Figure S3. Cell growth and specific activity of *E. coli* (SpEH-MVTA-GoSCR-BDHA) for conversion of **1c** to (*S*)-**4c**. ♦: Cell density, ■: Specific activity.

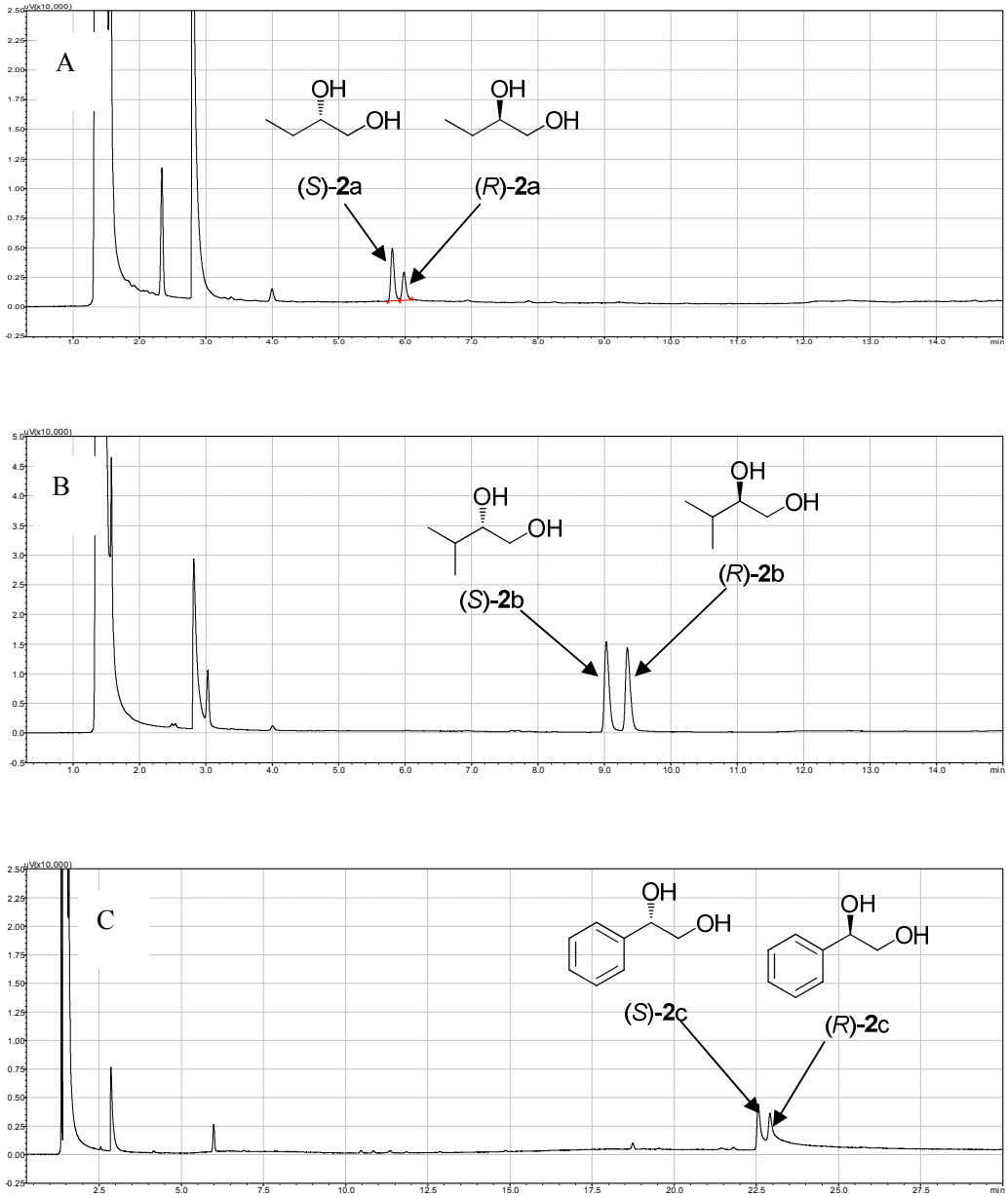
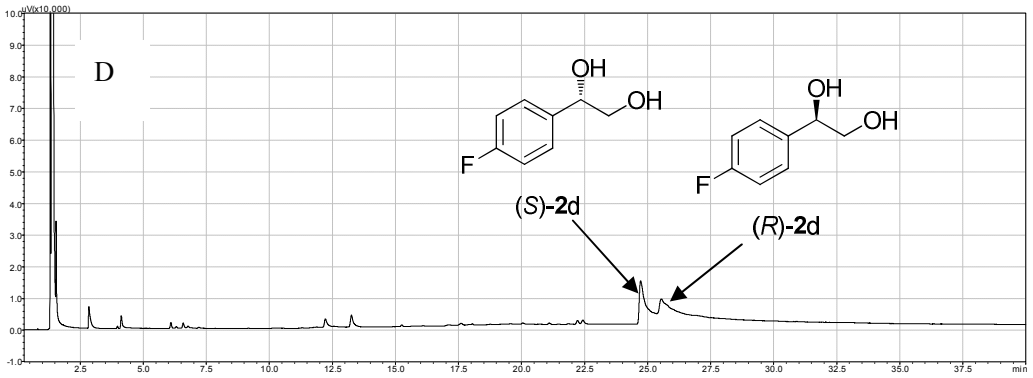
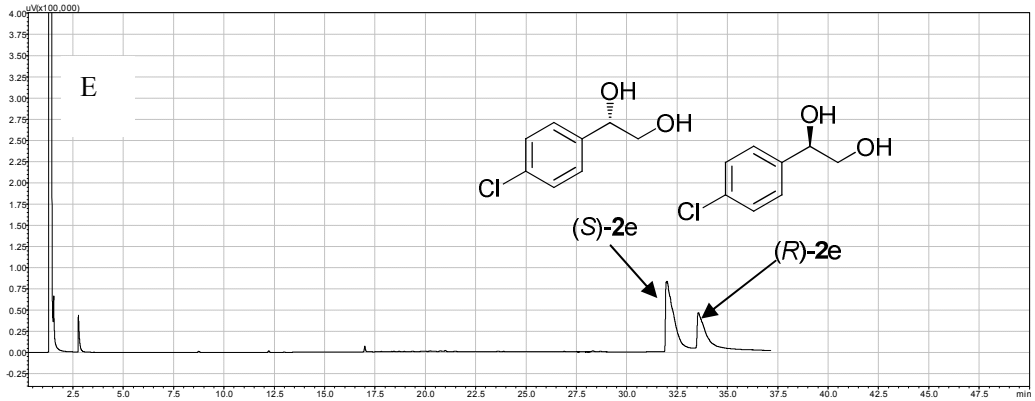


Figure S4. Chiral GC chromatograms of vicinal diol **2** produced from racemic epoxide **1**. A: **2**a produced by conversion of **1**a (20 mM) with cell free extract of *E. coli* (SpEH) at 2 h; B: **2**b produced by conversion of **1**b (20 mM) with cell free extract of *E. coli* (SpEH) at 2 h; C:**2**c produced by conversion of **1**c (20 mM) with cell free extract of *E. coli* (SpEH) at 3 h; D: **2**d produced by conversion of **1**d (20 mM) with cell free extract of *E. coli* (SpEH) at 3 h. E: **2**e produced by conversion of **1**e (20 mM) with cell free extract of *E. coli* (SpEH) at 3 h. F:**2**f produced by conversion of **1**f (20 mM) with cell free extract of *E. coli* (SpEH) at 3 h.





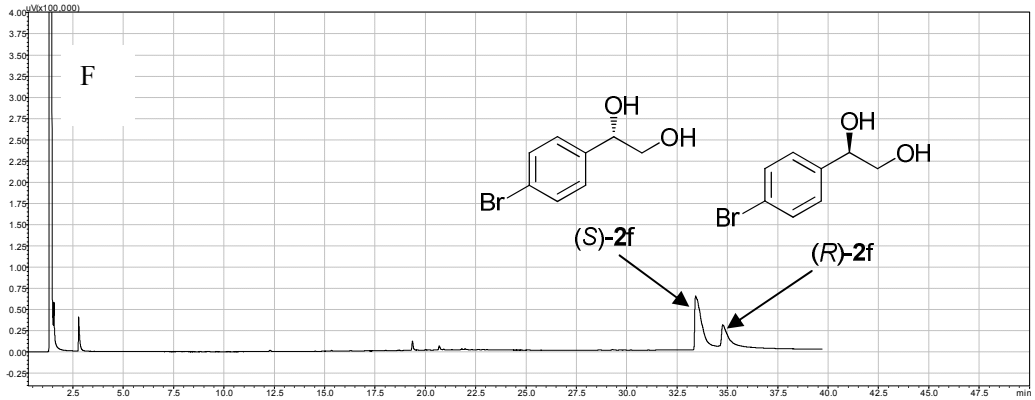


Figure S4. Continued.

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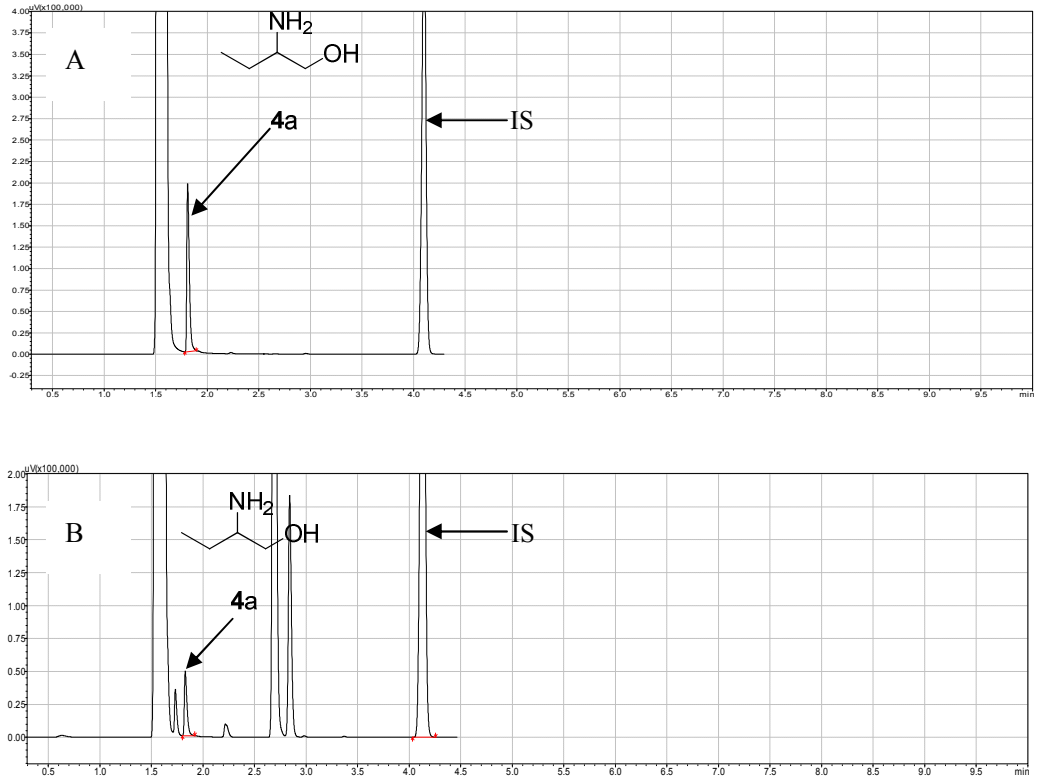

Figure S5. Achiral GC chromatograms of **4**a. A: **4**a standard. B: **4**a produced by conversion of **1**a (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (16 g cdw/L) at 12 h. IS: internal standard.

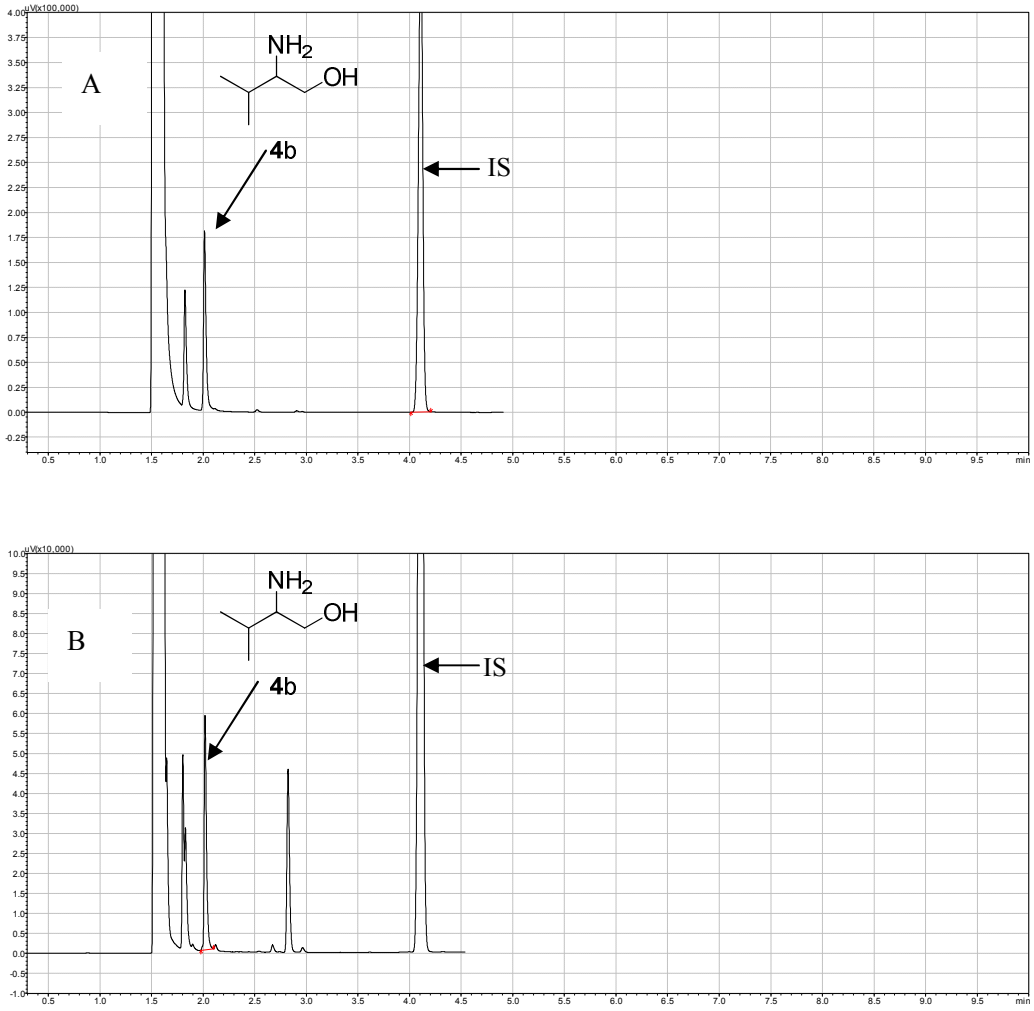

Figure S6. Achiral GC chromatograms of **4**b. A: **4**b standard. B: **4**b produced by conversion of **1**b (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (16 g cdw/L) at 12 h. IS: internal standard.

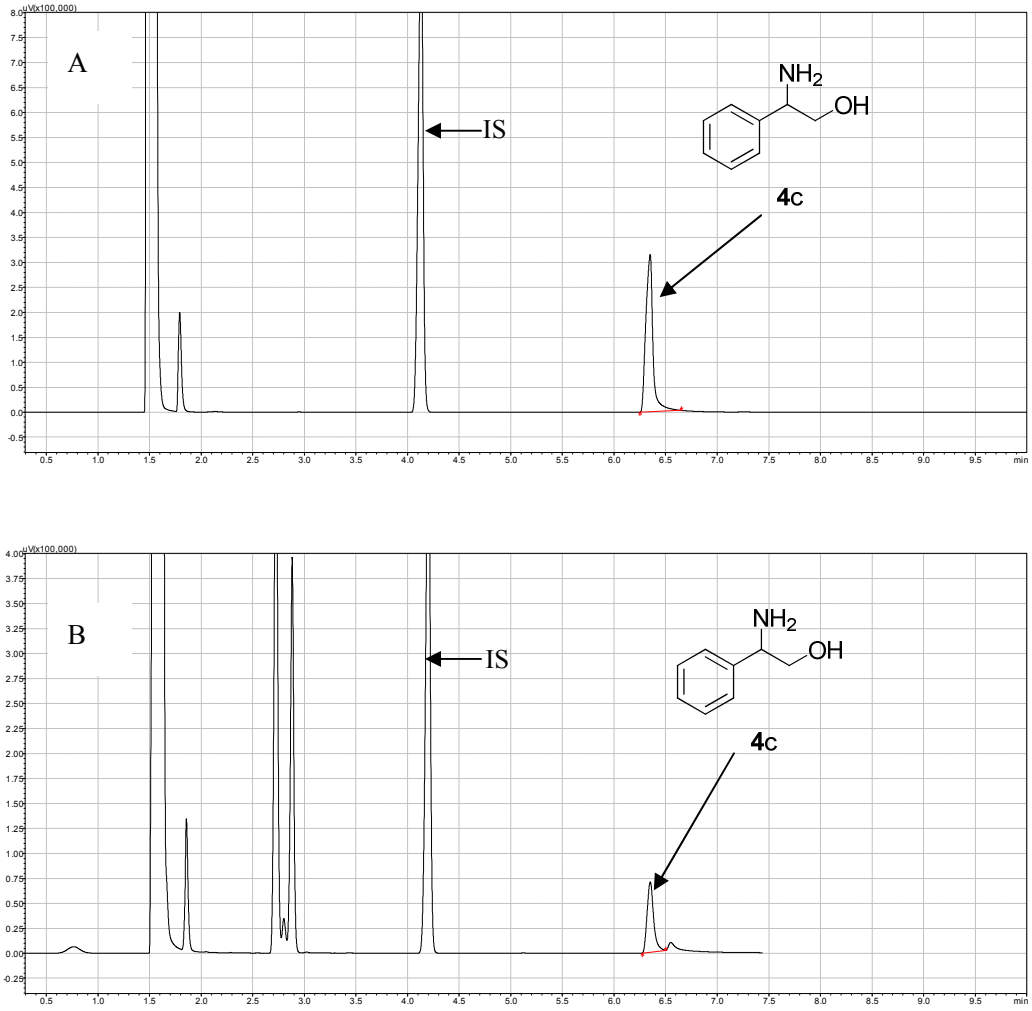

Figure S7. Achiral GC chromatograms of **4**c. A: **4**c standard. B: **4**c produced by conversion of **1**c (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (16 g cdw/L) at 12 h. IS: internal standard.

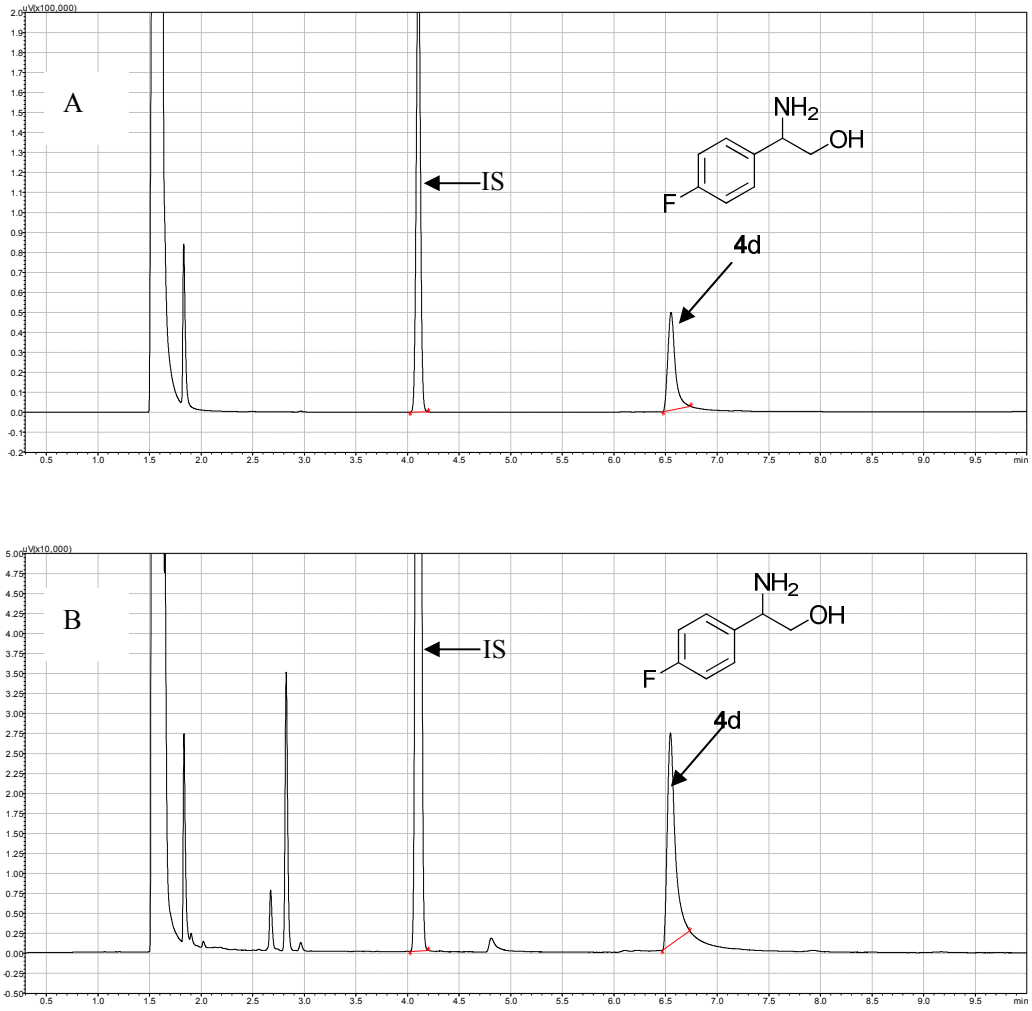

Figure S8. Achiral GC chromatograms of **4**d. A: **4**d standard. B: **4**d produced by conversion of **1**d (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (16 g cdw/L) at 12 h. IS: internal standard.

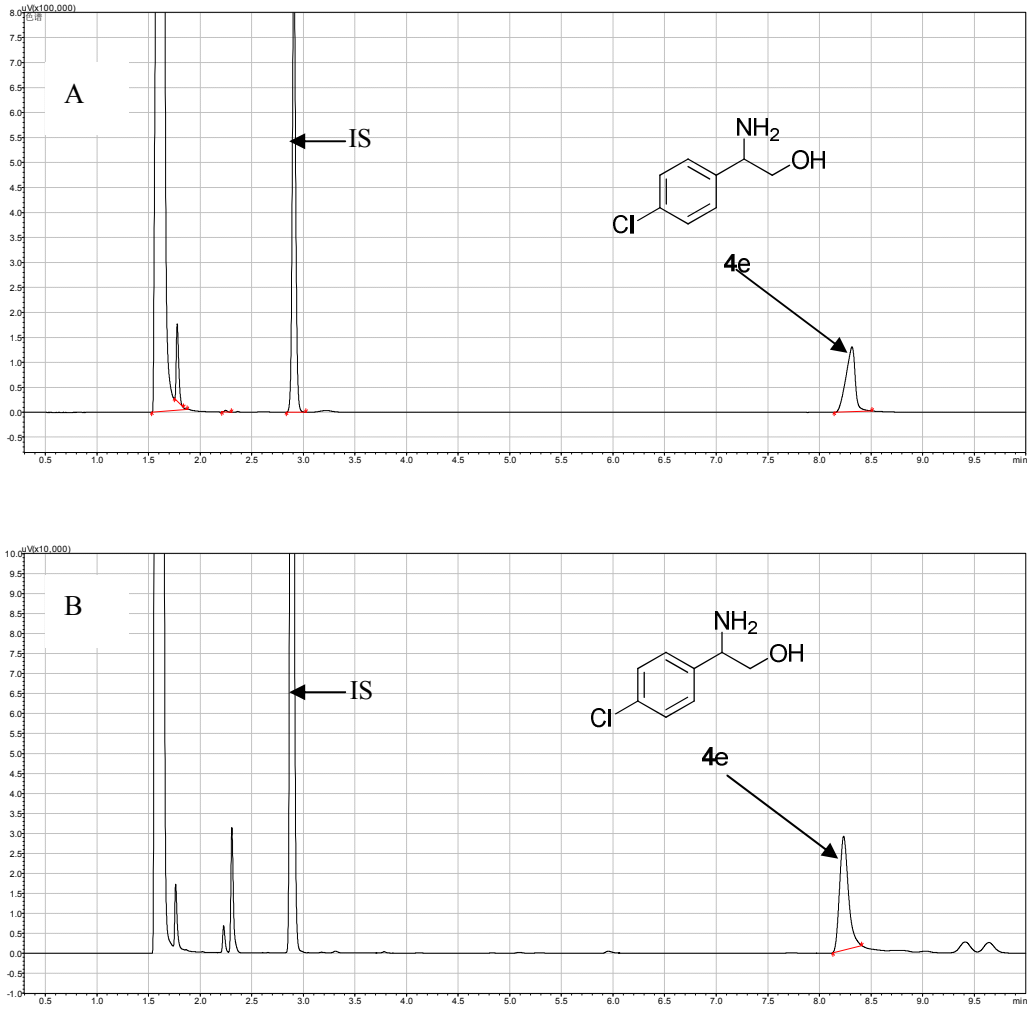

Figure S9. Achiral GC chromatograms of **4**e. A: **4**e standard. B: **4**e produced by conversion of **1**e (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (16 g cdw/L) at 12 h. IS: internal standard.

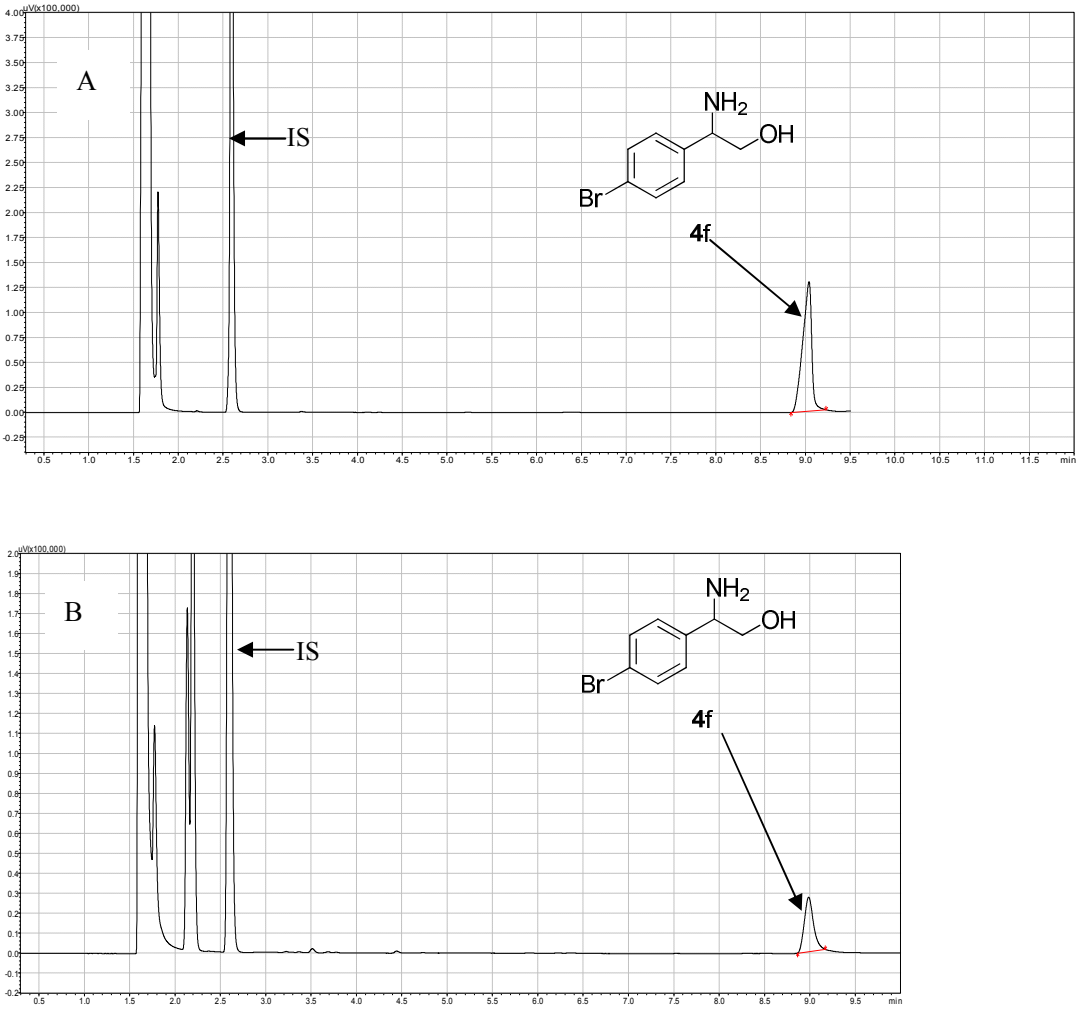

Figure S10. Achiral GC chromatograms of **4**f. A: **4**f standard. B: **4**f produced by conversion of **1**f (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (16 g cdw/L) at 12 h. IS: internal standard.

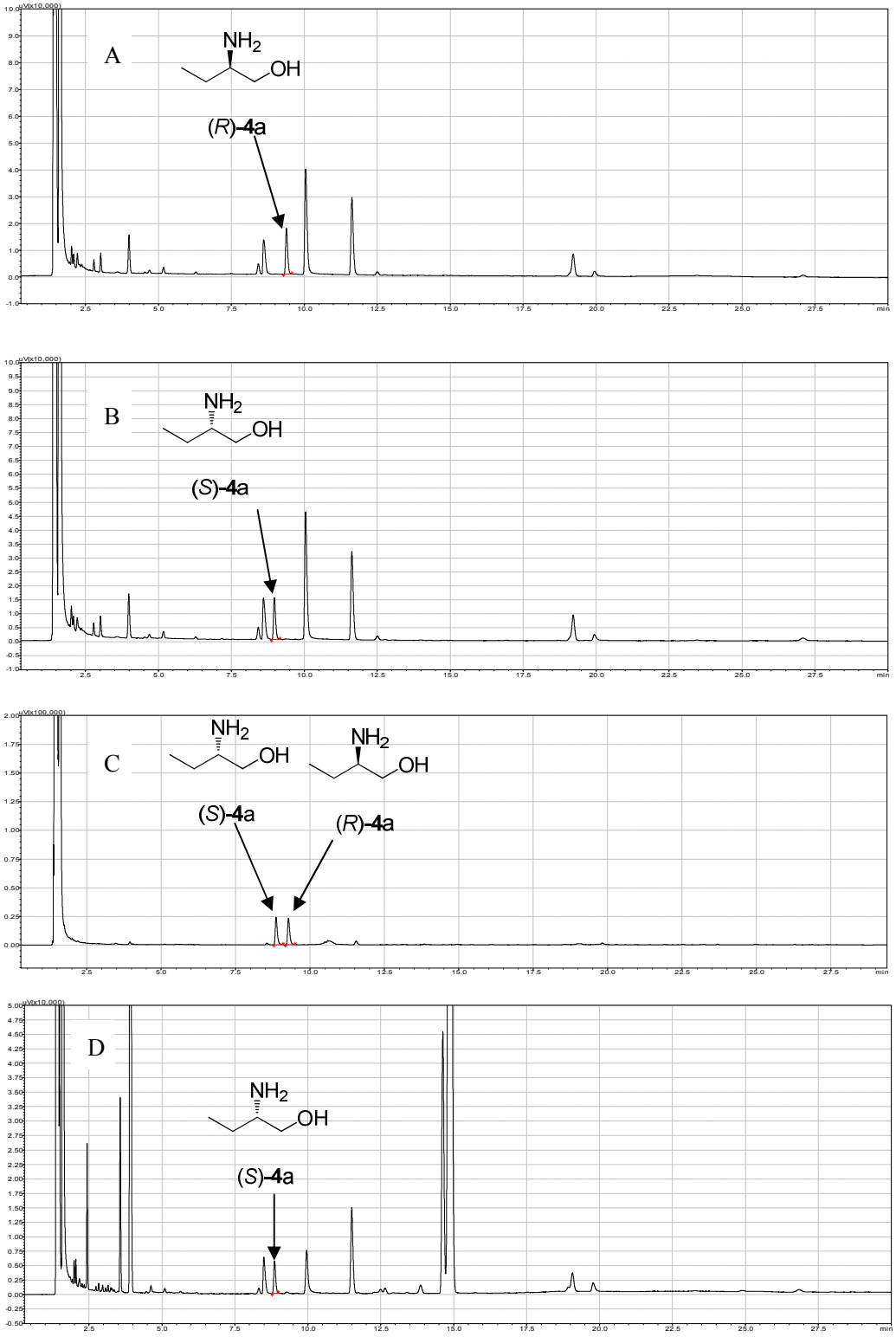

Figure S11. Chiral GC chromatograms of **4**a. A: (*R*)-**4**a standard. B: (*S*)-**4**a standard. C: (±)-**4**a standard. D: (*S*)-**4**a produced by conversion of **1a** (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (18 g cdw/L) at 5 h.

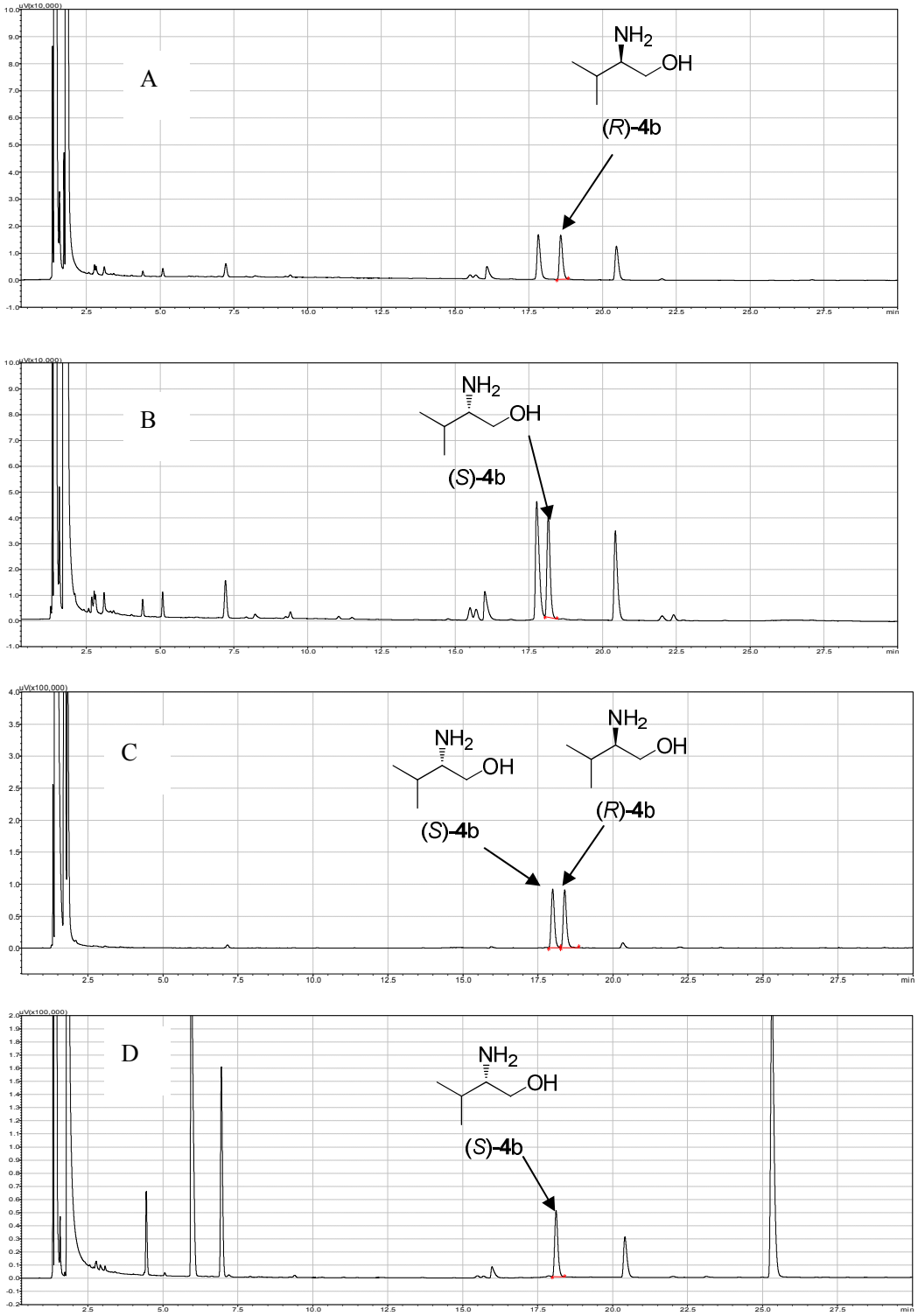

Figure S12. Chiral GC chromatograms of **4**b. A: (*R*)-**4**b standard. B: (*S*)-**4**b standard. C: (±)-**4**b standard. D: (*S*)-**4b** produced by conversion of **1b** (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (18 g cdw/L) at 5 h.

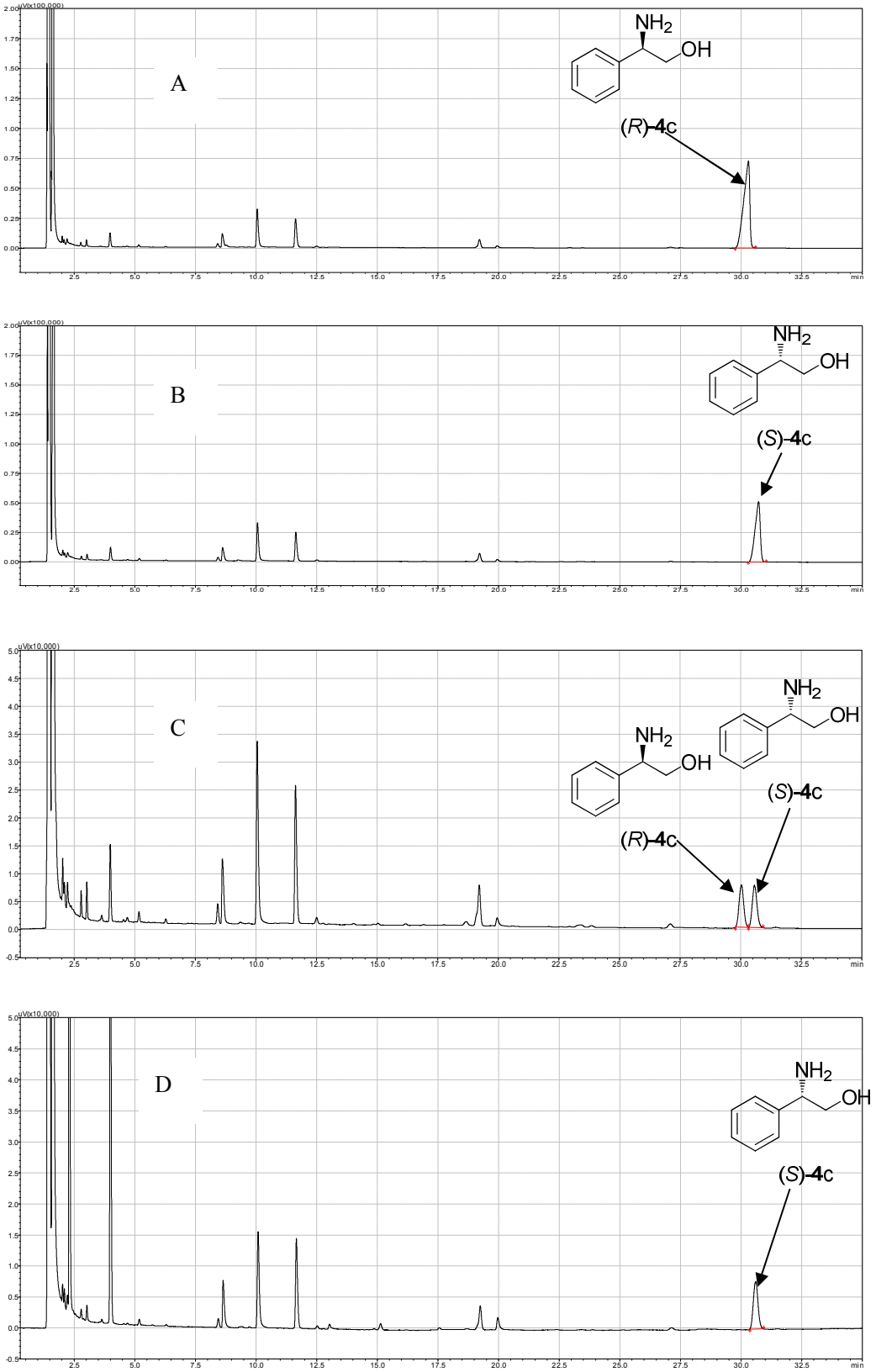

Figure S13. Chiral GC chromatograms of **4**c. A: (*R*)-**4**c standard. B: (*S*)-**4**c standard. C: (±)-**4**c standard. D: (*S*)-**4c** produced by conversion of **1c** (50 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (20 g cdw/L) at 12 h.

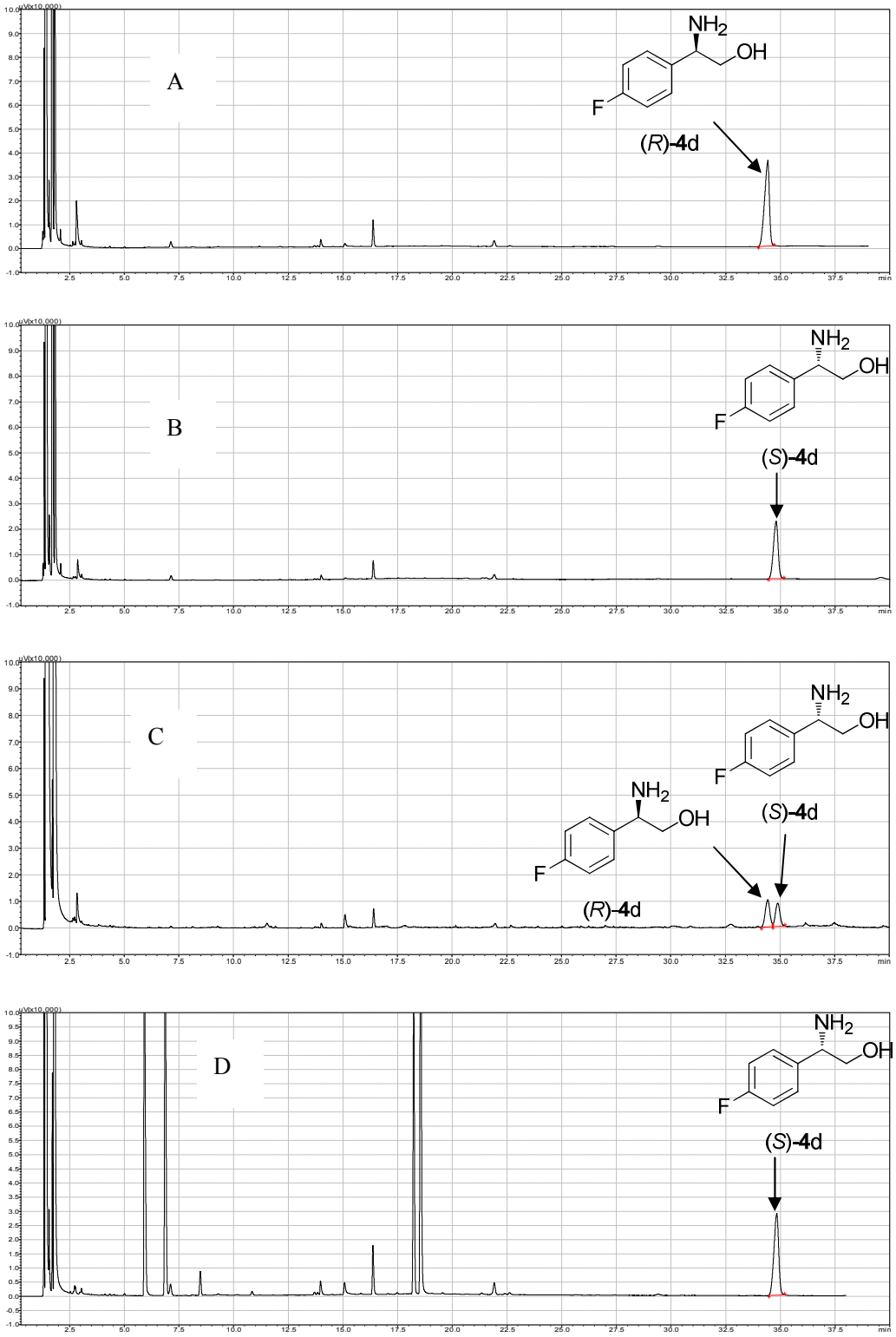

Figure S14. Chiral GC chromatograms of **4**d. A: (*R*)-**4**d standard. B: (*S*)-**4**d standard. C: (±)-**4**d standard. D: (*S*)-**4d** produced by conversion of **1d** (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (18 g cdw/L) at 5 h.

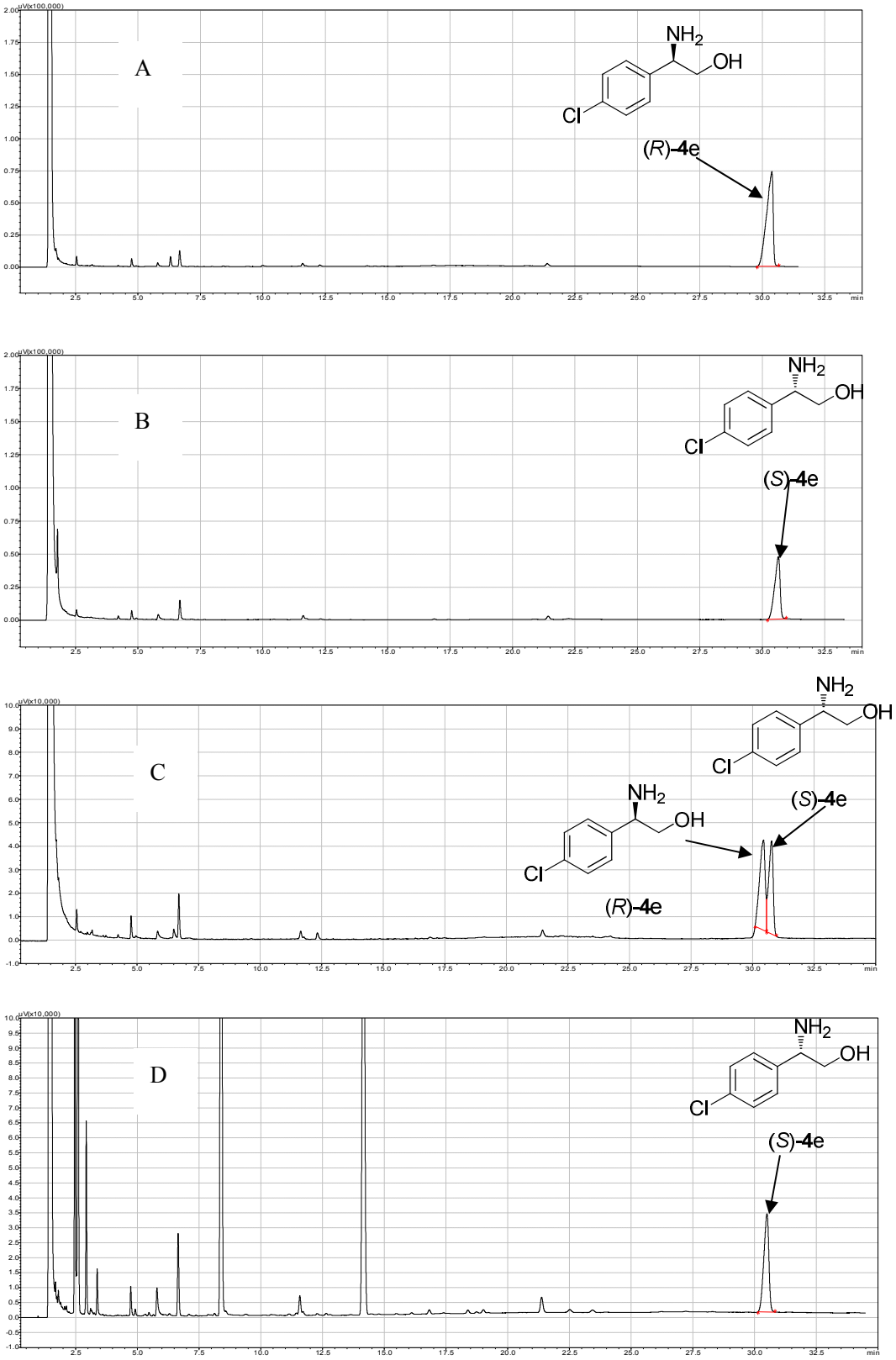

Figure S15. Chiral GC chromatograms of **4**e. A: (*R*)-**4**e standard. B: (*S*)-**4**e standard. C: (±)-**4**e standard. D: (*S*)-**4e** produced by conversion of **1e** (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (18 g cdw/L) at 5 h.

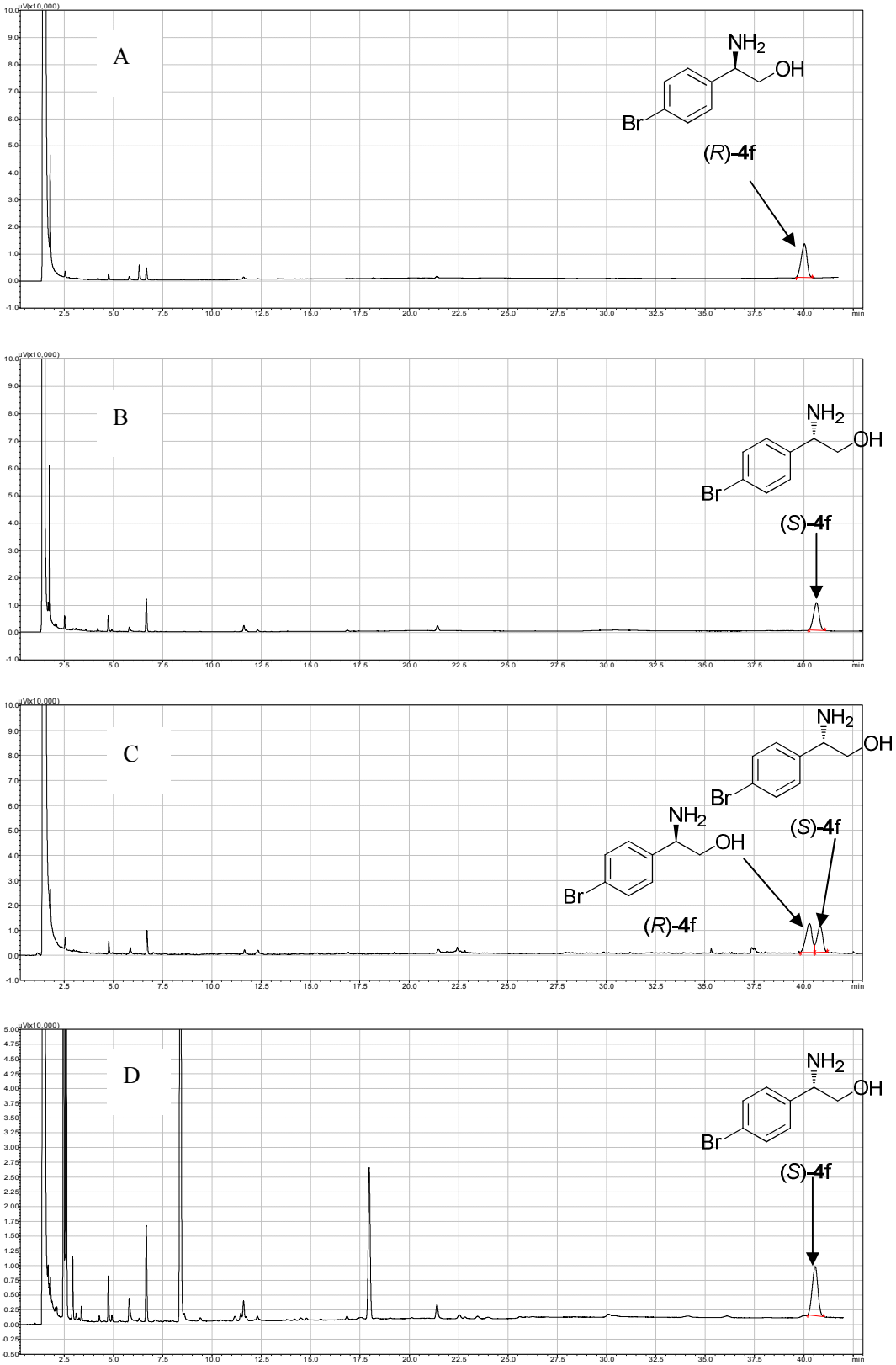

Figure S16. Chiral GC chromatograms of **4**f. A: (*R*)-**4**f standard. B: (*S*)-**4**f standard. C: (±)-**4**f standard. D: (*S*)-**4f** produced by conversion of **1f** (20 mM) with resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA) (18 g cdw/L) at 5 h.

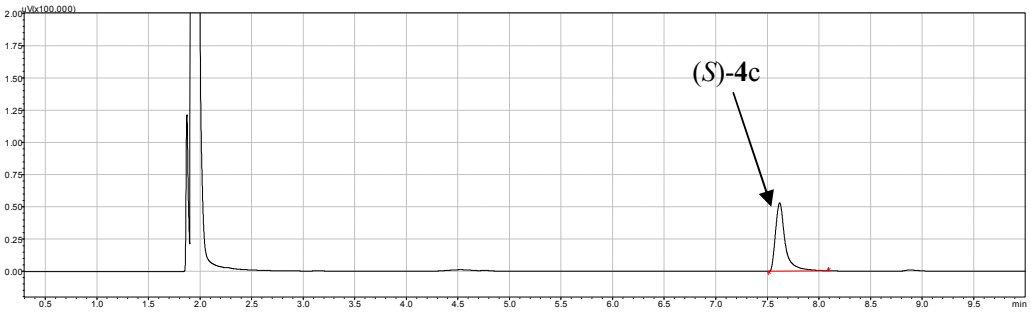

Figure S17. The purity analysis of (*S*)-2-amino-2-phenylethanol (**4c**).

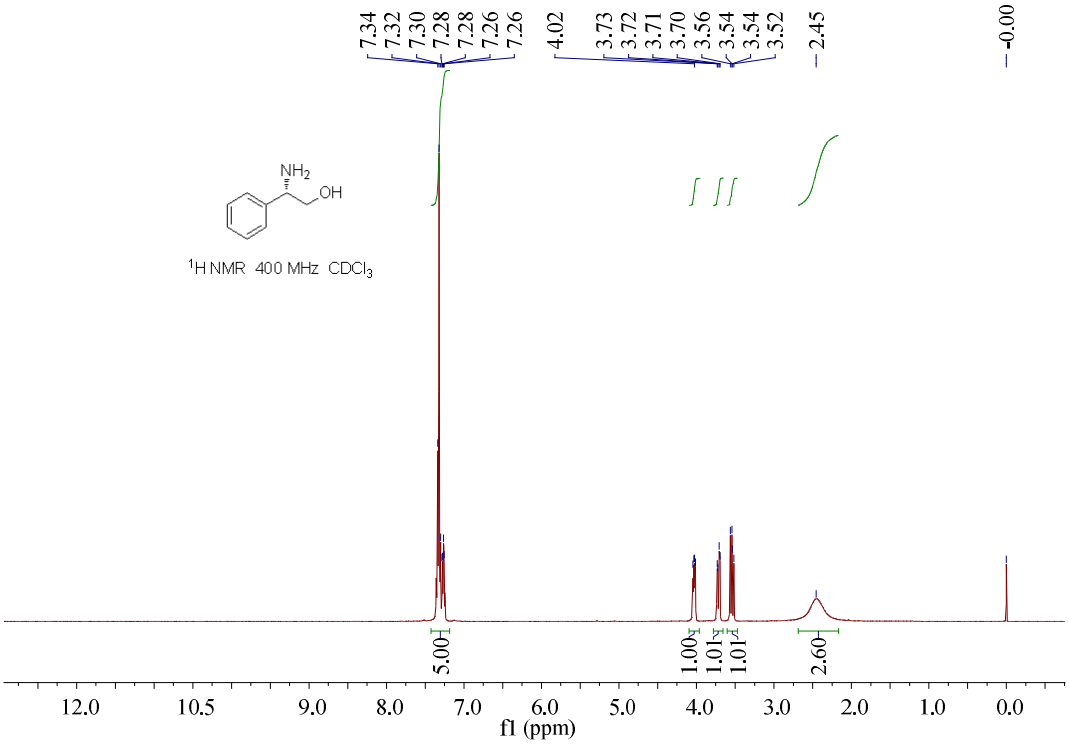

Figure S18. ^{1}H NMR spectra analysis of (*S*)-2-amino-2-phenylethanol (**4c**) prepared from conversion of (**1c**) (50 mM) with the resting cells of *E. coli* (SpEH-BDHA-GoSCR-MVTA). (*S*)-**4c**: White solid, ^{1}H NMR (400 MHz, 298K, $CDCl_3$) δ_H 7.30−7.18 (5H, m, Ph), 3.97 (1H, dd, $^{3}J_{HH}$ = 4.0 Hz, 8.5Hz, CH), 3.66 (^{1}H, dd, $^{2}J_{HH}$ = 11.0Hz, $^{3}J_{HH}$ = 4.0 Hz, CH_2), 3.48 (1H, dd, $^{2}J_{HH}$ = 11.0Hz, $^{3}J_{HH}$ = 8.5 Hz, CH_2), 2.05 (2H, s, NH_2).

Reference

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