Supplementary Material

Biocatalytic Formal Regio- and Enantioselective Markovnikov Hydroamination of Aryl Alkenes to Chiral Amines

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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, streptomycin and kanamycin were from Sigma Aldrich. 4-Vinylphenol (**1**a), 2-fluoro-4-vinylphenol (**1**b), 2-chloro-4-vinylphenol (**1**c), 2-bromo-4-ethenylphenol (**1**d), 2-methoxy-4-vinylphenol (**1**e), 2,6-dimethoxy-4-vinylphenol (**1**f), and pyridoxal-5'-phosphate (PLP) were from Energy Chemical (Shanghai, China). 4-(1-hydroxyethyl)phenol (**2**a), 2-fluoro-4-(1-hydroxyethyl)phenol (**2**b), 2-chloro-4-(1-hydroxyethyl)phenol (**2**c), 2-bromo-4-(1-hydroxyethyl)phenol (**2**d), 1-(4-hydroxy-3-methoxyphenyl)ethanol (**2**e), 4-(1-hydroxyethyl)-2,6-dimethoxyphenol (**2**f), 4'-hydroxy-acetophenon (**3**a), 1-(3-fluoro-4-hydroxyphenyl)ethanone (**3**b), 1-(3-chloro-4-hydroxyphenyl)ethanone (**3**c), 1-(3-bromo-4-hydroxyphenyl)ethanone (**3**d), 1-(4-hydroxy-3-methoxyphenyl)ethanone (**3**e), 1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone (**3**f), 4-(1-aminoethyl)phenol (**4**a), 4-(1-aminoethyl)-2-fluorophenol (**4**b), 4-(1-aminoethyl)-2-chlorophenol (**4**c), 4-(1-aminoethyl)-2-bromophenol (**4**d), 4-(1-aminoethyl)-2-methoxyphenol (**4**e), 4-(1-aminoethyl)-2,6-dimethoxyphenol (**4**f) were from Labter Pharmatech (Beijing, China). All other chemicals were of analytical grade and were commercially available.

2. Bacterial strains, vectors and culture conditions

E. coli T7 supercompetent cells was obtained from New England Biolabs (Beijing, China). They were routinely grown in Luria-Bertani (LB) medium at 37°C unless stated otherwise. Ampicillin (100 μg/mL) kanamycin (50 μg/mL) and streptomycin (50 μg/mL) were used for the selection of recombinant strains. The plasmids pET28a (+), pETduet-1, pCDFduet-1 and pRSFduet-1 for the heterogeneous expression studies were obtained from Novagen (Shanghai, China). Recombinant *E. coli* (pET28a-CV2025) [1], *E. coli* (pET28a-MVTA) [2], *E. coli* (pET28a-AlaDH) [3], *E. coli* (pET28a-GDH) [4] and *E. coli* (pET28a-NOX) [5] were constructed as our previously described.

3. Construction of the recombinant E. coli strains

All the primers used in this study were synthesized by Tsingke (Beijing, China) and list in Additional

file 1: Table S1. All the constructed plasmids and recombinant *E. coli* cells were list in Additional file 1: Table S2.

For *E. coli* (pET28a-FDC*), the gene of ferulic acid decarboxylase from *Enterobacter* sp. (FDC_Es) with a single-point mutation of valine 46 to glutamate (FDC*) [6] was synthesized by Tsingke (Beijing, 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 1min and followed by a final extension at 72°C for 10 min. The PCR product of FDC* genes were isolated and digested with corresponding restriction endonucleases (*Nde* I, *Xho* I), cloned into the pET28a vector to form the recombinant plasmid pET28a-FDC*. The recombinant plasmid pET28a-FDC* was transformed into the competent *E. coli* T7 to form *E. coli* (pET28a-FDC*).

For *E. coli* (pET28a-RaADH), the gene of alcohol dehydrogenase (RaADH) from *Rhodococcus aetherivorans* was synthesized by Tsingke (Beijing, 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 68°C for 40 s, extension at 72°C for 1 min and followed by a final extension at 72°C for 10 min. The PCR product of RaADH genes were isolated and digested with corresponding restriction endonucleases (*BamH*I, *Xho*I), cloned into the pET28a vector to form the recombinant plasmid pET28a-RaADH. The recombinant plasmid pET28a-RaADH was transformed into the competent *E. coli* T7 to form *E. coli* (pET28a-RaADH).

For *E. coli* (RDR), the gene of alcohol dehydrogenase (RDR) from *Devosia riboflavina* IFO 13584 [7] was synthesized by Tsingke (Beijing, 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 min and followed by a final extension at 72°C for 10 min. The PCR product of RDR genes were isolated and digested with corresponding restriction endonucleases (*Nde*I, *Xho*I), cloned into the pET28a vector to form the recombinant plasmid pET28a-RDR. The recombinant plasmid pET28a-RDR was transformed into the competent *E. coli* T7 to form *E. coli* (pET28a-RDR).

E. coli (BsLDH), the gene of lactate dehydrogenase (BsLDH) from *Bacillus subtilis* [8] was synthesized by Tsingke (Beijing, 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 BsLDH genes were isolated and digested with corresponding restriction endonucleases (*Nco*I, *Hind*III), cloned into the pCDFduet vector to form the recombinant plasmid pCDFduet-BsLDH (named as CB). The sequence of the insert DNA was subsequently confirmed by sequencing. In addition, the recombinant plasmid pCDFduet-BsLDH was transformed into the competent *E. coli* T7 to form *E. coli* (pCDFduet-BsLDH) which was named *E. coli* (CB).

For *E. coli* (pRSFduet-FDC*-MVTA), the DNA fragments of FDC* and MVTA were amplified with PCR using the primers shown in Additional file 1: Table S1. The PCR product of FDC* genes were isolated and digested with corresponding restriction endonucleases (*Nco*I, *Hind*III), cloned into the pRSFduet-1 vector to form the recombinant plasmids pRSFduet-FDC*. The MVTA gene was then double-digested with *Nde*I and *Xho*I and ligated into the pRSFduet-FDC* at the *Nde*I/*Xho*I sites to form the recombinant pRSFduet-FDC*-MVTA (known as RFM). In addition, the recombinant plasmid pRSFduet-FDC*-MVTA was transformed into the competent *E. coli* T7 to form *E. coli* (pRSFduet-FDC*-MVTA) which was named *E. coli* (RFM).

For *E. coli* (pRSFduet-FDC*-CV2025), the DNA fragments of CV2025 enzymes were amplified with PCR using the primers shown in Additional file 1: Table S1. The CV2025 gene was then double-digested with *Nde*I and *Xho*I and ligated into the pRSFduet-FDC* at the *Nde*I/*Xho*I sites to form the recombinant pRSFduet-FDC*-CV2025 (known as RFC). In addition, the recombinant plasmid pRSFduet-FDC*-CV2025 was transformed into the competent *E. coli* T7 to form *E. coli* (pRSFduet-FDC*-CV2025) which was named *E. coli* (RFC).

For *E. coli* (pETduet-FDC*-MVTA), the DNA fragments of FDC* and MVTA were amplified with PCR using the primers shown in Additional file 1: Table S1. The PCR product of FDC* genes were isolated and digested with corresponding restriction endonucleases (*Nco*I, *Hind*III), cloned into the pETduet-1 vector to form the recombinant plasmids pETduet-FDC*. Then, the MVTA gene was double-digested with *Nde*I and *Xho*I and ligated into the pETduet-FDC* at the *Nde*I/*Xho*I sites to form the recombinant pETduet-FDC*-MVTA (known as EFM). In addition, the recombinant plasmid pETduet-FDC*-MVTA was transformed into the competent *E. coli* T7 to form *E. coli* (pETduet-FDC*-MVTA) which was named *E. coli* (EFM).

For *E. coli* (pETduet-FDC*-CV2025), the DNA fragments of CV2025 were amplified with PCR

using the primers shown in Additional file 1: Table S1. Then, the CV2025 gene was double-digested with *Nde*I and *Xho*I and ligated into the pETduet-FDC* at the *Nde*I/*Xho*I sites to form the recombinant plasmid pETduet-FDC*-CV2025 (known as EFC). In addition, the recombinant plasmid pETduet-FDC*-CV2025 was transformed into the competent *E. coli* T7 to form *E. coli* (pETduet-FDC*-CV2025) which was named *E. coli* (EFC).

For *E. coli* (pETduet-RaADH-RDR), the DNA fragments of RaADH and RDR were amplified with PCR using the primers shown in Additional file 1: Table S1. The PCR product of RaADH genes were isolated and digested with corresponding restriction endonucleases (*Nco*I, *Hind*III), cloned into the pETduet-1 vector to form the recombinant plasmids pETduet-RaADH. Then, the RDR gene was double-digested with *Nde*I and *Xho*I and ligated into the pETduet-RaADH at the *Nde*I/*Xho*I sites to form the recombinant plasmid pETduet-RaADH-RDR (known as ERR). In addition, the recombinant plasmid pETduet-RaADH-RDR was transformed into the competent *E. coli* T7 to form *E. coli* (pETduet-RaADH-RDR) which was named *E. coli* (ERR).

For *E. coli* (pRSFduet-RaADH-RDR), the DNA fragments of RaADH and RDR were amplified with PCR using the primers shown in Additional file 1: Table S1. The PCR product of RaADH genes were isolated and digested with corresponding restriction endonucleases (*Nco*I, *Hind*III), cloned into the pRSFduet-1 vector to form the recombinant plasmids pRSFduet-RaADH. Then, the PCR product of RDR gene was double-digested with *Nde*I and *Xho*I and ligated into the pRSFduet-RaADH at the *Nde*I/*Xho*I sites to form the recombinant pRSFduet-RaADH-RDR (known as RRR). In addition, the recombinant plasmid pRSFduet-RaADH-RDR was transformed into the competent *E. coli* T7 to form *E. coli* (pRSFduet-RaADH-RDR) which was named *E. coli* (RRR).

For *E. coli* (RFM-ERR-CB), the constructed recombinant plasmids pRSFduet-FDC*-MVTA and pETduet-RaADH-RDR and pCDFduet-BsLDH were simultaneously transformed into the competent *E. coli* T7 to form the recombinant *E. coli* (pRSFduet-FDC*-MVTA/pETduet-RaADH-RDR/ pCDFduet-BsLDH), designated as *E. coli* (RFM-ERR-CB).

For *E. coli* (EFM-RRR-CB), the constructed recombinant plasmids pETduet-FDC*-MVTA and pRSFduet-RaADH-RDR and pCDFduet-BsLDH were simultaneously transformed into the competent *E. coli* T7 to form the recombinant *E. coli* (pETduet-FDC*-MVTA/pRSFduet-RaADH-RDR/ pCDFduet-BsLDH), designated as *E. coli* (EFM-RRR-CB).

For *E. coli* (RFC-ERR-CB), the constructed recombinant plasmids pRSFduet-FDC*-CV2025 and

pETduet-RaADH-RDR and pCDFduet-BsLDH were simultaneously transformed into the competent *E. coli* T7 to form the recombinant *E. coli* (pRSFduet-FDC*-CV2025/pETduet-RaADH-RDR/ pCDFduet-BsLDH), designated as *E. coli* (RFC-ERR-CB).

For *E. coli* (EFC-RRR-CB), the constructed recombinant plasmids pETduet-FDC*-CV2025 and pRSFduet-RaADH-RDR and pCDFduet-BsLDH were simultaneously transformed into the competent *E. coli* T7 to form the recombinant *E. coli* (pETduet-FDC*-CV2025/pRSFduet-RaADH-RDR/ pCDFduet-BsLDH), designated as *E. coli* (EFC-RRR-CB).

The expression of all genes from above constructed recombinant *E. coli* strains was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and testing the activity of corresponding enzymes.

4. Protein expression and activity assay

The constructed recombinant *E. coli* cells were cultivated at 37°C for 12 h in 5 mL LB medium (10 g/L peptone, 5 g/L yeast extract and 10 g/L NaCl, pH7.0) containing appropriate antibiotics (100 μg/mL ampicillin or 50 μg/mL kanamycin or 0.1 mg/mL streptomycin or three of them). Two milliliter of seed culture was then inoculated into 50 mL of Terrific Broth (TB) medium (12 g/L tryptone, 24 g/L yeast extract, 4 g/L glycerol, 17 mM KH_2PO_4, 72 mM K_2HPO_4) supplemented with the appropriate antibiotics, and cultivated at 37°C for 2-3 h. When OD600 value of the recombinant *E. coli* cells reached 0.6−0.8, IPTG (0.5 mM) was then added to the medium to induce the protein expression. After incubation for 12−20 h at 20°C and 200 rpm, cells were harvested by centrifugation (8,000 rpm) at 4°C for 10 min, washed twice with cold saline and resuspended in sodium phosphate buffer (100 mM, pH 7.5). The cell pellets were put on ice and lysed by ultra-sonication for 90 times at 400 W for 4 s with 6 s of interval. The cell debris were removed by centrifugation (12,000 rpm, 30 min) at 4°C, and the soluble portion of cell lysate was stored at 4°C for further use. SDS-PAGE was carried out employing a 12% polyacrylamide gels, stained with Coomassie Brilliant Blue R250.

The activity of FDC* was measured by monitoring the product 4-(1-hydroxyethyl)phenol produced. The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 6.0), 10 mM 4-hydroxystyrenes substrate, and an appropriate amount of enzyme in a total volume of 1 mL. After incubation at 25 °C for 10 min, the reaction samples (300 μL) were saturated with sodium chloride, and extracted with ethyl acetate (EtOAc) containing 2 mM of n-dodecane (internal standard). The organic

phase was dried over anhydrous Na_2SO_4, and the concentration of 4-(1-hydroxyethyl)phenol was determined by GC analysis. 1 unit of activity refers to the amount of catalyst that catalyzed the conversion of 1 μmol 4-vinylphenol to 4-(1-hydroxyethyl)phenol per minute.

The oxidation activity of alcohol dehydrogenases was measured by monitoring the NADH concentration increase using UV absorbance at 340 nm. The assay mixture contained 1 mL potassium phosphate buffer (pH 7.5, 100 mM), including 0.2 mM NAD^+, 10 mM substrate racemic **2**a and 10 μL enzyme solution (5 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 activity of BsLDH was measured by monitoring the NADH concentration decrease using UV absorbance at 340 nm. The assay mixture contained 1 mL potassium phosphate buffer (pH 7.5, 100 mM), including 0.2 mM NADH, 10 mM pyruvate and 10 μL enzyme solution (5 mg/mL). Reactions were started by addition of the enzyme solution and measured over a period of 1 min. The reaction mixture without NADH 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 decrease per minute under standard conditions (25°C, pH 7.5).

The activity of transaminase was assayed by monitoring the product amine **4**a produced. Briefly, the reaction mixture (1 mL) contained 100 mM potassium phosphate buffer (pH 8.0), 10 mM ketone **3**a substrate, 200 mM Ala, 10% DMSO, 0.1 mM pyridoxal 5'-phosphate (PLP) and an appropriate amount of enzyme. After the enzyme solution was added, the reaction mixture was immediately incubated at 30°C for 15 min, the reaction samples (300 μL) were saturated with sodium chloride, and extracted with ethyl acetate (EtOAc) containing 2 mM of *n*-dodecane (internal standard). The organic phase was dried over anhydrous Na_2SO_4, and the concentration of **4**a was determined by GC analysis. 1 unit of activity refers to the amount of enzyme that catalyzed the conversion of 1 μmol ketone to amine per min.

The activity of recombinant *E. coli* (RFM-ERR-CB), *E. coli* (EFM-RRR-CB), *E. coli* (RFC-ERR-CB) and *E. coli* (EFC-RRR-CB) cells was measured by testing the formation of amine **4**a from **1**a. The reaction mixture consisted of 100 mM sodium phosphate buffer (pH 7.5), 10 mM 4-vinylphenol **1**a, 200 mM Ala, 0.1 mM pyridoxal 5'-phosphate (PLP) and 25 g cell dry weight (cdw)/L recombinant *E. coli* cells in a total volume of 3.0 mL, the reaction was performed at 25°C and 200 rpm for 10 min. The

concentration of product amine **4**a was determined by GC analysis. 1 Unit of activity refers to the amount of catalyst that catalyzed the conversion of 1 μmol **1**a to **4**a per min.

The protein concentration was determined by the Bradford method.

5. General procedure for conversion of 4-hydroxystyrenes 1 to ketones 3 with the mixtures of lyophilized cell-free extract

The freshly prepared cells of *E. coli* (pET28a-FDC*), *E. coli* (pET28a-RaADH), *E. coli* (pET28a-RDR), *E. coli* (pET28a-NOX) 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 8,000 ×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°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* (FDC*), *E. coli* (RaADH), *E. coli* (RDR) and *E. coli* (NOX) were mixed at different ratios. The standard reaction mixture was potassium phosphate buffer (100 mM, pH 8.0) containing 10 mM **1**a-f, 0.05-1 mM NAD^+, 10 mg/mL FDC*, 20 mg /mL RaADH, 20 mg /mL RDR, 20 mg /mL NOX. The reactions were carried out in 5 mL sodium phosphate buffer at 30°C and 200 rpm in capped 50 mL conical flask. To quantitate the formation of **3**, 300 μL aliquots were taken out at different time points, saturated with NaCl and extracted with 0.3 mL ethyl acetate (EtOAc) containing 2 mM of *n*-dodecane as an internal standard. The organic phase was dried over anhydrous sodium sulfate and subjected to GC analysis. All experiments were performed in duplicate.

6. General procedure for conversion of 4-hydroxystyrenes 1 to chiral amines 4 with the mixtures of lyophilized cell-free extract

The freshly prepared cells of *E. coli* (FDC*), *E. coli* (RaADH), *E. coli* (RDR), *E. coli* (CV2025) or *E. coli* (MVTA), *E. coli* (AlaDH), *E. coli* (GDH) and *E. coli* (BsLDH) 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 8,000 ×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°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* (FDC*), *E. coli* (RaADH), *E. coli* (RDR), *E. coli* (CV2025), *E. coli* (MVTA) *E. coli* (AlaDH), *E. coli* (GDH) and *E. coli* (BsLDH) were mixed at different ratios. The standard reaction mixture was potassium phosphate buffer (100 mM, pH 8.0) containing 10 mM **1**a, 1 mM NAD^+, 10 mg/mL FDC, 20 mg /mL RaADH, 20 mg /mL RDR, 20 mg /mL MVTA or CV2025, 15 mg /mL AlaDH, 10 mg /mL GDH, 20 mg /mL BsLDH, 0.1 mM PLP, L-Ala or D-Ala (200 mM) was also added as the amino donor. The reactions were carried out in 5 mL sodium phosphate buffer at 30°C and 200 rpm in capped 50 mL conical flask. To quantitate the formation of **4**, 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 0.3 mL ethyl acetate (EtOAc) containing 2 mM of *n*-dodecane as an internal standard. The organic phase was dried over anhydrous sodium sulfate and subjected to GC analysis. All experiments were performed in duplicate.

7. General procedure for conversion of 4-hydroxystyrenes 1a-f to chiral amines 4a-f with the resting cells of *E. coli* (RFM-ERR-CB) and *E. coli* (RFC-ERR-CB)

The freshly prepared cells of *E. coli* (RFM-ERR-CB) and *E. coli* (RFC-ERR-CB) were resuspended in 5 mL 100 mM potassium phosphate buffer (pH 8.0) to a cell density of 20-45 g cdw/L, respectively. The standard reaction mixture was containing 10-50 mM 4-hydroxystyrenes **1**a-f, 0.1 mM PLP, 200-1000 mM L- or D-Ala, 10-50 mM glucose, and the mixtures were shaken at 200 rpm at 35°C. 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.

8. Preparation of (*S*)-4a or (*R*)-4a from 1a by cascade biocatalysis with the resting cells of *E. coli* (RFM-ERR-CB) and *E. coli* (RFC-ERR-CB)

For preparation of (*S*)-**4**a and (*R*)-**4**a, the reaction was conducted in 100 mL potassium phosphate buffer (100 mM, pH 8.0) containing 10 mM (120.0 mg) **1**a, 40 g cdw/L *E. coli* (RFC-ERR-CB) or *E. coli* (RFM-ERR-CB), 10 mM glucose, 0.1 mM PLP and 200 mM D-/L-Ala. The reactions were performed at 35°C and 200 rpm for 30 h. After the reactions were finished, the reaction mixtures were basified (pH > 10)

by adding NaOH (10 N), and extracted with EtOAc for three times (3 × 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4. The solvent was removed by evaporation and the crude products were purified by flash chromatography on a silica gel column to give (*S*)-**4**a or (*R*)-**4**a as a white solid in 54.8% (75.1 mg) and 59.2% yields (81.1 mg) and >99% *ee*, respectively. ^{1}H NMR (400 MHz, 298K, $CDCl_3$) 7.2-7.1 (m, J = 8.5 Hz, 2H), 6.75-6.65 (m, J = 8.5 Hz, 2H), 3.95-3.80 (td, J = 5.8, 11.9 Hz, 1H), 1.25-1.13 (d, J = 6.8 Hz, 3H).

9. Assay method

The concentrations of alcohols and ketones 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 GC analysis conditions were as follows: injector temperature, 250°C; detector temperature, 275 °C; temperature program: column temperature, 120 °C, hold 10 min; gradient 2 °C min^{-1} up to 140 °C, hold 5 min.

The concentrations of amines were measured by two methods. **Method A**: the concentrations of **4**a-d and **4**f 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 DB-WAX column (30 m × 0.320 mm × 0.30 mm; Agilent Technologies, lnc.). The analytical conditions were as follows: injector temperature, 250°C; detector temperature, 275 °C; temperature program: column temperature, 90 °C，hold 5 min.; gradient 10 °C min^{-1} up to 140 °C, hold 5 min；gradient 20 °C min^{-1} up to 240 °C, hold 30 min. **Method B**: the concentrations of 4-(1-aminoethyl)-2-methoxyphenol **4**e were determined by using a Shimadzu HPLC on a C18 (4.6*250 mm,5 μm) column (Phenomenex, Shanghai). Detection: UV at 210 nm; Eluent: acetonitrile : water (80:20); Flow rate: 0.3 mL/min.

The enantiomeric excesss of amines were determined by GC with a chiral column (CP-Chirasil-Dex CB, 25 m x 0.32 mm x 0.25 μm; Agilent Technologies, lnc.) after derivative with 4-dimethylamino pyridine (DMAP) and acetic anhydride [9]. The acetylation was performed at 40°C in an Eppendorf orbital shaker (700 rpm) for 4 h. The reactions were quenched with a saturated NH_4Cl solution (500 μL) and the supernatant dried by anhydrous Na_2SO_4 and analysed via chiral GC measurement. The GC analysis conditions were as follows: injector temperature, 250°C; detector temperature, 275 °C; temperature

program: column temperature, 100 °C, hold 10 min; gradient 5°C min^{-1} up to 180 °C, hold 40 min.

10. Gene sequences

Gene sequence of FDC-Es Val46Glu (FDC*):

ATGAACACCTTCGATAAACATGATCTGAGCGGTTTTGTTGGTAAACATCTGGTGTATACCT
ATGATAATGGCTGGGAGTATGAGATCTATGTGAAAAATGAAAACACCCTGGATTATCGCA
TTCATAGCGGTCTGGAAGGTAATCGTTGGGTTAAAGATCAGCAGGCATATATTGTTCGTGT
GGGTGAAAGCATCTATAAAATCAGCTGGACCGAACCGACCGGCACCGATGTTAGCCTGAT
TGTTAATCTGGGTGATAGCCTGTTTCATGGCACCATCTTTTTTCCGCGTTGGGTGATGAATA
ATCCGGAAAAAACCGTTTGCTTTCAGAACGATCATATTCCGCTGATGAATAGCTATCGTGA
TGCAGGTCCGGCATATCCGACCGAAGTTATTGATGAATTTGCCACCATTACCTTTGTTCGT
GATTGTGGTGCAAATAACGAAAGCGTTATTGCATGTGCAGCAAGCGAACTGCCGAAAAAC
TTTCCGGATAATCTGAAATAA

Gene sequence of RaADH:

ATGAAGGCCGTTCAGTATACCGAAATTGGCAGCGAACCGGTGGTTGTGGACATTCCGACC
CCGACCCCGGGCCCTGGTGAAATTCTGCTGAAAGTGACCGCCGCCGGCCTGTGTCACAGC
GATATTTTTGTTATGGACATGCCGGCAGCACAGTATGCGTATGGATTACCGTTAACCCTTG
GCCATGAAGGTGTGGGTACCGTTGCAGAATTAGGCGAAGGTGTTACCGGTTTTGGCGTTG
GTGATGCAGTTGCAGTTTATGGACCGTGGGGGTGTGGTGCATGTCATGCGTGTGCTCGTGG
TCGTGAGAATTATTGTACCCGTGCGGCGGATCTGGGGATTACGCCGCCGGGTCTGGGTAG
TCCGGGTAGTATGGCCGAATATATGATTGTTGATAGTGCCCGTCATCTGGTTCCTATTGGT
GATCTGGACCCTGTTGCAGCAGCACCGCTTACAGACGCAGGTCTGACCCCTTATCACGCTA
TTTCACGTGTTTTACCTTTACTGGGTCCGGGGAGTACCGCCGTTGTTATTGGTGTTGGTGGT
TTAGGTCATGTTGGTATTCAGATTCTGAGAGCAGTTAGCGCAGCGCGGGTTATTGCGGTTG
ATTTAGATGATGATCGTCTGGCACTGGCACGTGAGGTTGGTGCGGATGCGGCGGTTATGA
GTGGTGCAGGGGCGGCAGACGCAATTCGCGAGCTGACCGGTGGTCAGGGTGCGACGGCA
GTTTTTGATTTTGTTGGTGCACAGAGTACAATTGATACGGCACAGCAGGTTGTTGCAGTTG
ATGGGCATATTAGCGTTGTTGGGATTCATGCGGGAGCACATGCAAAGGTTGGCTTTTTTAT
GATTCCGTTTGGTGCAAGCGTGGTTACCCCGTATTGGGGTACCCGTGCGGAACTGATGGA
AGTTGTTGCACTGGCCCGTGCCGGTCGTCTGGATATTCATACCGAGACATTTACTCTGGAC

GAAGGTCCGGCAGCATATCGTAGACTGCGTGAGGGTAGCATTAGAGGACGTGGTGTTGTT
GTTCCGTAA

Gene sequence of RDR:

ATGTCCCAGGATTTCAGCGGCAAAGTGGCCTTTGTGACCGGTGGCGCGAGCGGCATTGGT
GAAGCCGTGGTGAAACAGCTGGCGGCGCGTGGTGCGAAAGTTGTGGTTGCGGATCTGAAA
CTGGAAGGTGCGCAGGCCGTGGCGGATGCGGTGAAAGCGGCGGGTGGTGAAGCCGCGGC
CGTGGCGGTGGATGTGGCCAAAGCGGATCAGGTGGAAAAAGCGGTTCAGTTTGCGGTGGA
TACCTTTGGTGCGCTGCATCTGGCGGTGAACAATGCGGGCATTGGTGGCGCGAGCGCGCC
GCTGGGTGATTATAGCTTTGATGATTGGCATCGTGTGATCGATGTGAACCTGAACAGCGTG
TTTTATAGCATGAAATATGAAATTGTTGCCATGCTGCGCGCGGGTGGCGGCGCCATTGTGA
ACATGGCGAGCATTCTGGGTAGCGTGACCTTTCCGAATGCGCCGGCCTATGTTACCGCGA
AACACGGCGTGGTGGGCATGACCAAAAGCGCCGCCGTTGATTATGCCAAAAAAGGCATCC
GCGTTACCGCGGTTGGCCCGGGCTTTATTGATACCCCGCTGCTGAGCGCCCTGCCGAAAGA
AACCCTGGATTATCTGAAAAGCGTGCATCCGATTGGTCGCCTGGGCACCAGCGATGAAGT
GGCCGCGCTGACCGCGTTTCTGCTGAGCGATGCGGCCAGCAACATCACCGGCAGCTATCA
TCTGGTGGATGGTGGTTATGTGGCCCAGTAG

Gene sequence of CV2025:

ATGCAGAAGCAACGTACGACCAGCCAATGGCGCGAACTGGATGCCGCCCATCACCTGCAT
CCGTTCACCGATACCGCATCGCTGAACCAGGCGGGCGCGCGCGTGATGACGCGCGGAGAG
GGCGTCTACCTGTGGGATTCGGAAGGCAACAAGATCATCGACGGCATGGCCGGACTGTGG
TGCGTGAACGTCGGCTACGGCCGCAAGGACTTTGCCGAAGCGGCGCGCCGGCAGATGGAA
GAGCTGCCGTTCTACAACACCTTCTTCAAGACCACCCATCCGGCGGTGGTCGAGCTGTCCA
GCCTGCTGGCTGAAGTGACGCCGGCCGGTTTCGACCGCGTGTTCTATACCAATTCCGGTTC
CGAATCGGTGGACACCATGATCCGCATGGTGCGCCGCTACTGGGACGTGCAGGGCAAGCC
GGAGAAGAAGACGCTGATCGGCCGCTGGAACGGCTATCACGGCTCCACCATCGGCGGCGC
CAGCCTGGGCGGCATGAAGTACATGCACGAGCAGGGCGACTTGCCGATTCCGGGCATGGC
CCACATCGAGCAGCCTTGGTGGTACAAGCACGGCAAGGACATGACGCCGGACGAGTTCGG
CGTGGTGGCCGCGCGCTGGCTGGAAGAGAAGATTCTGGAAATCGGCGCCGACAAGGTGG

CCGCCTTCGTCGGCGAACCCATCCAGGGCGCCGGCGGCGTGATCGTCCCGCCGGCCACCT

ACTGGCCGGAAATCGAGCGCATTTGCCGCAAGTACGACGTGCTGCTGGTGGCCGACGAAG

TGATCTGCGGCTTCGGGCGTACCGGCGAATGGTTCGGCCATCAGCATTTCGGCTTCCAGCC

CGACCTGTTCACCGCCGCCAAGGGCCTGTCCTCCGGCTATCTGCCGATAGGCGCGGTCTTT

GTCGGCAAGCGCGTGGCCGAAGGCCTGATCGCCGGCGGCGACTTCAACCACGGCTTCACC

TACTCCGGCCACCCGGTCTGCGCCGCCGTCGCCCACGCCAACGTGGCGGCGCTGCGCGAC

GAGGGCATCGTCCAGCGCGTCAAGGACGACATCGGCCCGTACATGCAAAAGCGCTGGCGT

GAAACCTTCAGCCGTTTCGAGCATGTGGACGACGTGCGCGGCGTCGGCATGGTGCAGGCG

TTCACCCTGGTGAAGAACAAGGCGAAGCGCGAGCTGTTCCCCGATTTCGGCGAGATCGGC

ACGCTGTGCCGCGACATCTTCTTCCGCAACAACCTGATCATGCGGGCATGCGGCGACCAC

ATCGTGTCGGCGCCGCCGCTGGTGATGACGCGGGCGGAAGTGGACGAGATGCTGGCGGTG

GCGGAACGCTGTCTGGAGGAATTCGAGCAGACGCTGAAGGCGCGCGGGCTGGCTTAG

Gene sequence of MVTA:

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GACACACCGGCAGGCAGCGTCATCCAGTATTCCGATTACGAGATTGATTACAGCTCGCCG

TTCGCGGGCGGCGTGGCGTGGATTGAGGGTGAGTACCTGCCGGCCGAAGATGCGAAAATT

AGCATCTTCGACACCGGTTTCGGCCACTCGGACCTGACCTACACGGTGGCGCACGTTTGGC

ACGGCAACATTTTCCGCTTGGGCGATCACCTGGACCGCCTGCTCGACGGCGCACGGAAGC

TCCGCCTGGACAGCGGCTACACCAAAGACGAGCTCGCCGACATCACCAAGAAGTGCGTGA

GCCTGAGCCAGCTGCGTGAAAGCTTCGTGAACCTGACCATCACCCGCGGGTACGGCAAGC

GCAAGGGGGAAAAGGACCTGAGCAAGCTGACCCACCAAGTGTACATTTACGCCATTCCGT

ATCTGTGGGCGTTCCCCCCGGCGGAGCAGATCTTCGGCACCACCGCGGTGGTGCCGCGCC

ACGTGCGCCGCGCCGGCCGCAATACCGTGGACCCGACCATCAAGAACTACCAGTGGGGCG

ACCTGACCGCGGCCAGCTTCGAGGCTAAGGATCGGGGCGCGCGCACCGCCATTCTGATGG

ACGCGGACAACTGCGTCGCGGAGGGCCCGGGATTCAACGTGTGCATTGTGAAGGACGGCA

AGCTGGCAAGCCCGTCCCGCAACGCGCTGCCGGGCATCACCCGCAAGACCGTGTTCGAGA

TTGCGGGAGCCATGGGAATTGAAGCGGCGCTCCGCGACGTGACCTCCCATGAGCTGTACG

ACGCCGACGAGATTATGGCGGTGACCACCGCGGGCGGCGTGACCCCGATTAACACCCTGG

ACGGCGTGCCGATCGGCGACGGCGAGCCGGGTCCGGTGACCGTGGCGATTCGCGACCGCT

TCTGGGCGCTGATGGACGAGCCGGGGCCACTGATTGAAGCGATTCAGTAC

Gene sequence of BsLDH:

ATGATGAACAAACATGTAAATAAAGTAGCTTTAATCGGAGCGGGTTTTGTTGGAAGCAGT
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AAGAAAAAGCAATGGGCGATGTGATGGATTTAAACCACGGAAAGGCGTTTGCGCCACAA
CCGGTCAAAACATCTTACGGAACATATGAAGACTGCAAGGATGCTGATATTGTCTGCATTT
GCGCCGGAGCAAACCAAAAACCTGGTGAGACACGCCTTGAATTAGTAGAAAAGAACTTG
AAGATTTTCAAAGGCATCGTTAGTGAAGTCATGGCGAGCGGATTTGACGGCATTTTCTTAG
TCGCGACAAATCCGGTTGATATCCTGACTTACGCAACATGGAAATTCAGCGGCCTGCCAA
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ACACAGAGCTTCCTGTTTGGAGCCACGCGAATGTCGGCGGTGTGCCGGTCAGTGAACTCG
TTGAGAAAAACGATGCGTACAAACAAGAGGAGCTGGACCAAATTGTAGATGATGTGAAA
AACGCAGCTTACCATATCATTGAGAAAAAAGGCGCGACTTATTATGGGGTTGCGATGAGT
CTTGCTCGCATTACAAAAGCCATTCTTCATAATGAAAACAGCATATTAACTGTCAGCACAT
ATTTGGACGGGCAATACGGTGCAGATGACGTGTACATCGGTGTGCCGGCTGTCGTGAATC
GCGGAGGGATCGCAGGTATCACTGAGCTGAACTTAAATGAGAAAGAAAAAGAACAGTTC
CTTCACAGCGCCGGCGTCCTTAAAAACATTTTAAAACCTCATTTTGCAGAACAAAAAGTCA
ACTAA

Table S1. Primers used in this study.

Entry	Names	Primers(5'-3')	REA[a]
1	FDC*-*Nde*I-F	GGGAATTCCATATGAACACCTTCGATAAA CATGATCTGAGCGG	*Nde*I
2	FDC*-*Xho*I-R	CCGCTCGAGTTATTTCAGATTATCCGGAA AGTTTTTCGGCAGTTCGC	*Xho*I
3	RaADH-*BamH*I-F	CGCGGATCCGATGAAGGCCGTTCAGTATA CCGAAATTGGC	*BamH*I
4	RaADH-*Xho*I-R	CCGCTCGAGTTACGGAACAACAACACCAC GTCCTCTAATGC	*Xho*I
5	RDR-*Nde*I-F	GGGAATTCCATATGTCCCAGGATTTCAGC GGCAAAGTGGC	*Nde*I
6	RDR-*Xho*I-R	CCGCTCGAGCTACTGGGCCACATAACCAC CATCCACC	*Xho*I
7	FDC*-*Nco*I-F	CATGCCATGGGCATGAACACCTTCGATAA ACATGATCTGAGCGG	*Nco*I
8	FDC*-*Hind*III-R	CCCAAGCTTTTATTTCAGATTATCCGGAA AGTTTTTCGGCAGTTCGC	*Hind*III
9	RaADH-*Nco*I-F	CATGCCATGGGCATGAAGGCCGTTCAGTA TACCGAAATTGGC	*Nco*I
10	RaADH-*Hind*III-R	CCCAAGCTTTTACGGAACAACAACACCAC GTCCTCTAATGC	*Hind*III
11	MVTA-*Nde*I-F	GGGAATTCCATATGGGCATCGACACTGGC ACCTCGAACC	*Nde*I
12	MVTA-*XhoI*-R	TGCACTGCAGTTAAGCACCCGCCACAG	*Xho*I
13	CV2025-*Nde*I-F	GGGAATTCCATATGCAGAAGCAACGTACG ACCAGCCAATGGCGCG	*Nde*I
14	CV2025-*Xho*I-R	CCGCTCGAGCTAAGCCAGCCCGCGCG	*Xho*I
15	BsLDH-*Nco*I-F	CATGCCATGGGCATGATGAACAAACATGT AAATAAAGTAGCTTTAATC	*Nco*I
16	BsLDH-*Hind*III-R	CCCAAGCTTTTAGTTGACTTTTTGTTCTGC AAAATGAGGTTTTAAAATG	*Hind*III

[a] REA: restriction endonuclease

Table S2. Recombinant strains constructed in this study.

Recombinant *E. coli*	Recombinant plasmid	Enzyme expressed	Reference
E. coli (FDC*)	pET28a-FDC*	FDC* (*Enterobacter* sp.)	[6]
E. coli (RaADH)	pET28a-RaADH	RaADH (*Rhodococcus aetherivorans*)	This study
E. coli (RDR)	pET28a-RDR	RDR (*Devosia riboflavina*)	[7]
E. coli (MVTA)	pET28a-MVTA	MVTA (*Mycobacterium vanbaalenii*)	[2]
E. coli (CV2025)	pET28a-CV2025	CV2025 (*Chromobacterium violaceum*)	[1]
E. coli (BsLDH)	pET28a-BsLDH	BsLDH (*Bacillus subtilis*)	This study
E. coli (AlaDH)	pETduet-AlaDH	AlaDH *(Bacillus subtilis)*	[3]
E. coli (NOX)	pET28a-NOX	NOX (*Lactobacillus pentosus*)	[5]
E. coli (GDH)	pETduet-GDH	GDH (*Bacillus subtilis*)	[4]
E. coli (RFM)	pRSFduet-FDC*-MVTA	FDC* and MVTA	This study
E. coli (EFM)	pETduet-FDC*-MVTA	FDC* and MVTA	This study
E. coli (CB)	pCDFduet-BsLDH	BsLDH	This study
E. coli (RFC)	pRSFduet-FDC*-CV2025	FDC* and CV2025	This study
E. coli (EFC)	pETduet-FDC*-CV2025	FDC* and CV2025	This study
E. coli (RRR)	pRSFduet-RaADH-RDR	RaADH and RDR	This study
E. coli (ERR)	pETduet-RaADH-RDR	RaADH and RDR	This study
E. coli (RFM-ERR-CB)	pRSFduet-FDC*-MVTA pETduet-RaADH-RDR pCDFduet-BsLDH	FDC*, MVTA, RaADH, RDR and BsLDH	This study
E. coli (RFC-ERR-CB)	pRSFduet-FDC*-CV2025 pETduet-RaADH-RDR pCDFduet-BsLDH	FDC*, CV2025, RaADH, RDR and BsLDH	This study
E. coli (EFM-RRR-CB)	pETduet-FDC*-MVTA pRSFduet-RaADH-RDR pCDFduet-BsLDH	FDC*, MVTA, RaADH, RDR and BsLDH	This study
E. coli (EFC-RRR-CB)	pETduet-FDC*-CV2025 pRSFduet-RaADH-RDR pCDFduet-BsLDH	FDC*, CV2025, RaADH, RDR and BsLDH	This study

Table S3. Retention times for the amine products analyzed by achiral GC.

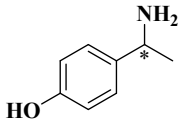
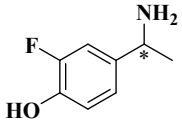
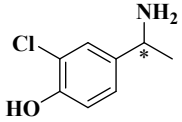
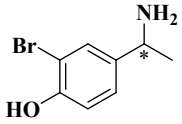
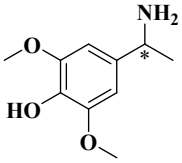
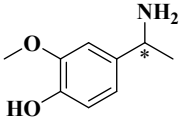
Product	method	[min]
 4-(1-Aminoethyl)phenol (**4**a)	A	24.7
 4-(1-aminoethyl)-2-fluorophenol (**4**b)	A	21.9
 4-(1-aminoethyl)-2-chlorophenol (**4**c)	A	22.6
 4-(1-aminoethyl)-2-bromophenol (**4**d)	A	23.8
 4-(1-aminoethyl)-2,6-dimethoxyphenol (**4**f)	A	26.9
 4-(1-aminoethyl)-2-methoxyphenol (**4**e)	B	8.7

Table S4. Retention times for the amines analyzed by chiral GC.

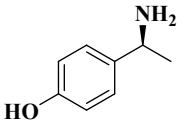
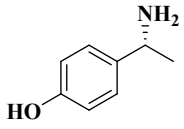
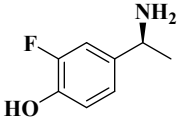
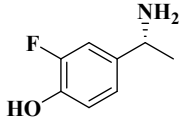
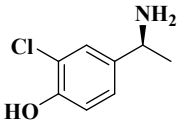
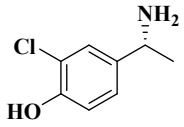
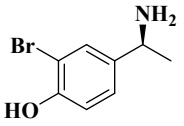
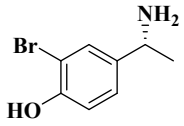
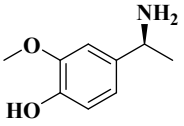
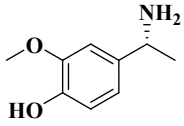
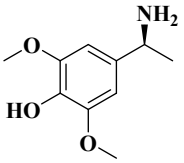
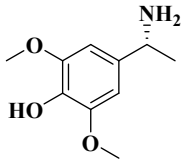
Product	[min]	Product	[min]
 (*S*)-**4**a	34.3	 (*R*)-**4**a	34.6
 (*S*)-**4**b	33.5	 (*R*)-**4**b	34.0
 (*S*)-**4**c	43.6	 (*R*)-**4**c	44.4
 (*S*)-**4**d	52.3	 (*R*)-**4**d	53.4
 (*S*)-**4**e	43.3	 (*R*)-**4**e	43.9
 (*S*)-**4**f	46.7	 (*R*)-**4**f	47.4

Table S5. Hydratase FDC* catalyzed asymmetric hydration of 4-hydroxystyrenes **1** to alcohols **2**.

Substrate	Time (h)	Conversion (%)	2 ee (%)
1a	12	90	91 (*S*)
1b	12	92	66 (*S*)
1c	24	78	75 (*S*)
1d	12	92	77 (*S*)
1e	24	52	55 (*S*)
1f	24	50	53 (*S*)

Reaction conditions: 100 mM potassium phosphate buffer (pH 6.0), 10 mg/mL FDC*, 10 mM **1**, incubation for 12-24 h at 25 °C and 200 rpm shaking. Conversion was determined by GC, error limit: <2% of the stated values; ee was determined by chiral GC.

Table S6. Enantioselective oxidation of racemic **2** to ketone **3** with the resting cells of *E. coli* (RaADH) and *E. coli* (RDR).

Sub.	*E. coli* (RaADH) (g cdw/L)	*E. coli* (RDR) (g cdw/L)	Time (h)	Conv. to 3 (%)	2 ee (%)
2a	10		6	50.0	>99 (*S*)
2b	10		6	50.2	>99 (*S*)
2c	10		6	50.5	>99 (*S*)
2d	10		6	50.1	>99 (*S*)
2e	15		6	49.8	>99 (*S*)
2f	15		6	49.6	>99 (*S*)
2a		10	6	50.1	>99 (*R*)
2b		10	6	50.2	>99 (*R*)
2c		10	6	50.5	>99 (*R*)
2d		10	6	50.0	>99 (*R*)
2e		15	6	50.1	>99 (*R*)
2f		15	6	50.1	>99 (*R*)
2a	10	10	6	99.5	-
2b	10	10	6	99.7	-
2c	10	10	6	99.9	-
2d	10	10	6	99.8	-
2e	15	15	6	99.5	-
2f	15	15	6	99.7	-

Reaction conditions: 100 mM potassium phosphate buffer (pH 8.0), 10-15 g cdw/L of *E. coli* (RaADH) or *E. coli* (RDR), 10 mM *rac*-**2**, incubation for 6 h at 30 °C and 200 rpm shaking. Conversion was determined by GC, error limit:<2% of the stated values; ee was determined by chiral GC.

Table S7. Enantioselective reduction amination of ketones **3** to chiral amines **4** with the resting cells of *E. coli* (MVTA) and *E. coli* (CV2025).

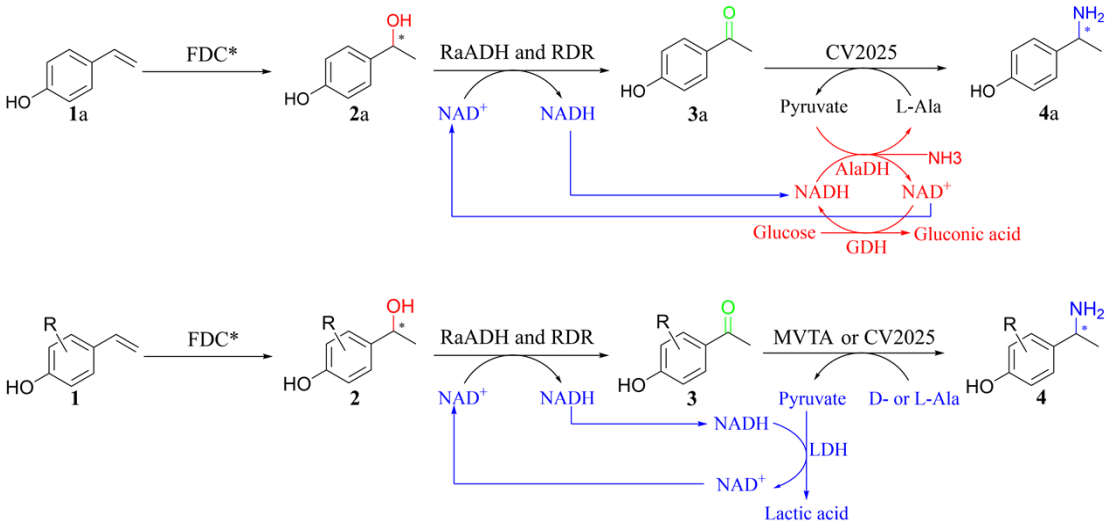
Substrate	*E. coli* (MVTA) (g cdw/L)	*E. coli* (CV2025) (g cdw/L)	Time (h)	Conv. to 4 (%)	4 ee (%)
3a	10		12	51.0	>99 (*R*)
3b	10		12	49.8	>99 (*R*)
3c	10		12	28.4	>99 (*R*)
3d	10		12	44.3	>99 (*R*)
3e	15		24	24.1	>99 (*R*)
3f	15		24	25.8	>99 (*R*)
3a		10	12	40.1	>99 (*S*)
3b		10	12	30.0	>99 (*S*)
3c		10	12	27.1	>99 (*S*)
3d		10	12	36.2	>99 (*S*)
3e		15	24	22.6	>99 (*S*)
3f		15	24	20.7	>99 (*S*)

Reaction conditions: 100 mM potassium phosphate buffer (pH 8.0), 10-15 g cdw/L of resting cell of *E. coli* (MVTA) and *E. coli* (CV2025), 10 mM substrates **3**, 10% DMSO, 0.1 mM PLP, 200 mM D-/L-Ala, incubation for 12-24 h at 30 °C, and 200 rpm shaking. Conversion was determined by GC, error limit:<2% of the stated values; ee was determined by chiral GC.

Table S8. The specific activity of enzymes used in this study.

Enzyme	Specific activity (U/mg)
FDC*	0.32
RaADH	0.46
RDR	0.96
BsLDH	8.9
GDH	3.7
NOX	5.2
AlaDH	1.2
CV2025	4.17
MVTA	4.4

Table S9. *In vitro* asymmetric hydroamination of 4-hydroxystyrenes **1**a-f to (*S*)- or (*R*)-**4**a-f.



Entry	CV2025 (mg/mL)	MVTA (mg/mL)	LDH (mg/mL)	AlaDH (mg/mL)	GDH (mg/mL)	NAD^+ (mM)	Time (h)	Conv. to 4a (%)	4a ee (%)
1	10	-	-	-	-	1	24	38	99 (S)
2	10	-	-	15		1	48	50	99 (S)
3	10	-	-	15	10	1	48	55	99 (S)
4	10	-	20	-	-	1	24	73	99 (S)
4	10	-	20	-	-	0.5	24	73	99 (S)
4	20	-	20	-	-	0.05	24	67	99 (S)
5	-	10	-	-	-	1.0	24	42	99 (R)
6	-	10	20	-	-	1.0	24	80	99 (R)
6	-	10	20	-	-	0.5	24	75	99 (R)
6	-	20	20	-	-	0.05	24	74	99 (R)

Reaction conditions: 3 mL potassium phosphate buffer (100 mM, pH 8.0) including 10 mM **1**a, 200 mM L-/D-Ala, 500 mM NH_3/NH_4Cl, 50 mM glucose, 0.05-1.0 mM NAD^+, 0.1 mM PLP, 10 mg/mL FDC*, 20 mg/mL RaADH, 20 mg/mL RDR, 10-20 mg/mL CV2025, 10-20 mg/mL MVTA, 20 mg/mL LDH, 15 mg/mL AlaDH, 10 mg/mL GDH, incubation at 30°C, and 200 rpm for 24-48 h. Conversion was determined by GC, error limit:<2% of the stated values. ee values were determined by chiral GC.

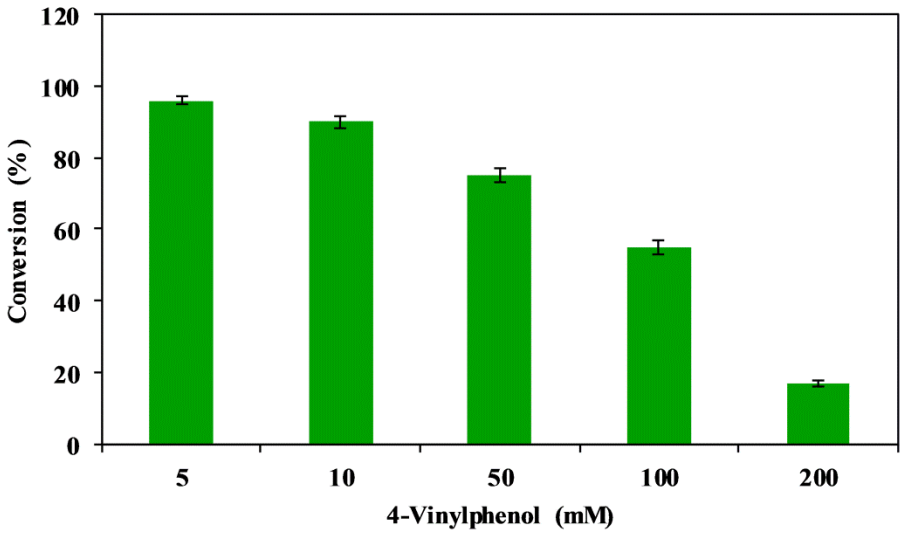


Figure S1. Conversion of 4-vinylphenol with the resting cells of *E. coli* (FDC*).

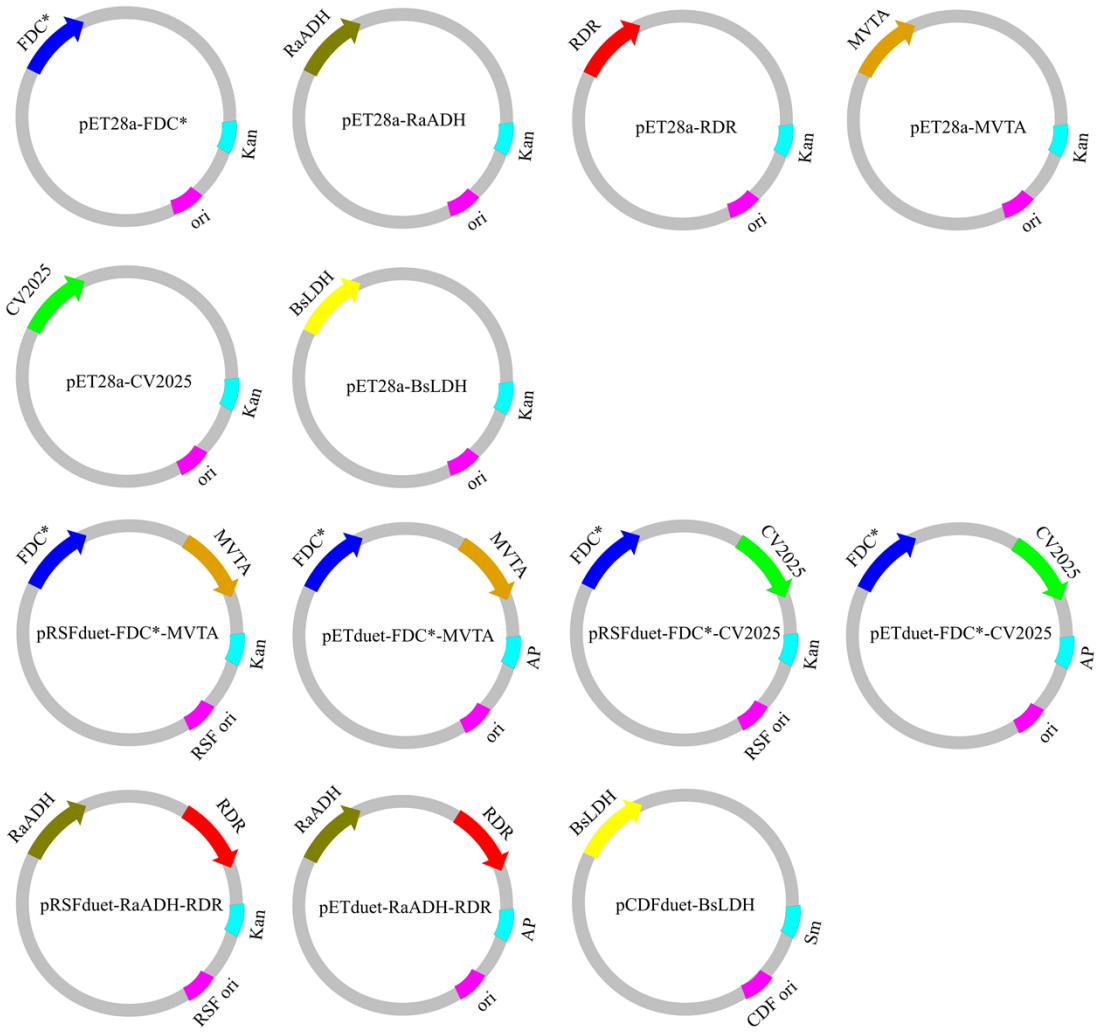


Figure S2. Recombinant plasmid constructed.

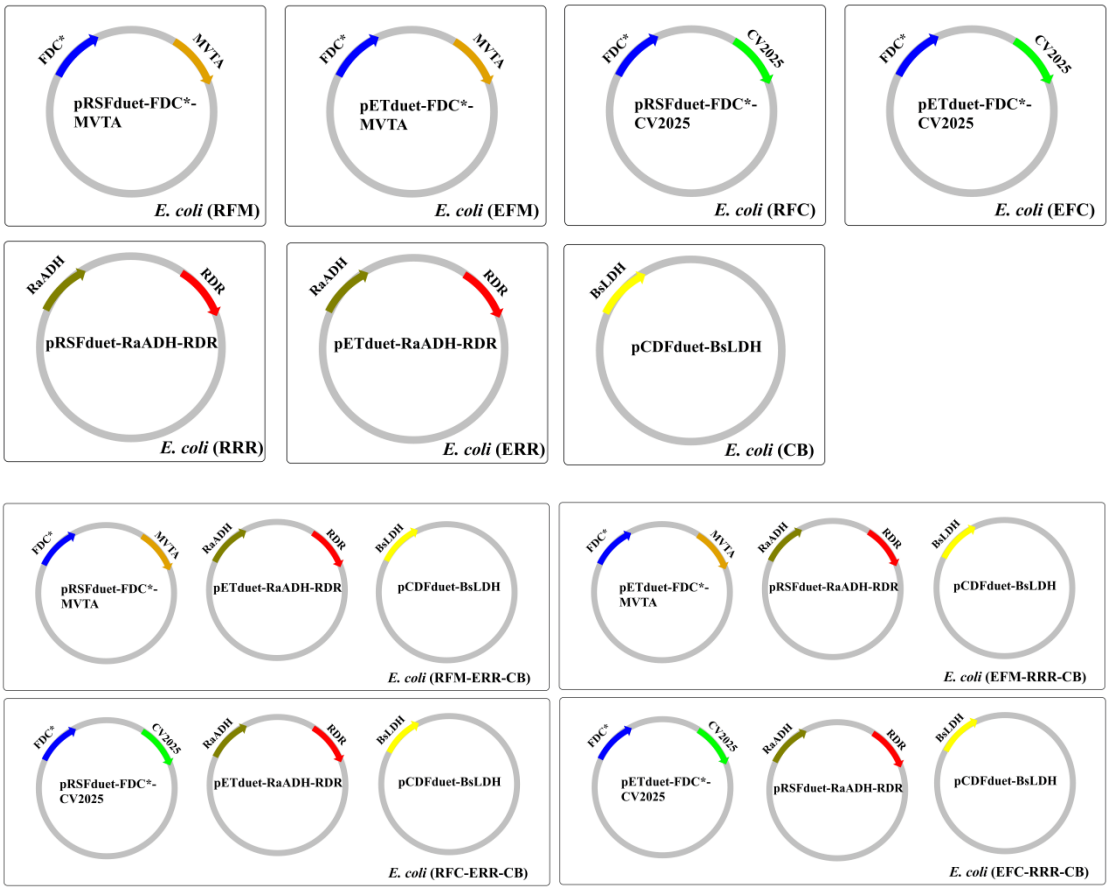


Figure S3. Recombinant *E. coli* cells co-expression of multiple enzymes.

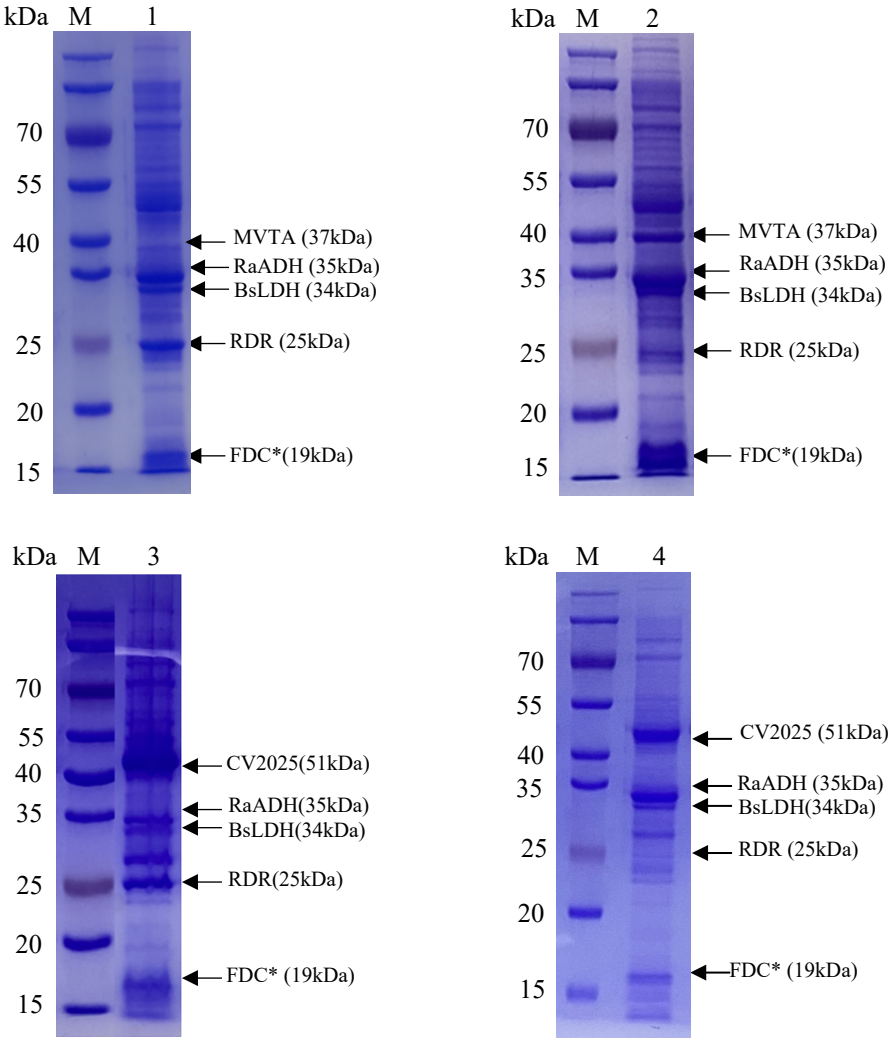


Figure S4. SDS-PAGE of the cell-free extracts of recombinant *E. coli* cells co-expression of multiple enzymes. Lane M：marker, lane 1: *E. coli* (RFM-ERR-CB), lane 2: *E. coli* (EFM-RRR-CB), lane 3: *E. coli* (RFC-ERR-CB), lane 4: *E. coli* (EFC-RRR-CB).

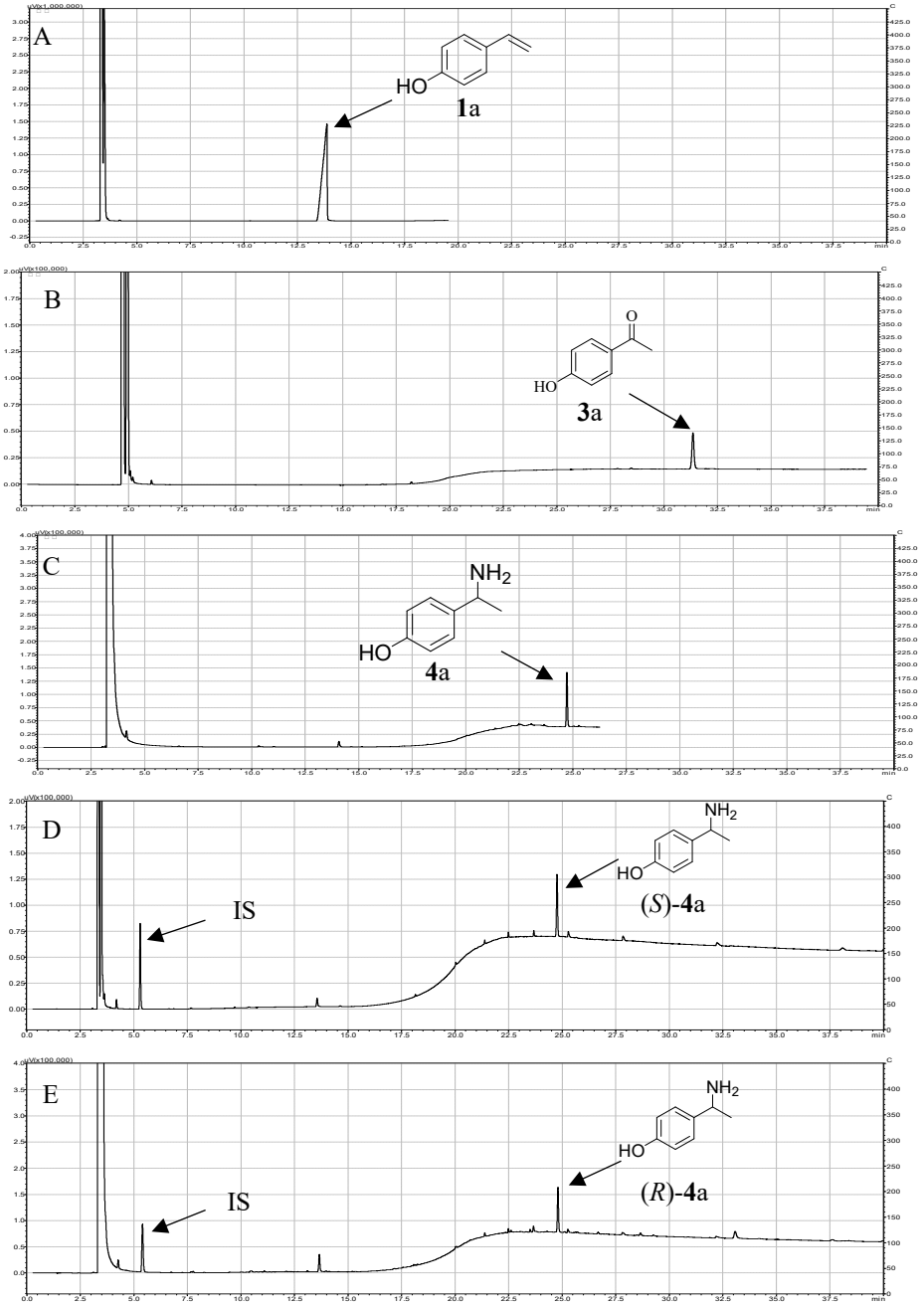


Figure S5. Achiral GC chromatograms of 4-(1-aminoethyl)phenol **4**a. A: 4-vinylphenol **1**a standard. B: 4'-hydroxy-acetophenon **3**a standard. C: 4-(1-aminoethyl)phenol **4**a standard. D: *(S)*-4-(1-aminoethyl)phenol **4**a produced by conversion of 4-vinylphenol **1**a (10 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. E: *(R)*-4-(1-aminoethyl)phenol **4**a produced by conversion of 4-vinylphenol **1**a (10 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. IS: Internal standard (*n*-dodecane), 5.5 min; 4-(1-Aminoethyl)phenol **4**a : 24.7 min.

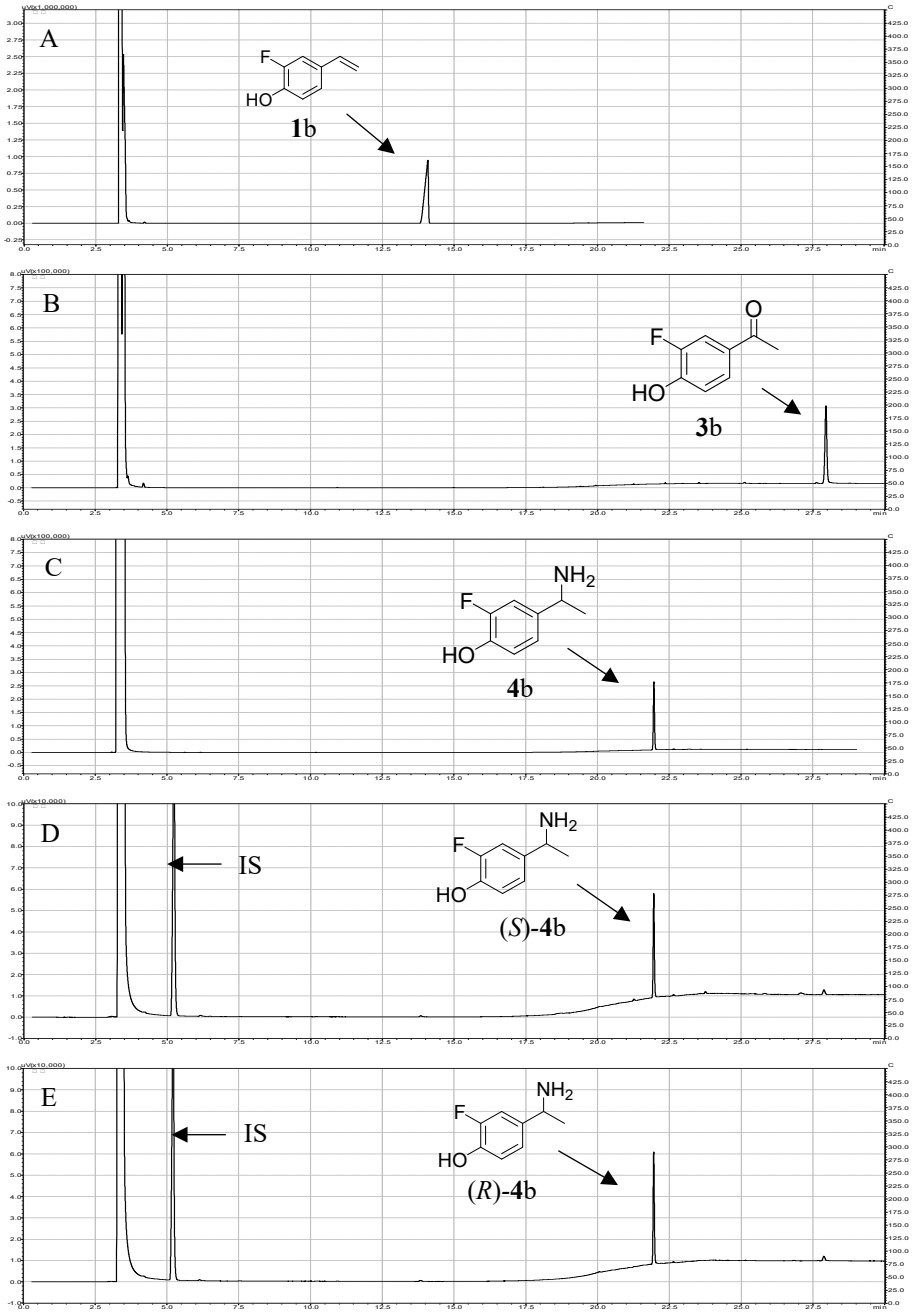


Figure S6. Achiral GC chromatograms of 4-(1-aminoethyl)-2-fluorophenol **4**b. A: 2-fluoro-4-vinylphenol **1**b standard. B: 1-(3-fluoro-4-hydroxyphenyl)-1-ethanone **3**b standard. C: 4-(1-aminoethyl)-2-fluorophenol **4**b standard. D: *(S)*-4-(1-aminoethyl)-2-fluorophenol **4**b produced by conversion of 2-fluoro-4-vinylphenol **1**b (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. E: *(R)*-4-(1-aminoethyl)-2-fluorophenol **4**b produced by conversion of 2-fluoro-4-vinylphenol **1**b (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. IS: Internal standard (*n*-dodecane), 5.5 min; 4-(1-aminoethyl)-2-fluorophenol **4**b : 21.976 min.

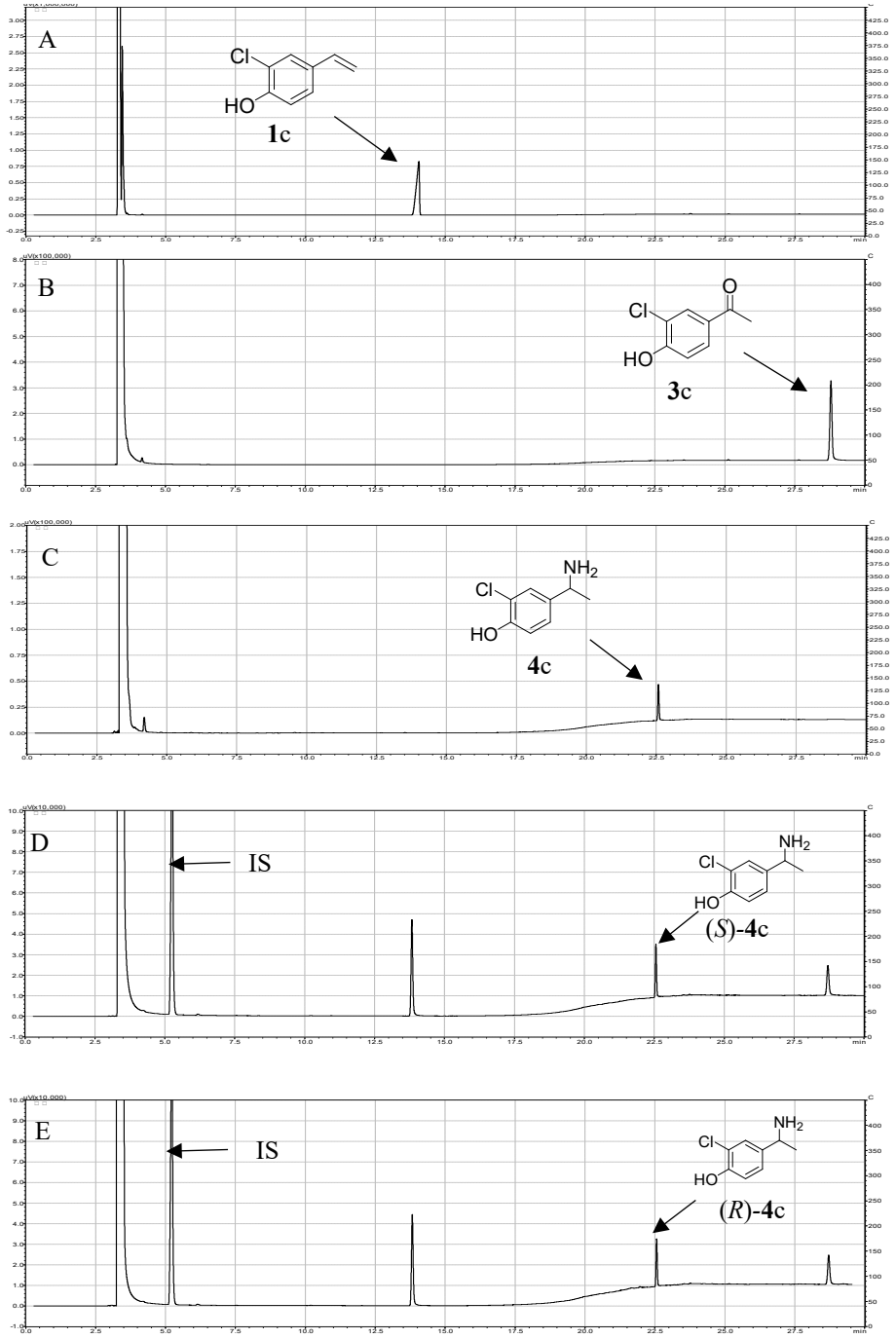


Figure S7. Achiral GC chromatograms of 4-(1-aminoethyl)-2-chlorophenol **4**c. A: 2-chloro-4-vinylphenol **1**c standard. B: 1-(3-chloro-4-hydroxyphenyl)-1-ethanone **3**c standard. C: 4-(1-aminoethyl)-2-chlorophenol **4**c standard. D: (*S*)-4-(1-aminoethyl)-2-chlorophenol **4**c produced by conversion of 2-chloro-4-vinylphenol **1**c (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. E: (*R*)-4-(1-aminoethyl)-2-chlorophenol **4**c produced by conversion of 2-chloro-4-vinylphenol **1**c (20 mM)

with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. IS: Internal standard (*n*-dodecane), 5.5 min; 4-(1-aminoethyl)-2-chlorophenol **4**c : 22.575 min.

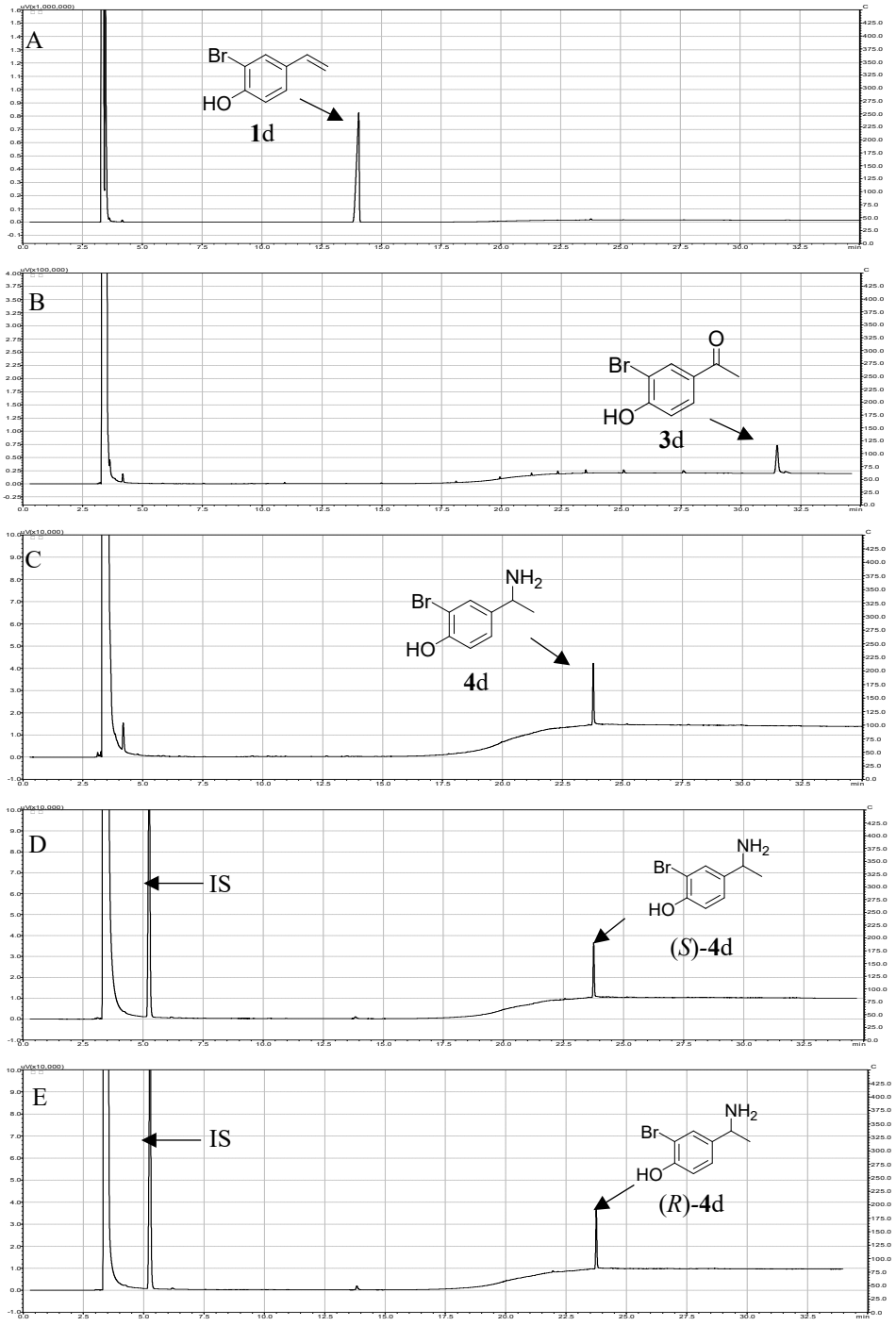


Figure S8. Achiral GC chromatograms of 4-(1-aminoethyl)-2-bromophenol **4**d. A: 2-bromo-4-ethenylphenol **1**d standard. B: 1-(3-Bromo-4-hydroxyphenyl)ethanone **3**d standard. C: 4-(1-aminoethyl)-2-bromophenol **4**d standard. D: (*S*)-4-(1-aminoethyl)-2-bromophenol **4**d produced by conversion of 2-bromo-4-ethenylphenol **1**d (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. E: (*R*)-4-(1-aminoethyl)-2-bromophenol **4**d produced by conversion of 2-bromo-4-ethenylphenol **1**d (20

mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. IS: Internal standard (*n*-dodecane), 5.5 min; 4-(1-aminoethyl)-2-bromophenol **4**d : 23.783 min.

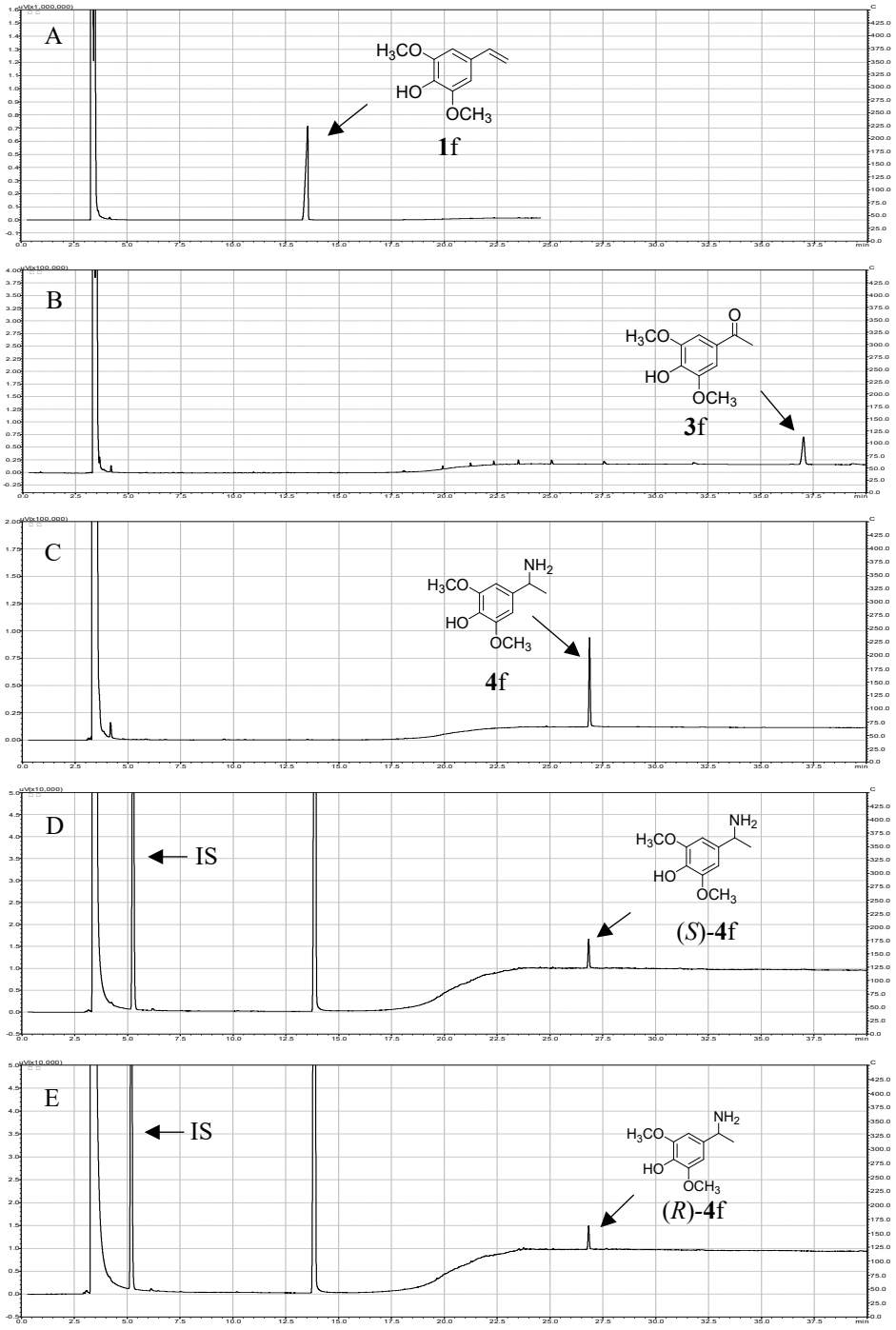


Figure S9. Achiral GC chromatograms of 4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f. A: 2,6-dimethoxy-4-vinylphenol **1**f standard. B: 1-(4-Hydroxy-3-methoxyphenyl)ethanone **3**f standard. C: 4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f standard. D: (*S*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f produced by conversion of 2,6-dimethoxy-4-vinylphenol **1**e (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. E: (*R*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f produced by

conversion of 2,6-dimethoxy-4-vinylphenol **1**e (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. IS: Internal standard (*n*-dodecane), 5.5 min; 4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f: 26.887 min.

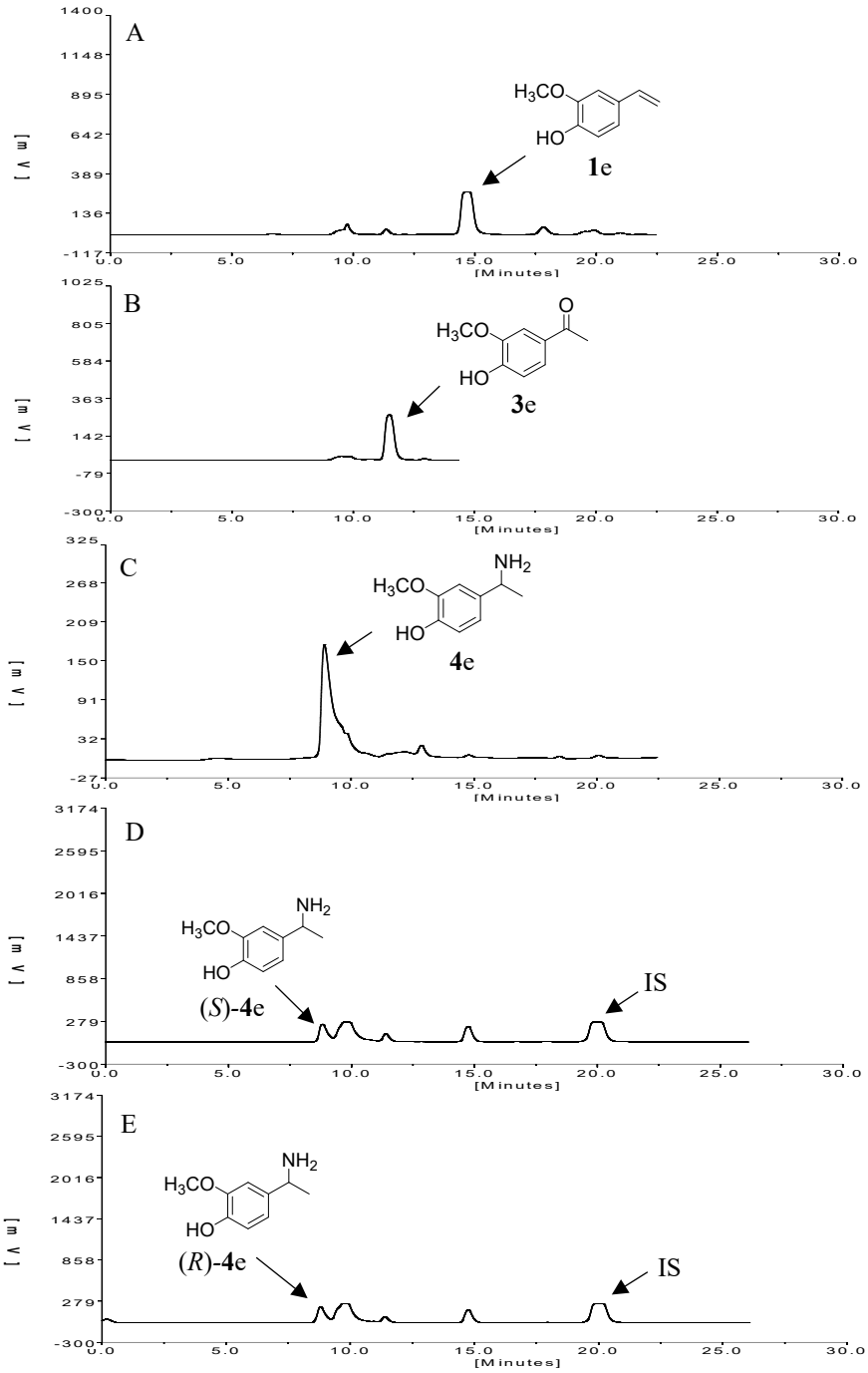


Figure S10. Achiral HPLC chromatograms of 4-(1-aminoethyl)-2-methoxyphenol **4**e. A: 2-methoxy-4-vinylphenol **1**e standard. B: 1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone **3**e standard. C: 4-(1-aminoethyl)-2-methoxyphenol **4**e standard. D: (*S*)-4-(1-aminoethyl)-2-methoxyphenol **4**e produced by conversion of 2-methoxy-4-vinylphenol **1**e (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. E: (*R*)-4-(1-aminoethyl)-2-methoxyphenol **4**e produced by conversion of 2-methoxy-4-vinylphenol **1**e (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. IS: Internal

standard (1-(p-Hydroxyphenyl)propane), 20.1 min; 4-(1-aminoethyl)-2-methoxyphenol **4**e : 8.67 min.

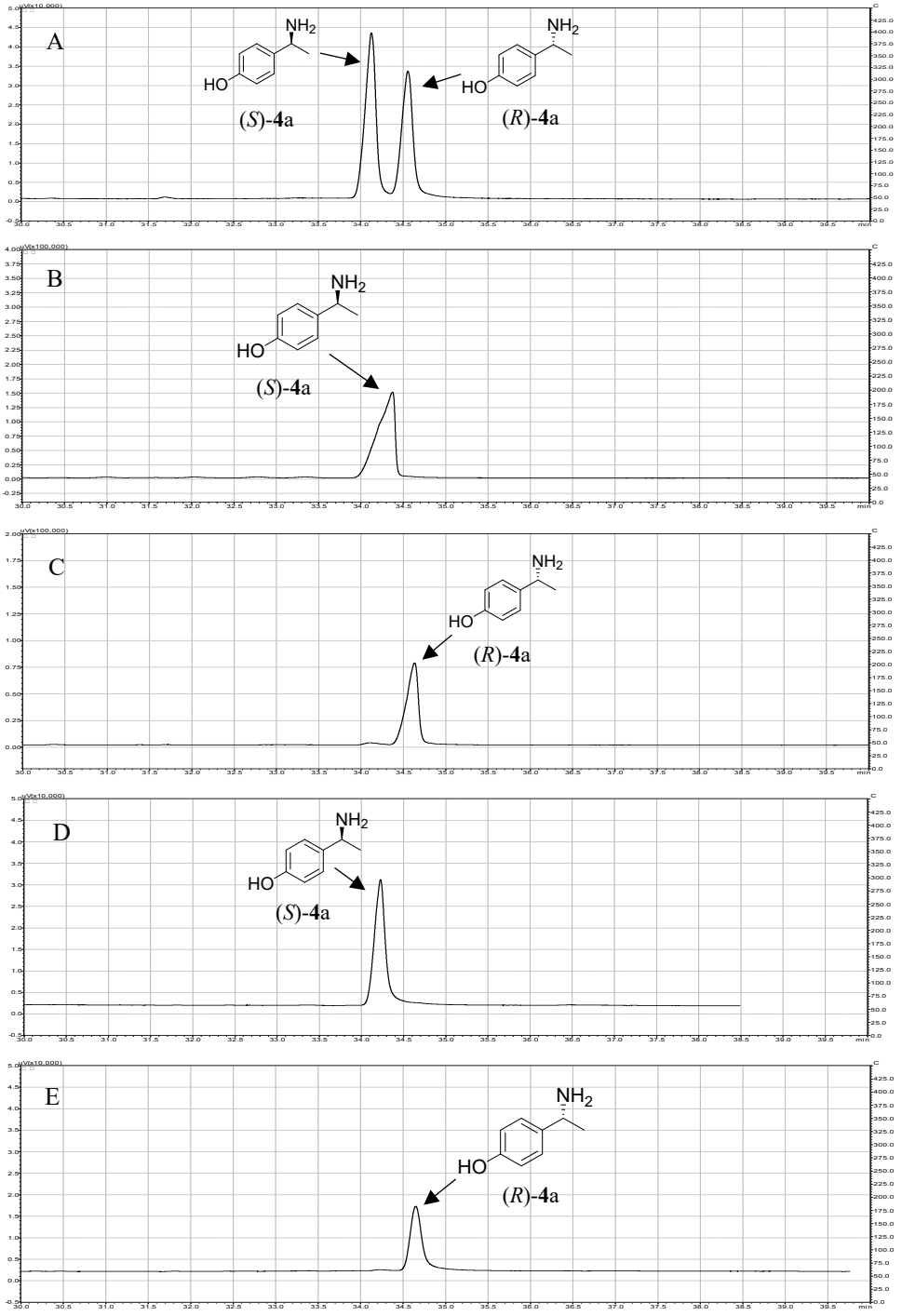


Figure S11. Chiral GC chromatograms of 4-(1-aminoethyl)phenol **4**a. A: *rac*-4-(1-aminoethyl)phenol **4**a standard. B: (*S*)-4-(1-aminoethyl)phenol **4**a standard. C: (*R*)-4-(1-aminoethyl)phenol **4**a standard. D: (*S*)-4-(1-Aminoethyl)phenol **4**a produced by conversion of 4-vinylphenol **1**a (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 30 h. E: (*R*)-4-(1-aminoethyl)phenol **4**a produced by conversion of 4-vinylphenol **1**a (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at

30 h. (*S*)-4-(1-aminoethyl)phenol **4**a: 34.3 min. (*R*)-4-(1-Aminoethyl)phenol **4**a: 34.6 min.

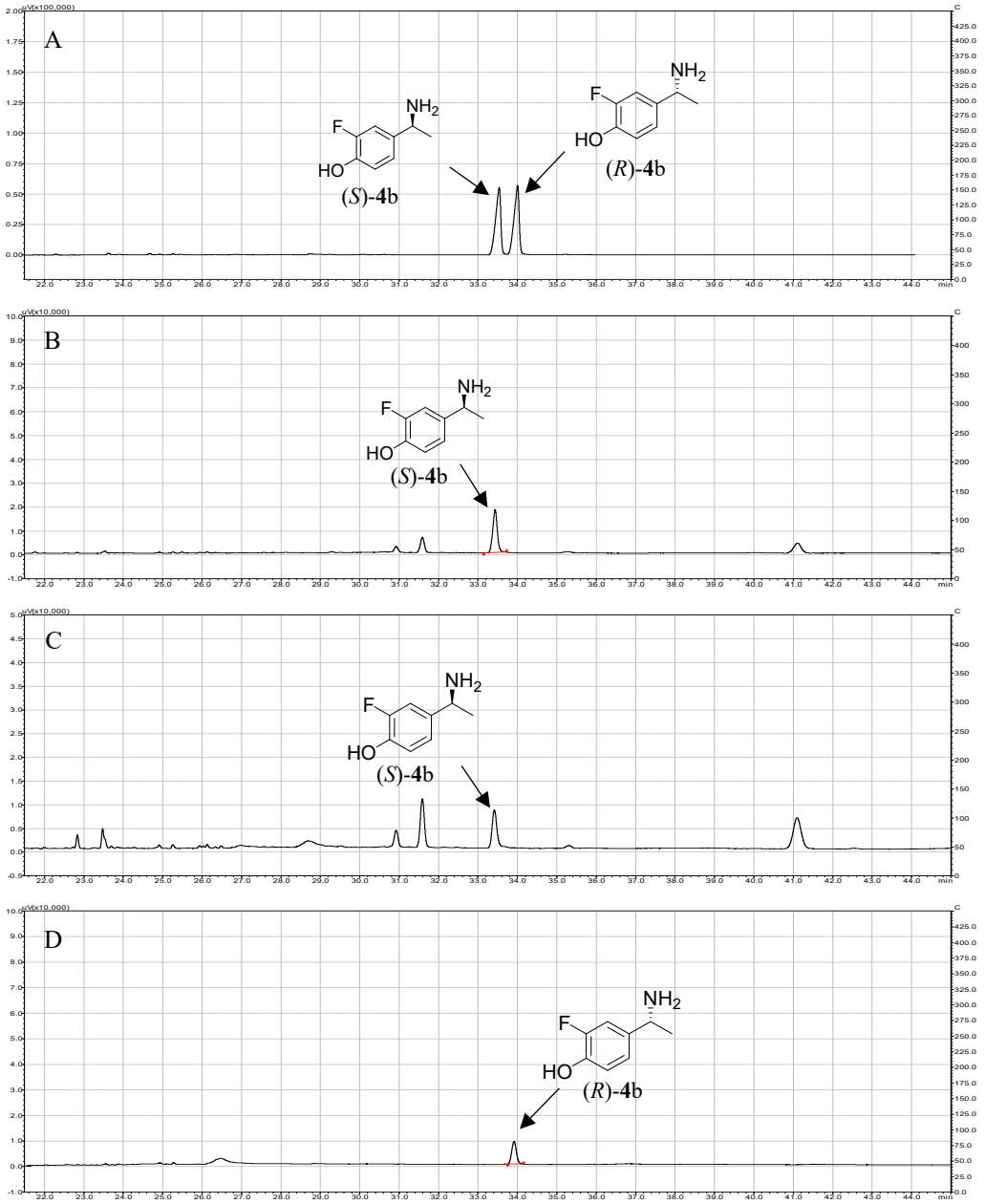


Figure S12. Chiral GC chromatograms of 4-(1-aminoethyl)-2-fluorophenol **4**b. A: rac-4-(1-aminoethyl)-2-fluorophenol **4**b standard. B: (*S*)-4-(1-aminoethyl)-2-fluorophenol **4**b standard. C: (*S*)-4-(1-aminoethyl)-2-fluorophenol **4**b produced by conversion of 2-fluoro-4-vinylphenol **1**b (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. D: (*R*)-4-(1-Aminoethyl)phenol **4**b produced by conversion of 2-fluoro-4-vinylphenol **1**b (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. (*S*)-4-(1-aminoethyl)-2-fluorophenol **4**b : 33.539 min. (*R*)-4-(1-aminoethyl)-2-fluorophenol **4**b : 34.004 min.

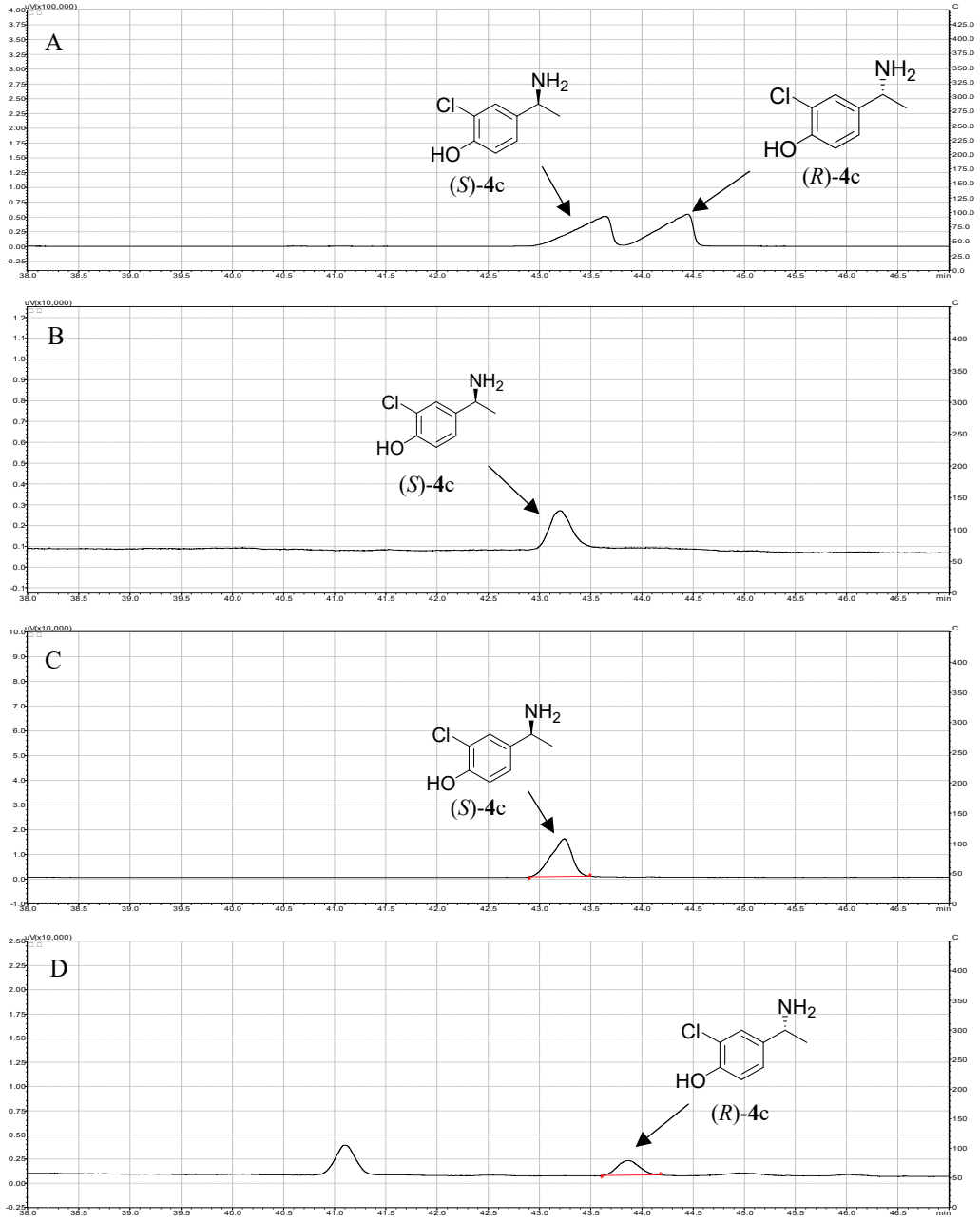


Figure S13. Chiral GC chromatograms of 4-(1-aminoethyl)-2-chlorophenol **4**c. A: rac-4-(1-aminoethyl)-2-chlorophenol **4**c standard. B: (S)-4-(1-aminoethyl)-2-chlorophenol **4**c standard. C: (*S*)-4-(1-aminoethyl)-2-chlorophenol **4**c produced by conversion of 2-chloro-4-vinylphenol **1**c (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. D: (*R*)-4-(1-aminoethyl)-2-chlorophenol **4**c produced by conversion of 2-chloro-4-vinylphenol **1**c (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. (*S*)-4-(1-aminoethyl)-2-fluorophenol **4**c : 43.638min. (*R*)-4-(1-aminoethyl)-2-fluorophenol **4**c : 44.445 min.

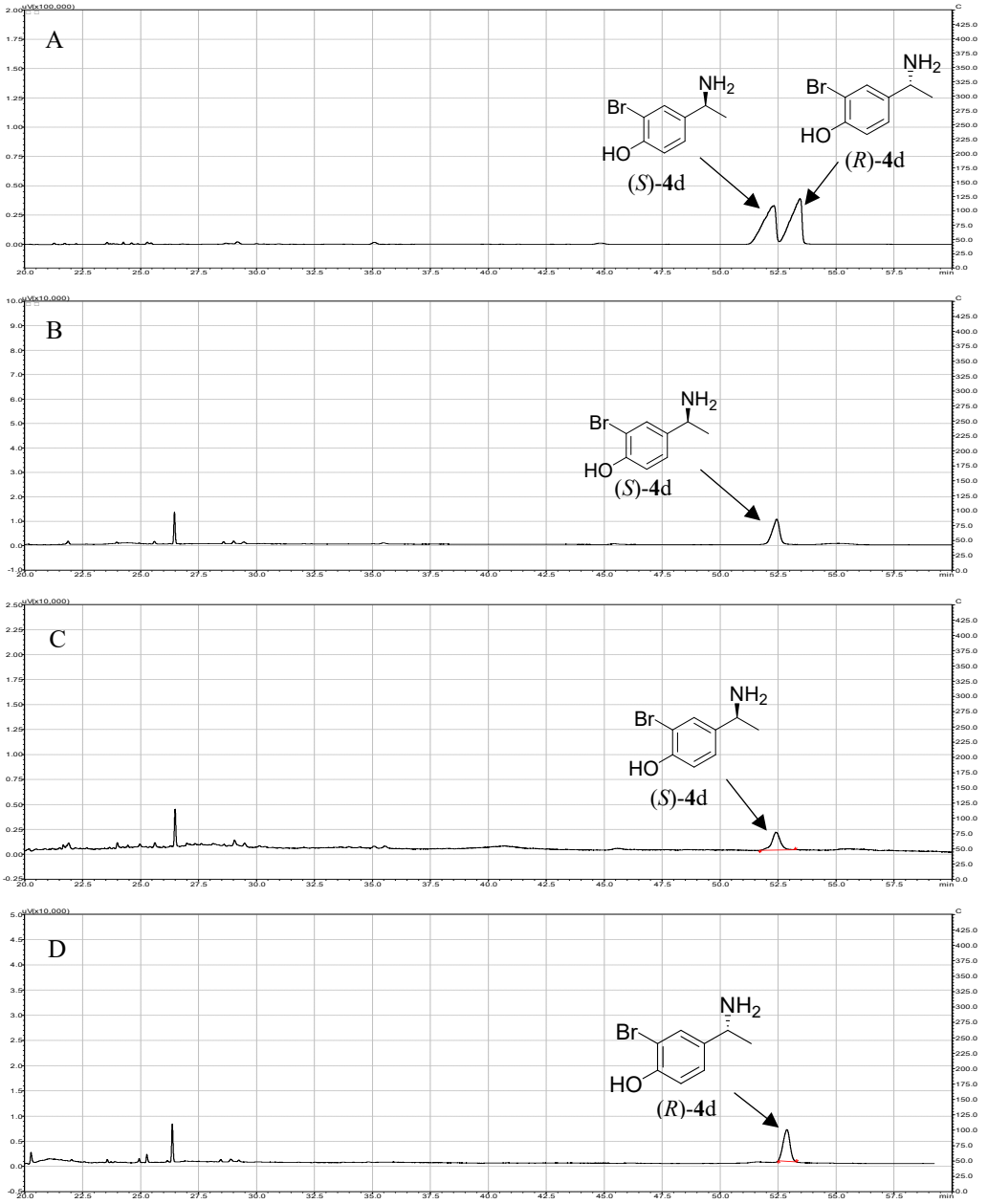


Figure S14. Chiral GC chromatograms of 4-(1-aminoethyl)-2-bromophenol **4**d. A: *rac*-4-(1-aminoethyl)-2-bromophenol **4**d standard. B: (*S*)-4-(1-aminoethyl)-2-bromophenol **4**d standard. C: (*S*)-4-(1-aminoethyl)-2-bromophenol **4**d produced by conversion of 2-bromo-4-ethenylphenol **1**d (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. D: (*R*)-4-(1-aminoethyl)-2-bromophenol **4**d produced by conversion of 2-bromo-4-ethenylphenol **1**d (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. (*S*)-4-(1-aminoethyl)-2-bromophenol **4**d : 52.324min. (*R*)-4-(1-aminoethyl)-2-bromophenol **4**d : 53.448min.

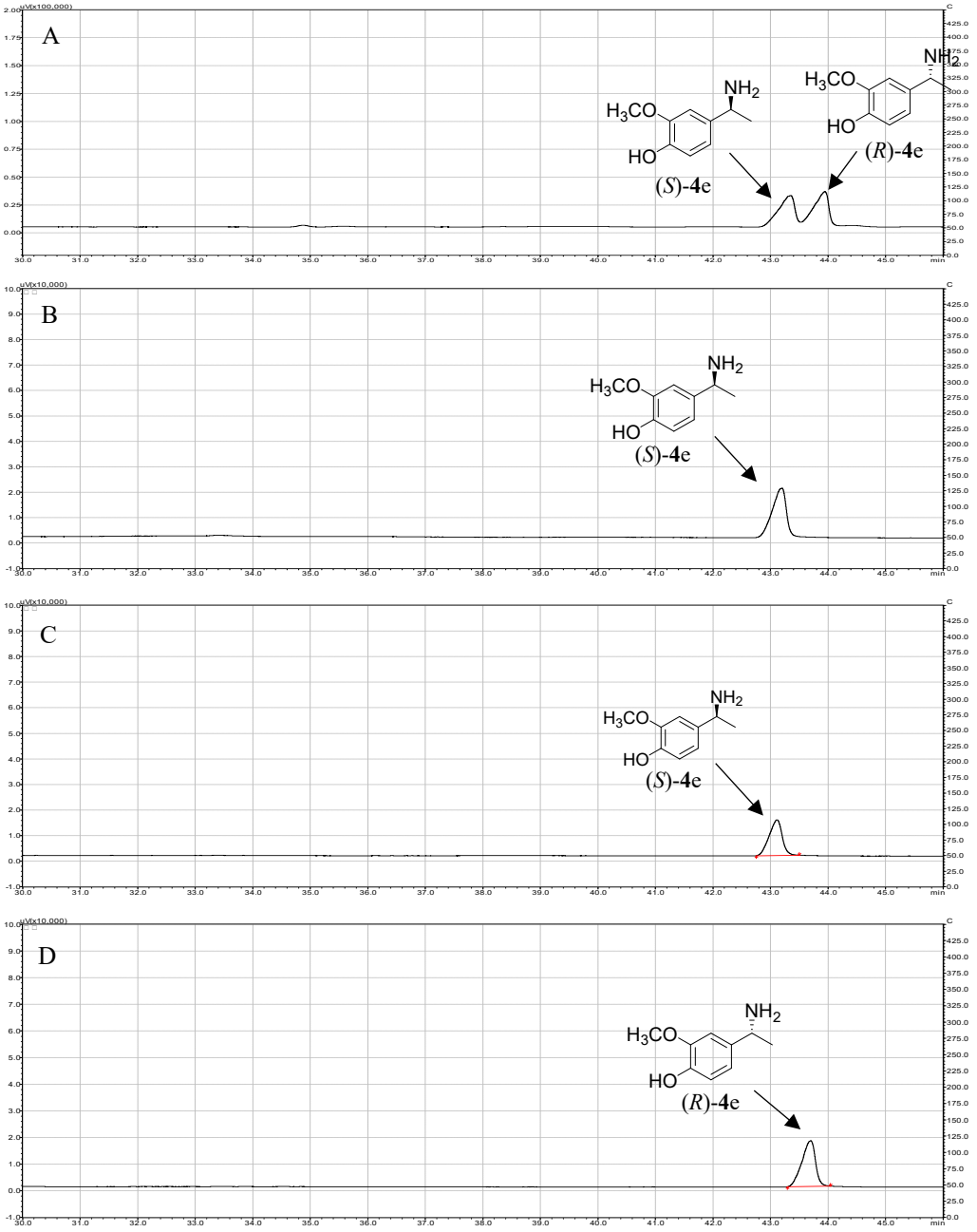


Figure S15. Chiral GC chromatograms of 4-(1-aminoethyl)-2-methoxyphenol **4**e. A: *rac*-4-(1-aminoethyl)-2-methoxyphenol **4**e standard. B: (*S*)-4-(1-aminoethyl)-2-methoxyphenol **4**e standard. C: (*S*)-4-(1-aminoethyl)-2-methoxyphenol **4**e produced by conversion of 2-methoxy-4-vinylphenol **1**e (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 24 h. D: (*R*)-4-(1-aminoethyl)-2-methoxyphenol **4**e produced by conversion of 2-methoxy-4-vinylphenol **1**e (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 24 h. (*S*)-4-(1-aminoethyl)-2-bromophenol **4**e : 43.357min. (*R*)-4-(1-aminoethyl)-2-bromophenol **4**e : 43.945 min.

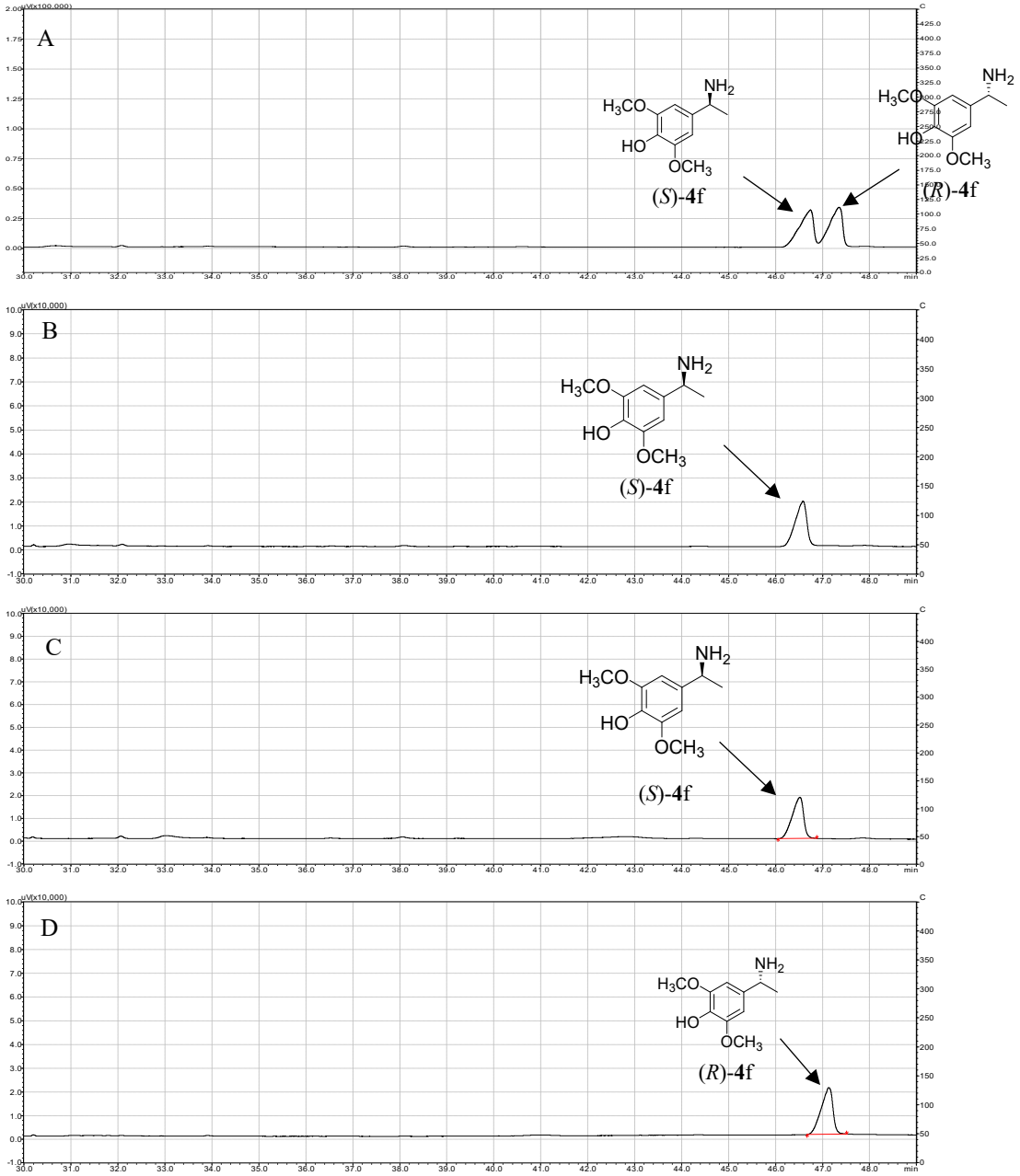


Figure S16. Chiral GC chromatograms of 4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f. A: *rac*-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f standard. B: (*S*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f standard. C: (*S*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f produced by conversion of 2,6-dimethoxy-4-vinylphenol **1**f (20 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 48 h. D: (*R*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f produced by conversion of 2,6-dimethoxy-4-vinylphenol **1**f (20 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 48 h. (*S*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f : 46.743min. (*R*)-4-(1-aminoethyl)-2,6-dimethoxyphenol **4**f : 47.350 min.

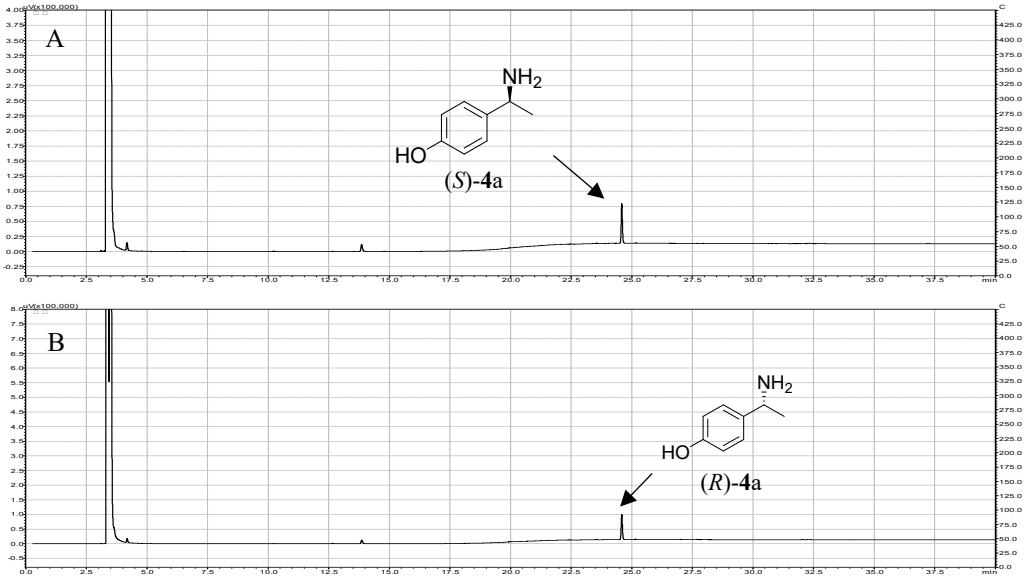


Figure S17. Achiral GC chromatograms of 4-(1-Aminoethyl)phenol **4**a prepared. A: (*S*)-4-(1-Aminoethyl)phenol **4**a prepared by conversion of 4-vinylphenol **1**a (10 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 30 h. B: (*R*)-4-(1-Aminoethyl)phenol **4**a prepared by conversion of 4-vinylphenol **1**a (10 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 30 h.

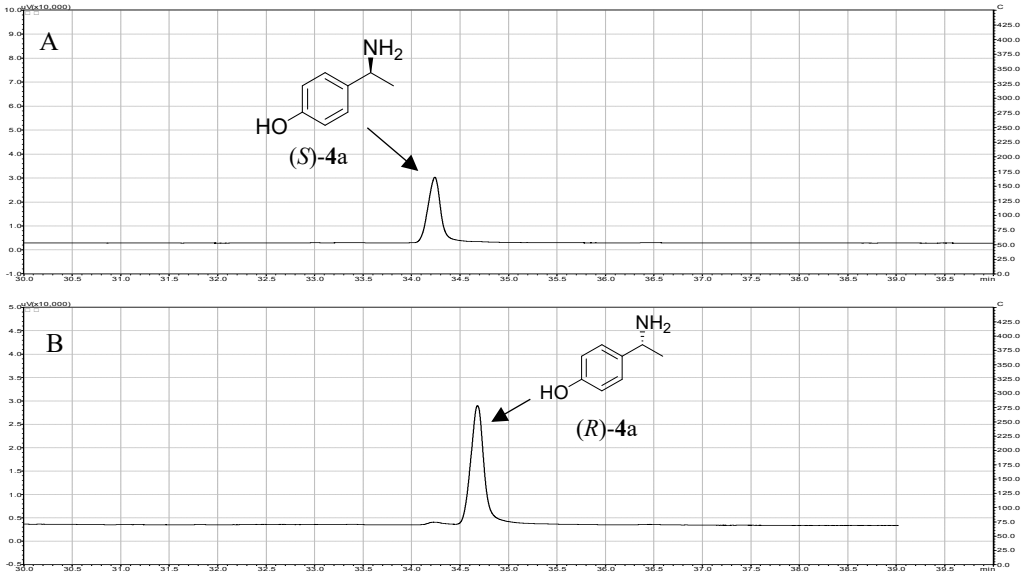
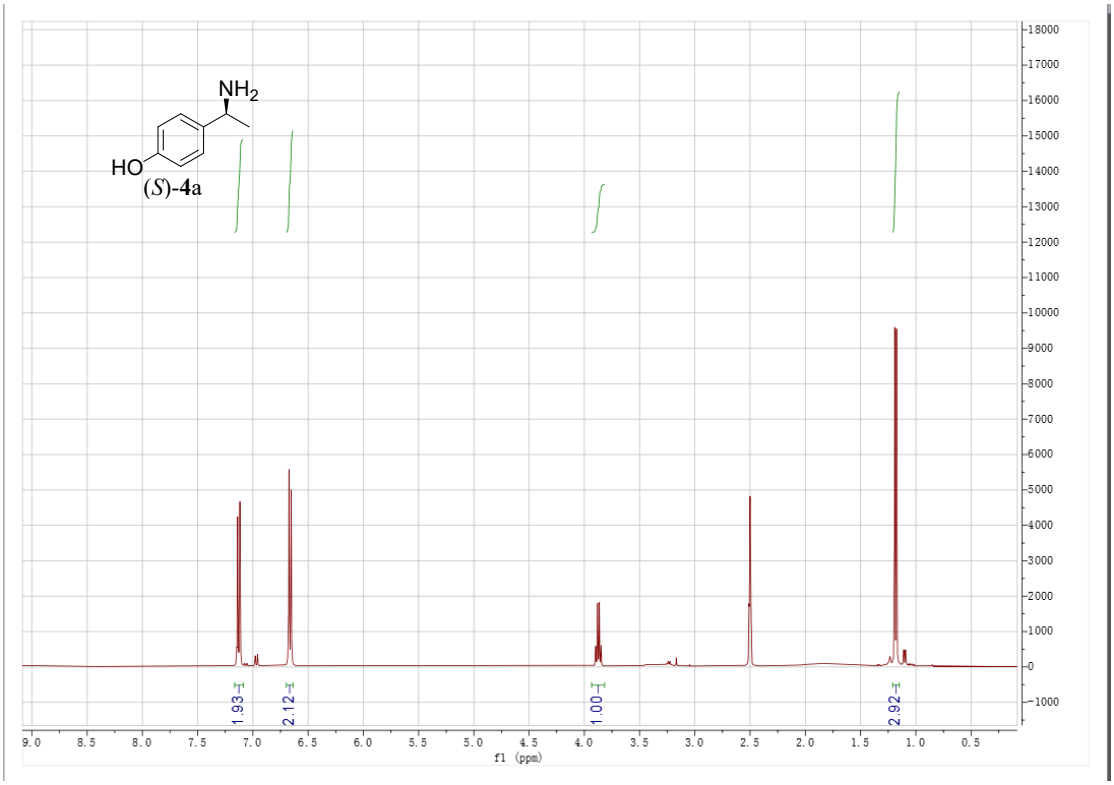


Figure S18. Chiral GC chromatograms of 4-(1-Aminoethyl)phenol **4**a prepared. A: (*S*)-4-(1-Aminoethyl)phenol **4**a prepared by conversion of 4-vinylphenol **1**a (10 mM) with resting cells of *E. coli* (RFC-ERR-CB) (40 g cdw/L) at 30 h. B: (*R*)-4-(1-Aminoethyl)phenol **4**a prepared by conversion of 4-vinylphenol **1**a (10 mM) with resting cells of *E. coli* (RFM-ERR-CB) (40 g cdw/L) at 30 h.


NH2
HO
(S)-4a
1.93
2.12
1.00
2.92
f1 (ppm)

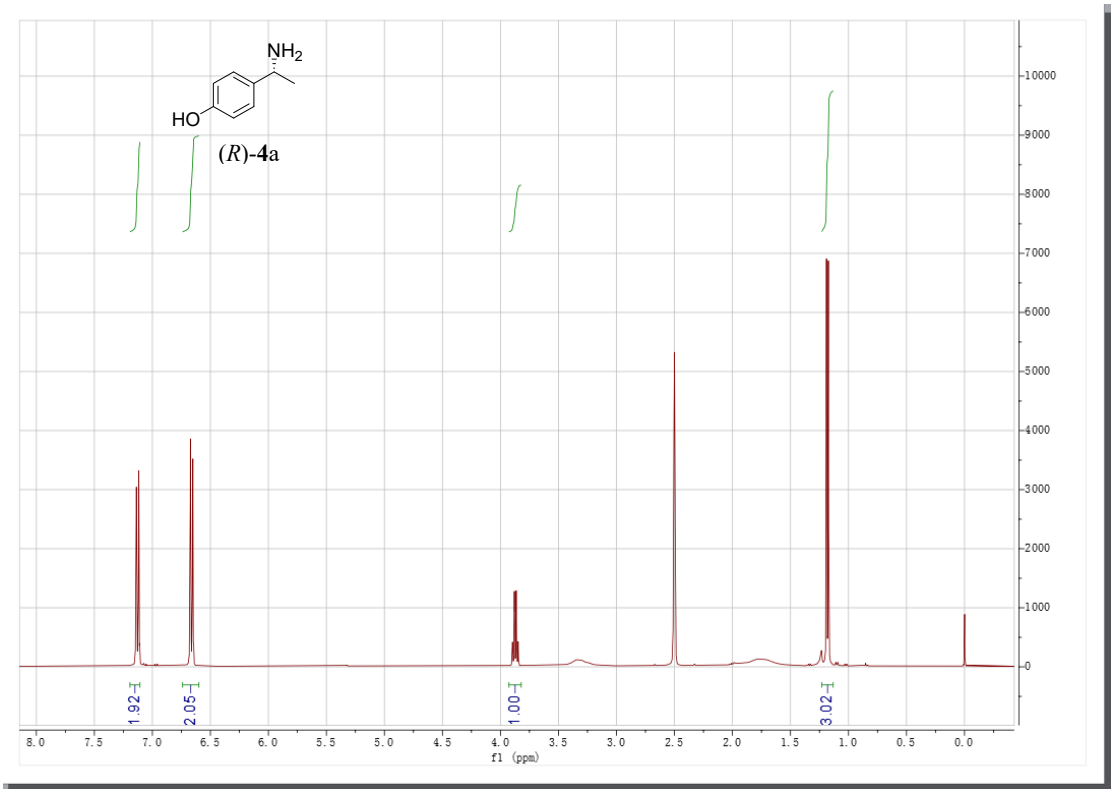

NH2
HO
(R)-4a
1.92
2.05
1.00
3.02
f1 (ppm)

Figure S19. ^{1}H NMR spectra analysis of (*S*)-4a and (*R*)-4a.

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