Supplementary Material

One-Pot Two-Stage Biocatalytic Upgrading of Biomass-Derived Aldehydes to Optically Active β-Amino Alcohols *via* Sequential Hydroxymethylation and Asymmetric Reduction Amination

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1. Molecular biology reagents and chemicals

The T4 DNA ligase, pfu DNA polymerase, restriction enzymes and isopropyl β-D-1-thiogalactopyranoside (IPTG) were acquired from TaKaRa (Dalian, China). Pyridoxal-5'-phosphate (PLP), kanamycin, furfural **1**a, benzaldehyde **1**b, 4-methoxybenzaldehyde **1**c, 3-bromobenzaldehyde **1**d, 4-chlorobenzaldehyde **1**e and 2-furanmethanamine **4**a were from Titan (Shanghai, China). 1-(2-Furyl)-2-hydroxyethan-1-one **2**a, 2-hydroxy-1-phenylethanone **2**b, 1-(4-methoxyphenyl)-2-hydroxyethanone **2**c 1-(3-bromophenyl)-2-hydroxyethanone **2**d and 1-(4-chlorophenyl)-2-hydroxyethanone **2**e were from Adamas-Beta (Shanghai, China). (*R*)-2-Amino-2-(furan-2-yl)ethanol **3**a, (*S*)-2-amino-2-(furan-2-yl)ethanol **3**a, (*R*)-2-amino-2-phenylethanol **3**b, (*S*)-2-amino-2-phenylethanol **3**b, (*R*)-2-amino-2-(4-methoxyphenyl)ethanol **3**c, (*S*)-2-amino-2-(4-methoxyphenyl)ethanol **3**c, (*R*)-2-amino-2-(3-bromophenyl)ethanol **3**d, (*S*)-2-amino-2-(3-bromophenyl)ethanol **3**d, (*R*)-2-amino-2-(4-chlorophenyl)ethanol **3**e and (*S*)-2-amino-2-(4-chlorophenyl)ethanol **3**e were acquired from Amatek Scientific (Suzhou, China). All the remaining reagents used were commercially available and of analytical grade.

2. Bacterial strains, plasmids and media

The *Escherichia coli* T7 super-competent cells were acquired from New England Biolabs (NEB) (Shanghai, China) and grown regularly in Luria-Bertani medium (LB) (Tryptone 10 g/L, Yeast extract 5 g/L, NaCl 10 g/L) at the temperature of 37°C and 200 rpm, with kanamycin (50 μg/mL) for plasmid selection. The plasmids pRSFduet-1 and pET28a (+) were obtained from Novagen (Shanghai, China) for heterogeneous expression studies. The previously described recombinant plasmids, pET28a-BMTA[1] and pET28a-MVTA,[2] are currently preserved in our lab.

3. Construction of recombinant *E. coli* cells

The benzaldehyde lyase (BAL, EC4.1.2.38) gene derived from *Pseudomonas fluorescens* Biovar I [3] was synthesized by Tsingke Biotechnology (Beijing, China). To amplify the BAL gene, the following primers were used: BAL-F forward primer (GGAATTCCATATGGCGATGATTACAGGCGGCGAACTGGTTG) and BAL-R reverse primer (CCGCTCGAGTCATGCGAAGGGGTCCATGCCGATC). The PCR amplification was performed with pfu 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 55 s, annealing at 67°C for 45 s, extension at 72°C for 1.0 min and followed by a final extension at 72°C for 10 min. The amplified DNA fragments were isolated and digested using *Nde*I and *Xho*I enzymes. Subsequently, they were ligated into the pRSFduet-1 vector, resulting in the creation of pRSFduet-BAL. The DNA sequencing confirmed the accuracy of the clone. The super-competent *E. coli* T7 cells were subsequently transformed with the pRSFduet-BAL *via* chemical transformation. The transformed strains were named *E. coli* (BAL).

The amine transaminase ArTA (accession number: 3WWH_A) gene derived from *Arthobacter,* the amine transaminase RbTA (accession number: 7DBE_A) gene derived from *Rhodobacter* sp.140A and the amine transaminase CepTA (accession number: AXP_007730450.1) gene derived from *Capronia epimyces* were synthesized by Tsingke Biotechnology (Beijing, China). They were ligated into the pET28a (+) vector, resulting in the creation of pET28a-ArTA, pET28a-RbTA and pET28a-CepTA. The super-competent *E. coli* T7 cells were subsequently transformed with the three recombinant vectors *via* chemical transformation. The transformed strains were named *E. coli* (ArTA), *E. coli* (RbTA) and *E. coli* (CepTA), respectively. All other recombinant *E. coli* cells expressing amine transaminases (MVTA, CV2025 and BMTA) were constructed previously and preserved in our lab.[1-2]

All the enzymes used in this study were listed in Table S1.

4. Cultivation of recombinant *E. coli* cells and enzyme activity analysis

Recombinant *E. coli* strains were cultured in 5 mL LB medium supplemented with 50 μg/mL kanamycin at 37°C for a duration of 12 hours. One milliliter of the seed culture was then transferred to a 250 mL flask containing 50 mL of Terrific Broth (TB) medium (Tryptone 12 g/L, Yeast extract 24 g/L, Glycerol 0.4%, K_2HPO_4 12.54 g/L, KH_2PO_4 2.32 g/L), along with the appropriate antibiotics. The cells were cultured at a temperature of 37°C for a period of 2 to 3 hours until they reached an OD600 of 0.6-0.8. Following this, they were induced with 0.8 mM IPTG. The cell growth was then continued at a temperature of 25°C (except for *E. coli* (BAL), which was kept at 37°C) and a stirring speed of 200 rpm for a duration of 12-16 hours. Afterwards, the recombinant *E. coli* cells were collected by centrifugation at 4,500×g for 10 minutes. They were washed with 0.9% saline water for two times and subsequently re-suspended in the KPi buffer (50 mM, pH 7.0). In order to obtain cell-free extracts, the cell suspension was chilled on ice and subjected to sonication for 120 cycles at 350 W for 6 s, with a 5 s interval. The supernatant of the cell lysate, obtained after centrifugation at 15,000×g and 4°C, was stored at -20°C for

future use. To analyze the protein samples, SDS-PAGE was performed using a 12% polyacrylamide gel, followed by staining with Coomassie Brilliant Blue R250.

The activity of BAL was detected as previously described by Demir et al.[3] The reaction mixture comprised 0.5 mL of 50 mM KPi buffer at pH 7.0. Additionally, it contained 5 mM 2-hydroxy-1-phenylethanone, 2.5 mM $MgSO_4$, 0.15 mM ThDP (thiamine diphosphate), and the necessary amount of enzyme. The reaction mixture was kept at a temperature of 30°C for a period of 10 minutes, 300 µL reaction mixture was saturated with NaCl, extracted with ethyl acetate (300 µL) containing 2 mM phenylacetone (internal standard), and dried over Na_2SO_4. The GC analysis method was used to determine the concentration of benzaldehyde. One unit (U) of activity is defined as the amount of enzyme that produces 1 µmol of benzaldehyde in 1 minute at a temperature of 30°C.

The transaminase activity was measured as previously described.[4]

5. Hydroxymethylation of furfural 1a into 1-(2-furyl)-2-hydroxyethan-1-one 2a with the *E. coli* (BAL) resting cells

The experiment was conducted in a 50-mL conical flask. A mixture of 3 mL KPi buffer (50 mM, pH 6.0-8.0), containing furfural **1**a (5-20 mM), formaldehyde (15-60 mM), $MgSO_4$ (2.5 mM), ThDP (thiamine diphosphate) (0.15 mM), and *E. coli* (BAL) resting cells with a cell dry weight of 10-30 g/L, was prepared. Additionally, 5-40% DMSO (v/v) was added as a cosolvent. The reactions were carried out at a temperature of 30°C and a stirring speed of 200 rpm for a duration of 4-8 hours. To determine the conversion of 1-(2-furyl)-2-hydroxyethan-1-one **2**a, 100 µL samples were withdrawn at various time intervals. These samples were quenched *via* addition of 900 µL acetonitrile containing 2 mM benzaldehyde, which served as the internal standard. After shaking and centrifuging at a speed of 16,000×g for a duration of 10 minutes, the supernatant was filtered through a 0.2 µm membrane filter. The filtered solution was then subjected to HPLC analysis to quantify the formation of **2**a. All experiments were conducted in duplicate.

6. Asymmetric reduction amination of 1-(2-furyl)-2-hydroxyethan-1-one 2a to enantiopure 2-amino-2-(furan-2-yl)ethanol 3a with *E. coli* (MVTA) or *E. coli* (BMTA) resting cells

The experiments were carried out using 1 mL KPi buffer (50 mM, pH 6.0-10.0). The buffer contained 5-20 mM of 1-(2-furyl)-2-hydroxyethan-1-one (**2**a), 0-30% DMSO (v/v), 0.1 mM PLP, and 100-400 mM

D- or L-alanine as the amino donor. Additionally, 20 g cdw/L *E. coli* (TAs) resting cells were included in the reaction mixture. The reactions were conducted for a duration of 9 hours at a temperature of 30°C and a speed of 200 rpm. To quantify the formation of 2-amino-2-(furan-2-yl)ethanol (**3**a), samples of 100 µL were collected at different time intervals. These samples were mixed with 900 µL ddH_2O (perchloric acid, pH 1.5) containing 2 mM 2-hydroxy-1-phenylethanone as an internal standard. The resulting suspension centrifuged (16000×g, 10 minutes) to precipitate cellular materials, the supernatant was filtered through a 0.2 µm membrane filter and analyzed by HPLC. Each experiment was performed twice.

7. One-pot concurrent hydroxymethylation and asymmetric reduction amination of furfural 1a to enantiopure 2-amino-2-(furan-2-yl)ethanol 3a

In a reaction vessel with a volume of 50 mL, a reaction mixture of 5 mL was prepared. The mixture contained the following components: 50 mM KPi buffer with a pH of 7.0, furfural **1**a at a concentration of 10-20 mM, formaldehyde at a concentration of 30-60 mM, $MgSO_4$ at a concentration of 2.5 mM, ThDP at a concentration of 0.15 mM, PLP at a concentration of 0.1 mM, D- or L-Ala as an amino donor at a concentration of 200-400 mM, DMSO (20% v/v), both the recombinant *E. coli* (BAL) and recombinant *E. coli* (MVTA) or *E. coli* (BMTA) at a cell dosage of 20 g cdw/L. The reactions were carried out at a temperature of 30°C and a speed of 200 rpm for a duration of 12-24 hours. At specific time intervals, 100 µL samples were taken and mixed with 900 µL ddH_2O (perchloric acid, pH 1.5) containing 2 mM 2-hydroxy-1-phenylethanone (internal standard). The resulting suspension centrifuged (16000×g, 10 minutes) to precipitate cellular materials, the supernatant was filtered through a 0.2 µm membrane filter and analyzed by HPLC. Each experiment was performed twice.

8. One-pot two-stage bioconversion of 2-furaldehyde 1a to enantiopure 2-amino-2-(furan-2-yl)ethanol 3a

In a reaction vessel with a volume of 50 mL, a reaction mixture of 5 mL was prepared. The mixture contained the following components: 50 mM KPi buffer (pH 7.0), 10-20 mM furfural **1**a, 20% DMSO, 30-60 mM formaldehyde, 0.15 mM ThDP and 2.5 mM $MgSO_4$. Additionally, 20 g cdw/L *E. coli* (BAL) cells was introduced. The reactions were conducted at 30°Cand 200 rpm. Once the conversion of **2**a reached 99%, 200-400 mM D- or L-Ala, 0.1 mM PLP, and 20 g cdw/L of either *E. coli* (BMTA) or *E. coli* (MVTA) were added to the reaction mixture. The reactions were allowed to continue for 16 hours at

the temperature of 30°C. At different time points, 100 µL samples were taken, mixed with 900 µL ddH_2O (perchloric acid, pH 1.5) containing 2 mM 2-hydroxy-1-phenylethanone (internal standard). The resulting suspension centrifuged (16000×g, 10 minutes) to precipitate cellular materials, the supernatant samples were filtered through a 0.2 µm membrane filter and analyzed by HPLC. All experiments were performed in duplicate.

9. One-pot two-stage bioconversion of benzaldehydes 1b-e into chiral β-amino alcohols 3b-e

In a reaction vessel with a volume of 50 mL, a mixture was prepared containing 5 mL of KPi buffer (50 mM, pH 7.0), 10 mM benzaldehydes **1**b-e, 20% DMSO, 30 mM formaldehyde, 2.5 mM $MgSO_4$, and 0.15 mM ThDP. Additionally, 20 g cdw/L *E. coli* (BAL) was added to the reaction mixture, which was then shaken at 200 rpm and maintained at 30ºC. Once the conversion of **2**b-e reached its maximum, 200 mM L-Ala or 15 mM (*R*)-MBA, 0.1 mM PLP, along with 20 g cdw/L *E. coli* (BMTA) or *E. coli* (MVTA) resting cells, were introduced into the reaction mixture. The reactions were continued for 3-12 hours at 30ºC and 200 rpm. During specific time intervals, 0.5 mL aliquots were collected, saturated with sodium chloride, basified by adding 50 µL NaOH (10 N), then, add 0.5 mL ethyl acetate (10 mM dodecane as an internal standard), after a strong shake with Vortex-5 (Kylin-Bell), the resulting suspension was centrifuged at 16000×g for 10 minutes, the ethyl acetate phase was then dried using anhydrous Na_2SO_4, the concentration and enantiomeric excess (*ee*) of β-amino alcohols **3**b-e were determined through GC analysis. All experiments were conducted in duplicate.

10. Preparation experiments

In a reaction vessel with a volume of 250 mL, a mixture containing 50 mL of KPi buffer (50 mM, pH 7.0), 20 mM (96.0 mg) of **1**a, 20% DMSO, 60 mM of formaldehyde, 2.5 mM of $MgSO_4$, and 0.15 mM of ThDP was prepared. Additionally, 20 g cdw/L *E. coli* (BAL) was added to the reaction mixture, which was then shaken at the temperature of 30°C and 200 rpm for 8 hours. Once the conversion of **2**a reached 99%, 400 mM L-Ala or D-Ala, 0.1 mM PLP and 20 g cdw/L *E. coli* (BMTA) or *E. coli* (MVTA) were introduced to the reaction mixture. The reactions were allowed to continue for 16 hours at 30°C and 200 rpm. The cells were removed by centrifuging (4500×g, 4ºC for 10 minutes), the supernatant was saturated with NaCl, extracted three times with ethyl acetate (50 mL) by centrifugation (16000×g, 4ºC for 10 minutes). The combined ethyl acetate phase was dried over anhydrous Na_2SO_4. The solvent was removed

by rotary evaporation under reduced pressure, the crude products were purified through flash chromatography using a column packed with silica gel. This process yielded (*R*)-**3**a and (*S*)-**3**a as yellow oils in 60.4% (58 mg) and 64.6% yields (62 mg), respectively. ^{1}H NMR (600 MHz, D_2O) δ 7.58 (d, *J* = 1.2 Hz, 1H), 6.57 (d, *J* = 3.4 Hz, 1H), 6.50 (dd, *J* = 3.3, 1.9 Hz , 1H), 4.60 (dd, *J* = 7.3, 5.2 Hz, 1H), 4.02 (m, 2H).

11. Assay method

The concentration of 1-(2-furyl)-2-hydroxyethan-1-one **2**a was measured using a Shimadzu HPLC system equipped with a C18 column (4.6*250 mm, 5 μm) from Phenomenex, Shanghai. The detection was performed at 284 nm, and the eluent consisted of a mixture of acetonitrile and water in a 50:50 ratio. The flow rate was set at 1 mL/min.

The concentration and enantiomeric excess of 2-amino-2-(furan-2-yl)ethanol **3**a were analyzed using a Shimadzu HPLC system with a CROWNPAK CR (+) column (4*150 mm, 5 μm). The detection was carried out at 210 nm, and the eluent used was H_2O with perchloric acid at pH 1.5. The flow rate was set at 0.8 mL/min.

The concentrations of α-hydroxy ketones **2**b-e and β-amino alcohol **3**b-e were determined by using gas chromatography. The analysis was performed on a GC (GC-14C, Shimadzu, Japan) with a column (HP-5, 30 m × 0.320 mm × 0.25 mm, Agilent Technologies, Inc.) and an FID detector. The following GC analysis conditions were used: For **2**b and **3**b, the column temperature was set to 120°C for 10 minutes. For **2**c and **3**c, the column temperature was set to 150°C for 10 minutes. For **2**d and **3**d, the column temperature was set to 140°C for 10 minutes. For **2**e and **3**e, the column temperature was initially set to 120°C, then increased at a rate of 2°C per minute until reaching 140°C, and held for 5 minutes. The injection temperature was set at 250°C, and the detector temperature was set at 275°C.

The enantiomeric excesses of β-amino alcohols **3**b-e were analyzed by using gas chromatography (GC) based on a method outlined by Zhang et al. [1]

12. Amino acid sequences of enzymes used in this study

Amino acid sequence of BAL

MAMITGGELVVRTLIKAGVEHLFGLHGAHIDTIFQACLDHDVPIIDTRHEAAAGHAAEGYARA
GAKLGVALVTAGGGFTNAVTPIANAWLDRTPVLFLTGSGALRDDETNTLQAGIDQVAMAAPIT
KWAHRVMATEHIPRLVMQAIRAALSAPRGPVLLDLPWDILMNQIDEDSVIIPDLVLSAHGARPD
PADLDQALALLRKAERPVIVLGSEASRTARKTALSAFVAATGVPVFADYEGLSMLSGLPDAMR

GGLVQNLYSFAKADAAPDLVLMLGARFGLNTGHGSGQLIPHSAQVIQVDPDACELGRLQGIAL
GIVADVGGTIEALAQATAQDAAWPDRGDWCAKVTDLAQERYASIAAKSSSEHALHPFHASQVI
AKHVDAGVTVVADGALTYLWLSEVMSRVKPGGFLCHGYLGSMGVGFGTALGAQVADLEAGR
RTILVTGDGSVGYSIGEFDTLVRKQLPLIVIIMNNQSWGATLHFQQLAVGPNRVTGTRLENGSY
HGVAAAFGADGYHVDSVESFSAALAQALAHNRPACINVAVALDPIPPEELILIGMDPFA

Amino acid sequence of MVTA

MGIDTGTSKVALVEPGAIREDTPAGSVIQYSDYEIDYSSPFAGGVAWIEGEYLPAEDAKISIFDTG
FGHSDLTYTVAHVWHGNIFRLGDHLDRLLDGARKLRLDSGYTKDELADITKKCVSLSQLRESF
VNLTITRGYGKRKGEKDLSKLTHQVYIYAIPYLWAFPPAEQIFGTTAVVPRHVRRAGRNTVDPTI
KNYQWGDLTAASFEAKDRGARTAILMDADNCVAEGPGFNVCIVKDGKLASPSRNALPGITRKT
VFEIAGAMGIEAALRDVTSHELYDADEIMAVTTAGGVTPINTLDGVPIGDGEPGPVTVAIRDRF
WALMDEPGPLIEAIQY

Amino acid sequence of ArTA

MTSEIVYTHDTGLDYITYSDYELDPANPLAGGAAWIEGAFVPPSEARISIFDQGYLHSDVTYTV
FHVWNGNAFRLDDHIERLFSNAESMRIIPPLTQDEVKEIALELVAKTELREAFVSVSITRGYSSTP
GERDITKHRPQVYMYAVPYQWIVPFDRIRDGVHAMVAQSVRRTPRSSIDPQVKNFQWGDLIRA
VQETHDRGFEAPLLLDGDGLLAEGSGFNVVVIKDGVVRSPGRAALPGITRKTVLEIAESLGHE
AILADITLAELLDADEVLGCTTAGGVWPFVSVDGNPISDGVPGPITQSIIRRYWELNVESSSLLTP
VQY

Amino acid sequence of RbTA

MNQLTILEAGLDEIICETVPGEAIQYSRYSLDRTSPLAGGCAWIEGAFVPAAAARISIFDAGFGHS
DVTYTVAHVWHGNFFRLEDHVERFLAGAEKMRIPMPATKAEIMDLMRGCVSKSGLREAYVN
VCVTRGYGRKPGEKTLEALESQLYVYAIPYLWVFSPIRQIEGIDAVIAQSVRRSPANVMDPWIK
NYQWGDLVRATFEAQERGARTAFLLDSDGFVTEGPGFNVLMVKDGTVFTAARNVLPGITRRT
ALEIARDFGLQTVIGDVTPEMLRGADEIFAATTAGGVTPVVALDGAPVGAGVPGDWTRKIRTR
YWQMMDEPSDLIEPVSYI

Amino acid sequence of CepTA

MASMDKVFAGYQSRLRVLEASTNPLAQGVAWIEGELVPLSQARIPLMDQGFLHSDLTYDVPAV
WDGRFFRLDDHISRLEKSCSKLRLKLPLPRDEVKRVLVDMVARSGIRDAFVELIVTRGLTGVRG
AGRPEDLVNNLYMFLQPYLWVMPPETQLVGGSAVITRTVRRTPPGSMDPTVKNLQWGDLTRA
LLEASDRGASYPFLTDGDANITEGSGYNIVLIKDGAIHTPDRGVLEGVTRKTVFDIAKANGFEV
RLEVVPVELAYRADEIFMCTTAGGIMPITSLDGQPVNGGQIGPITKKIWDDYWALHYDPAFSFEI
KYDEAGASTNGVNGVHK

Amino acid sequence of BMTA

MSLTVQKINWEQVKEWDRKYLMRTFSTQNEYQPVPIESTEGDYLIMPDGTRLLDFFNQLYCVN
LGQKNQKVNAAIKEALDRYGFVWDTYATDYKAKAAKIIIEDILGDEDWPGKVRFVSTGSEAVE
TALNIARLYTNRPLVVTREHDYHGWTGGAATVTRLRSYRSGLVGENSESFSAQIPGSSYNSAVL
MAPSPNMFQDSDGNLLKDENGELLSVKYTRRMIENYGPEQVAAVITEVSQGAGSAMPPYEYIP
QIRKMTKELGVLWINDEVLTGFGRTGKWFGYQHYGVQPDIITMGKGLSSSSLPAGAVLVSKEIA

AFMDKHRWESVSTYAGHPVAMAAVCANLEVMMEENFVEQAKDSGEYIRSKLELLQEKHKSIG
NFDGYGLLWIVDIVNAKTKTPYVKLDRNFTHGMNPNQIPTQIIMKKALEKGVLIGGVMPNTM
RIGASLNVSRGDIDKAMDALDYALDYLESGEWQ

Amino acid sequence of CV2025

MQKQRTTSQWRELDAAHHLHPFTDTASLNQAGARVMTRGEGVYLWDSEGNKIIDGMAGLWC
VNVGYGRKDFAEAARRQMEELPFYNTFFKTTHPAVVELSSLLAEVTPAGFDRVFYTNSGSESV
DTMIRMVRRYWDVQGKPEKKTLIGRWNGYHGSTIGGASLGGMKYMHEQGDLPIPGMAHIEQ
PWWYKHGKDMTPDEFGVVAARWLEEKILEIGADKVAAFVGEPIQGAGGVIVPPATYWPEIERI
CRKYDVLLVADEVICGFGRTGEWFGHQHFGFQPDLFTAAKGLSSGYLPIGAVFVGKRVAEGLIA
GGDFNHGFTYSGHPVCAAVAHANVAALRDEGIVQRVKDDIGPYMQKRWRETFSRFEHVDDVR
GVGMVQAFTLVKNKAKRELFPDFGEIGTLCRDIFFRNNLIMRACGDHIVSAPPLVMTRAEVDE
MLAVAERCLEEFEQTLKARGLA

Table S1. Enzymes used in this study.

Entry	Enzyme	Accession number	Original strain	Ref.
1	MVTA	WP_011781668.1	*Mycobacterium vanbaalenii*	[2]
2	ArTA	3WWH_A	*Arthobacter*	[5]
3	RbTA	7DBE_A	*Rhodobacter sp.*140A	[6]
4	CepTA	XP_007730450.1	*Capronia epimyces*	This study
5	BMTA	-	*Bacillus megaterium* SC6394	[7]
6	CV2025	WP_011135573.1	*Chromobacterium violaceum*	[8]
7	BAL	2AG0_A	*Pseudomonas fluorescens* Biovar I	[3]

Table S2 Amine donors of transaminases for conversion of **2**a to **3**a.

Entry	Enzymes	Amine donor	Amine donor Conc. (mM)	Conv. (%)[a]	Product *ee* (%)[a]
1	MVTA	IPA	100	35.9	>99 (*R*)
2	MVTA	IPA	200	42.1	>99 (*R*)
3	MVTA	IPA	300	41.5	>99 (*R*)
4	MVTA	(*R*)-PEA	10	90.4	>99 (*R*)
5	MVTA	(*R*)-PEA	20	94.8	>99 (*R*)
6	MVTA	(*R*)-PEA	30	94.2	>99 (*R*)
7	MVTA	D-Ala	50	87.4	>99 (*R*)
8	MVTA	D-Ala	100	95.2	>99 (*R*)
9	MVTA	D-Ala	200	95.0	>99 (*R*)
10	BMTA	IPA	25	41.6	>99 (*S*)
11	BMTA	IPA	100	26.6	>99 (*S*)
12	BMTA	IPA	200	12.4	>99 (*S*)
13	BMTA	(*S*)-PEA	10	63.7	>99 (*S*)
14	BMTA	(*S*)-PEA	20	65.7	>99 (*S*)
15	BMTA	(*S*)-PEA	30	65.5	>99 (*S*)
16	BMTA	L-Ala	50	91.6	>99 (*S*)
17	BMTA	L-Ala	100	94.2	>99 (*S*)
18	BMTA	L-Ala	200	93.6	>99 (*S*)

[a]Reaction condition: 1 mL KPi buffer (50 mM, pH 8.0), including 5 mM substrate **2**a, 0.1 mM PLP, 10-300 mM amine donor, 20 g cdw/L *E. coli* (BMTA) or *E. coli* (MVTA), 30°C, 200 rpm for 9 h.

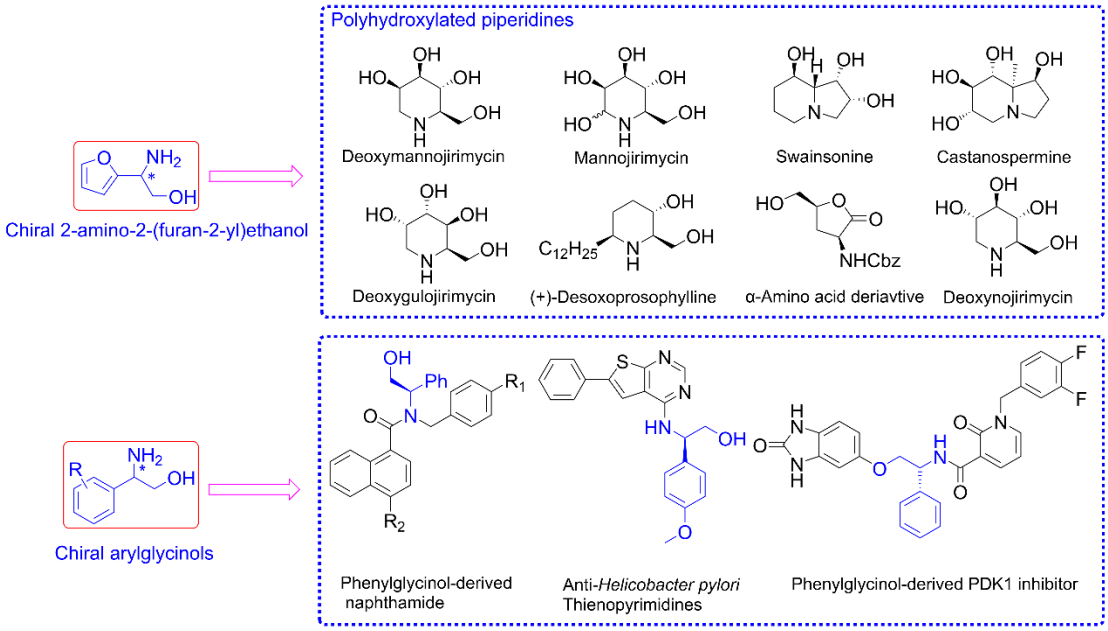
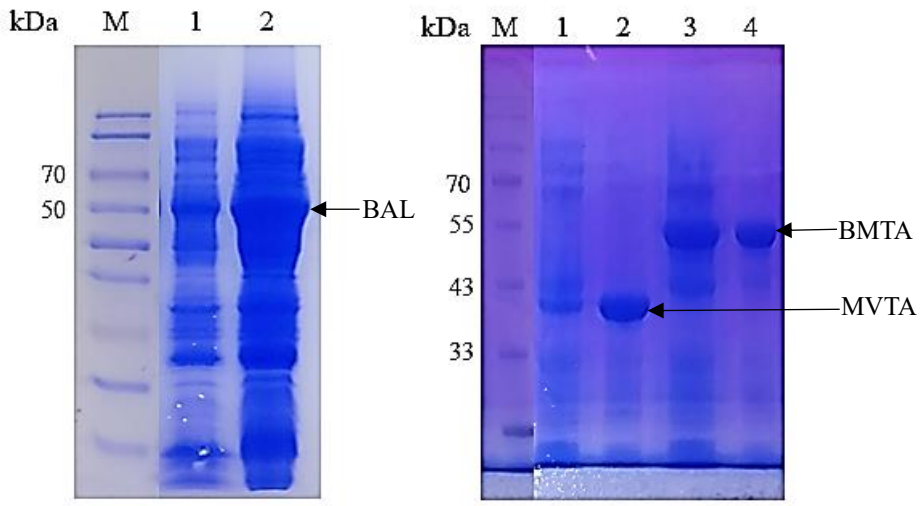


Figure S1. Examples of bioactive molecules derived from chiral β-amino alcohols.



(a) (b)

Figure S2. SDS-PAGE of *E. coli* (BAL), *E. coli* (BMTA) and *E. coli* (MVTA). (a): Lane M: protein marker, lane 1: cell of *E. coli* (BAL) (58.9 kDa), lane 2: cell-free extract of *E. coli* (BAL); (b): Lane M: protein marker, lane 1: cell of *E. coli* (MVTA), lane 2: cell-free extract of *E. coli* (MVTA) (36.7 kDa), lane 3: cell of *E. coli* (BMTA), lane 4: cell-free extract of *E. coli* (BMTA) (53.1 kDa).

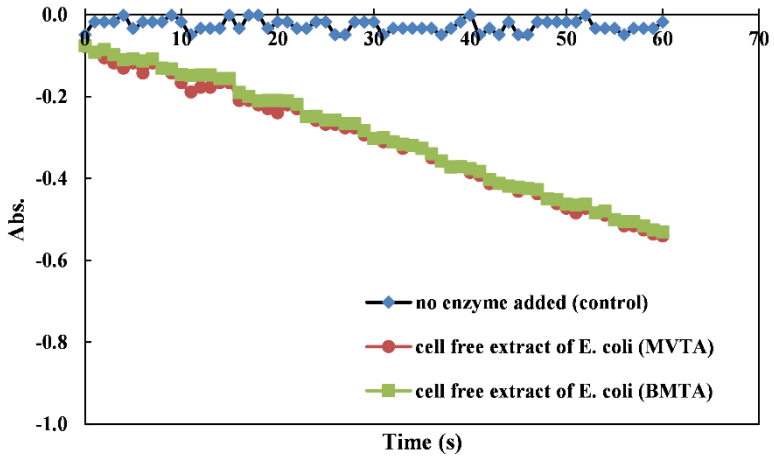


Figure S3. Determination of specific activity of lactate dehydrogenase (LDH) in cell free extracts of *E. coli* (MVTA) and *E. coli* (BMTA). The activity of LDH was measured by monitoring the NADH concentration reduction using UV absorbance at 340 nm. Reaction condition: 1 mL KPi buffer (100 mM, pH 8.0), including 5.0 mM pyruvate, 0.2 mM NADH, 10 µl cell free extracts of *E. coli* (BMTA) (10 g cdw/L) and *E. coli* (MVTA) (10 g cdw/L). Reactions were started by addition of the enzyme solution and measured over a period of 1 min. The reaction mixture without 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 8.0). Molar extinction coefficient of NADH (6.2 mM^{-1} cm^{-1}) was used to calculate enzyme activities. The specific activity of LDH in cell-free extracts of *E. coli* (MVTA) and *E. coli* (BMTA) reached 9.0 U/mg protein.

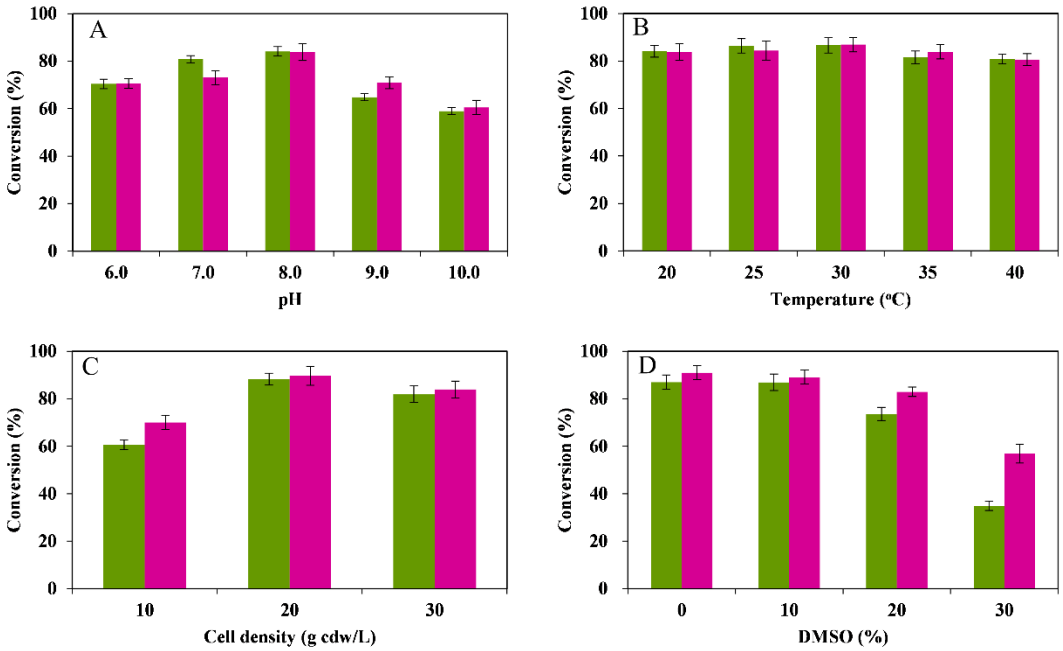


Figure S4. The optimized reaction conditions for *E. coli* (BMTA) (green bar) and *E. coli* (MVTA) (red purple bar). (A) pH; (B) temperature; (C) cell density; (D) DMSO concentration. Reaction condition: 1 mL KPi buffer (50 mM, pH 6.0-8.0) and glycine-NaOH buffer (100 mM, pH 9.0-10.0), including 10 mM substrate **2**a, 0.1 mM PLP, 200 mM D/L-Ala as amine donor, 10-30 g cdw/L *E. coli* (BMTA) or *E. coli* (MVTA), 0-30%(v/v) DMSO, 20-40°C, 200 rpm for 9 h.

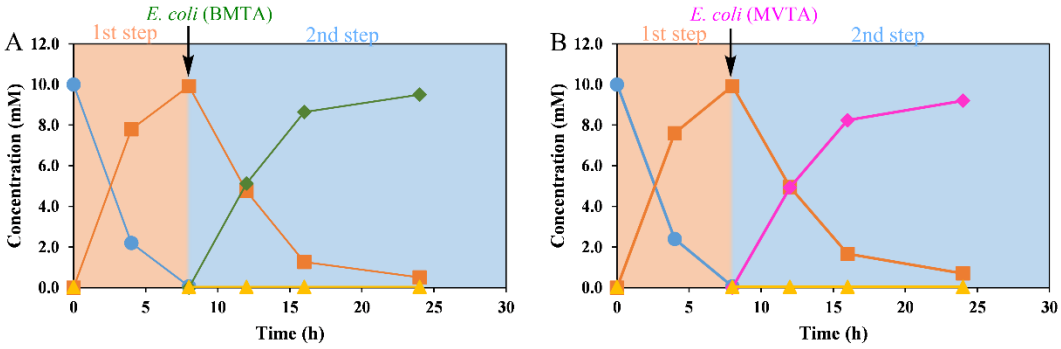


Figure S5. Time courses for conversion of **1**a to **3**a with the resting cells of *E. coli* (BAL) and *E. coli* (BMTA) or *E. coli* (MVTA) *via* one-pot two-stage cascade biocatalysis. A: *E. coli* (BAL) and *E. coli* (BMTA); B: *E. coli* (BAL) and *E. coli* (MVTA). ●: 1a; ■: 2a; ▲: 4a; ♦: (*S*)-3a; ♦: (*R*)-3a.

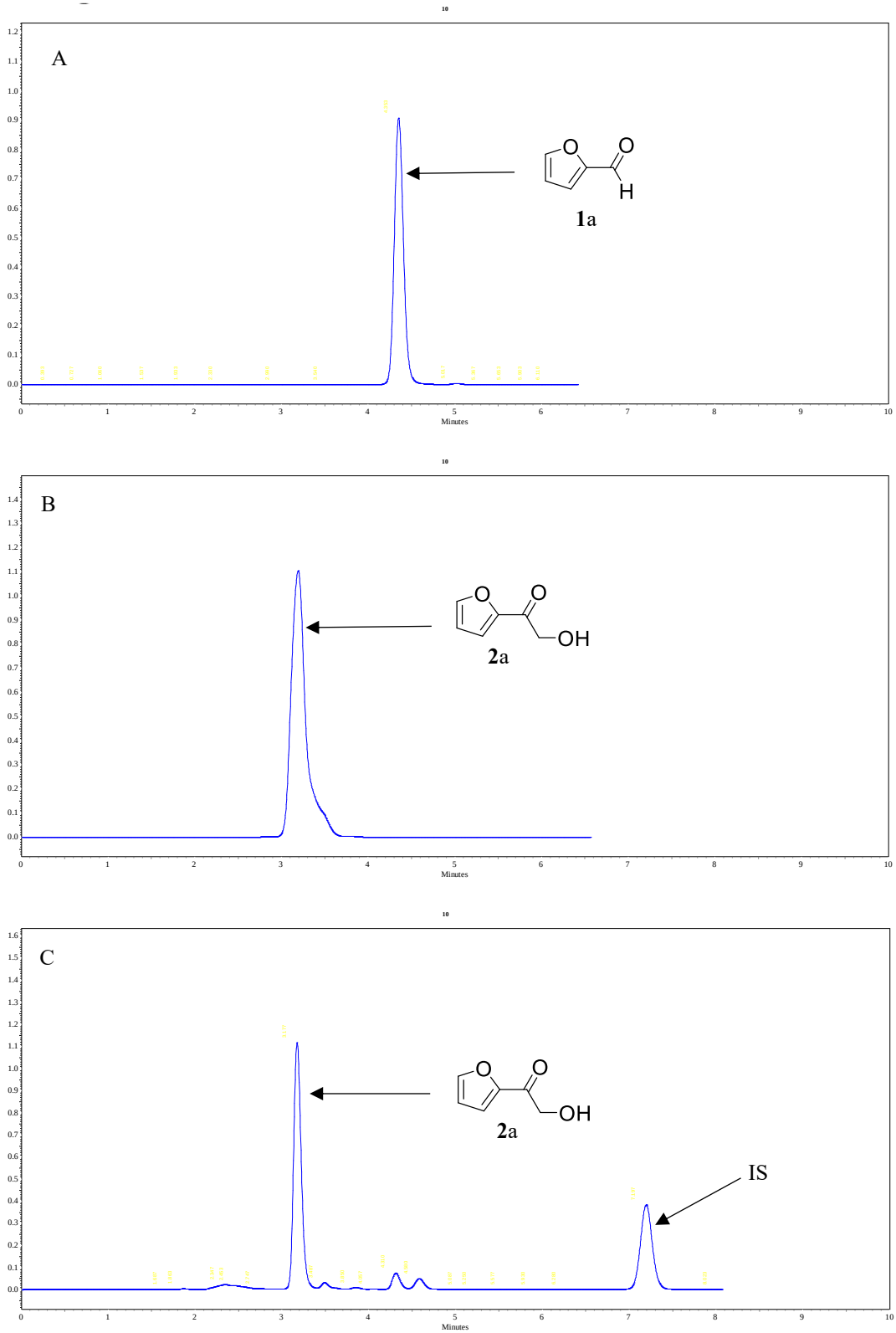


Figure S6. HPLC analysis of 1-(2-furyl)-2-hydroxyethan-1-one **2**a. A: Furfural **1**a standard; B:1-(2-furyl)-2-hydroxyethan-1-one **2**a standard; C: 1-(2-furyl)-2-hydroxyethan-1-one **2**a produced from furfural **1**a (10 mM) with 20 g CDW/L *E. coli* (BAL) at 4 h. IS: internal standard (benzaldehyde).

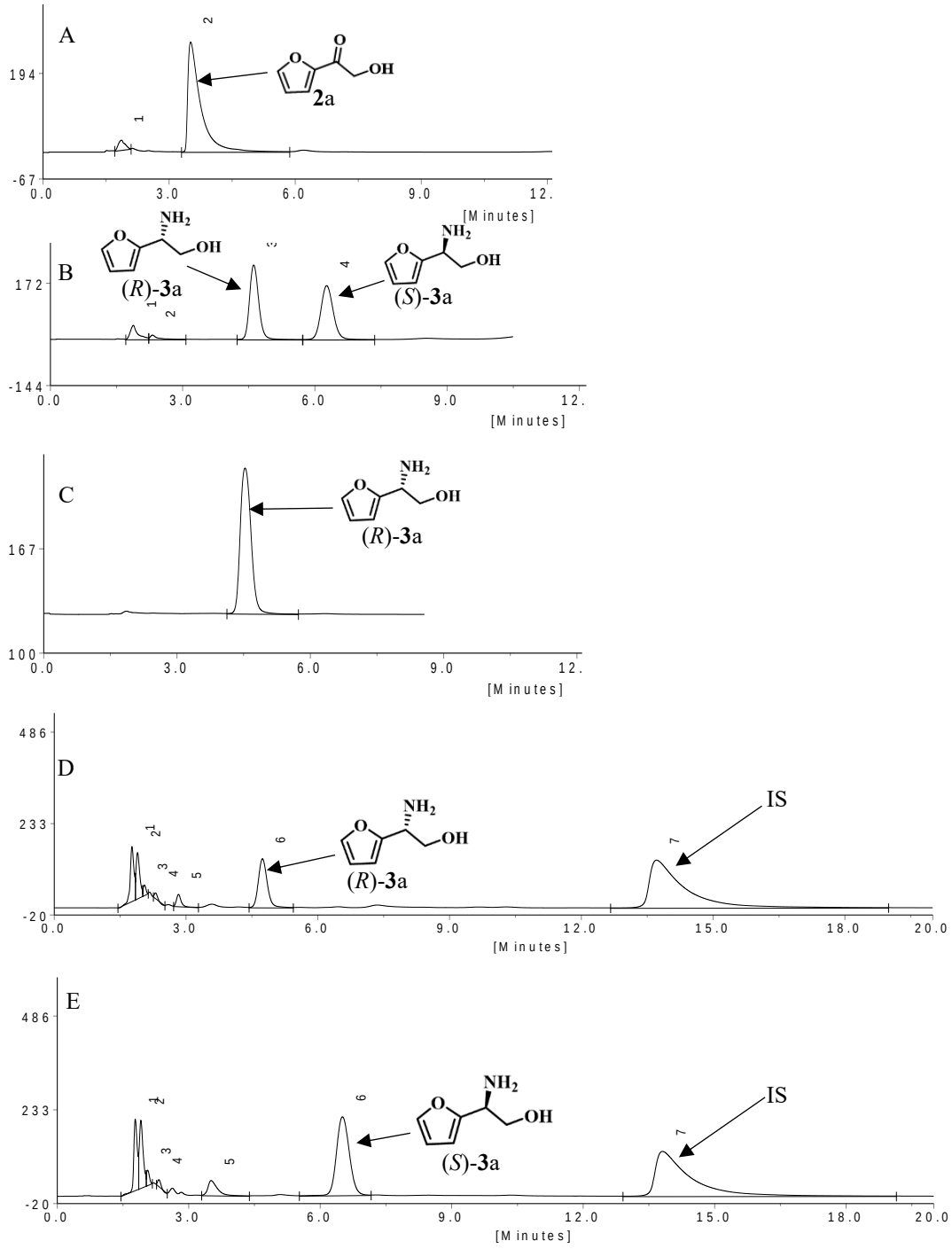


Figure S7. Chrial HPLC analysis of chiral 2-amino-2-(2-furyl)ethan-1-ol **3**a produced from **2**a. A: **2**a standard; B: racemic **3**a standard; C: (*R*)-**3**a standard; D: (*R*)-**3**a produced by reduction amination of **2**a (10 mM) with *E. coli* (MVTA) at 9 h; E: (*S*)-**3**a produced by reduction amination of **2**a (10 mM) with *E. coli* (BMTA) at 9 h. IS: internal standard (2-hydroxy-1-phenylethanone).

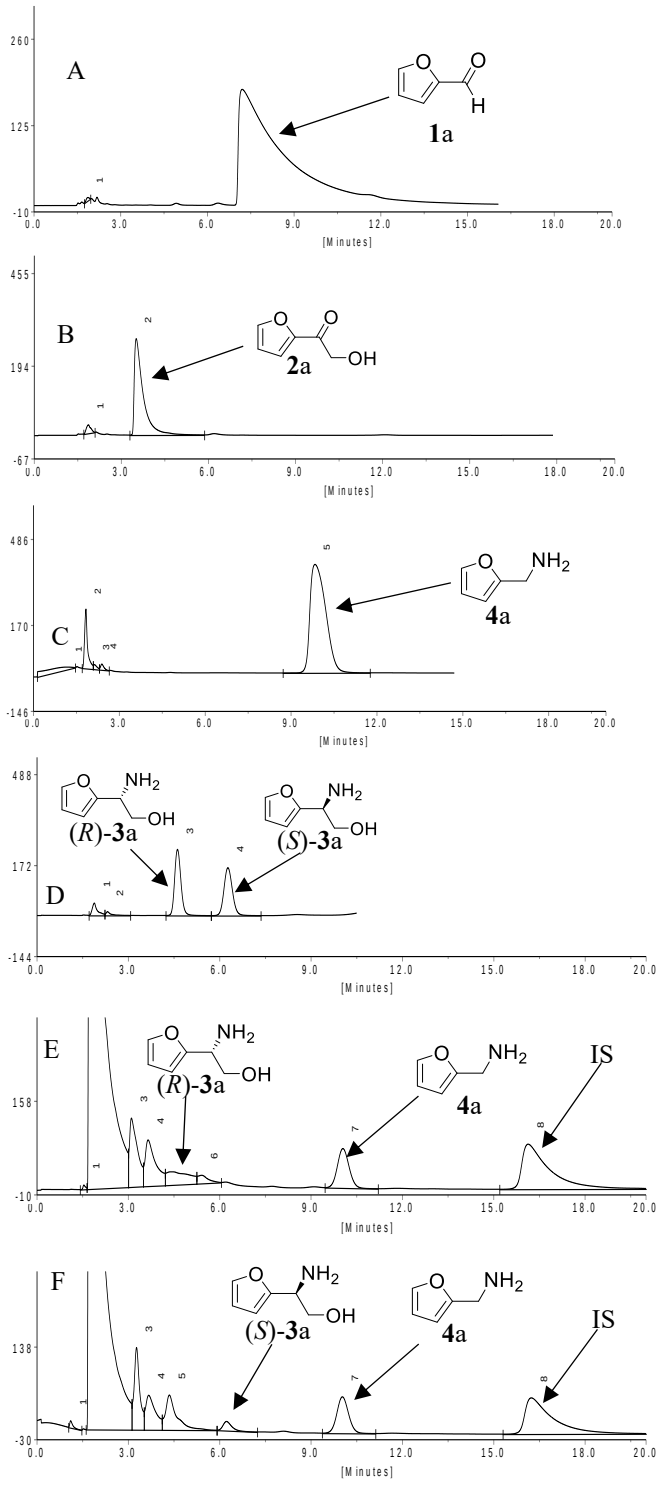


Figure S8. Chiral HPLC analysis of **3**a produced from **1**a *via* one-pot concurrent hydroxymethylation and asymmetric reduction amination. A: **1**a standard; B: **2**a standard; C: **4**a standard; D: racemic **3**a standard; E: One-pot concurrent hydroxymethylation and asymmetric reduction amination of **1**a (10 mM) to (*R*)-**3**a with *E. coli* (BAL) and *E. coli* (MVTA) at 12 h; F: One-pot concurrent hydroxymethylation and asymmetric reduction amination of **1**a (10 mM) to (*S*)-**3**a with *E. coli* (BAL) and *E. coli* (BMTA) at 12 h. IS: internal standard (2-hydroxy-1-phenylethanone).

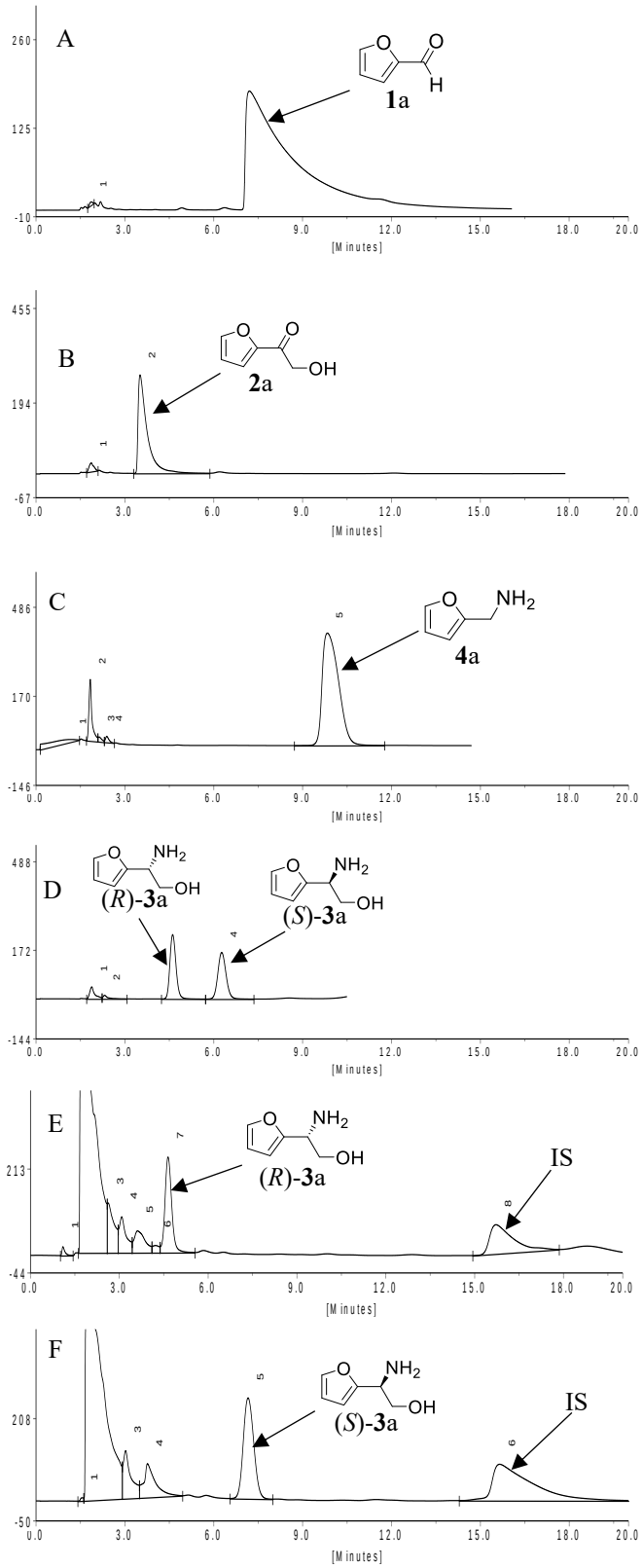


Figure S9. Chiral HPLC analysis of chiral **3**a produced from **1**a *via* one-pot two-stage hydroxymethylation and asymmetric reduction amination. A: **1**a standard; B: **2**a standard; C: **4**a standard; D: racemic **3**a standard; E: One-pot two-stage bioconversion of **1**a to (*R*)-**3**a with *E. coli* (BAL) and *E. coli* (MVTA) at 24 h. F: One-pot two-stage bioconversion of **1**a to (*S*)-**3**a with *E. coli* (BAL) and *E. coli* (BMTA) at 24 h. IS: internal standard (2-hydroxy-1-phenylethanone).

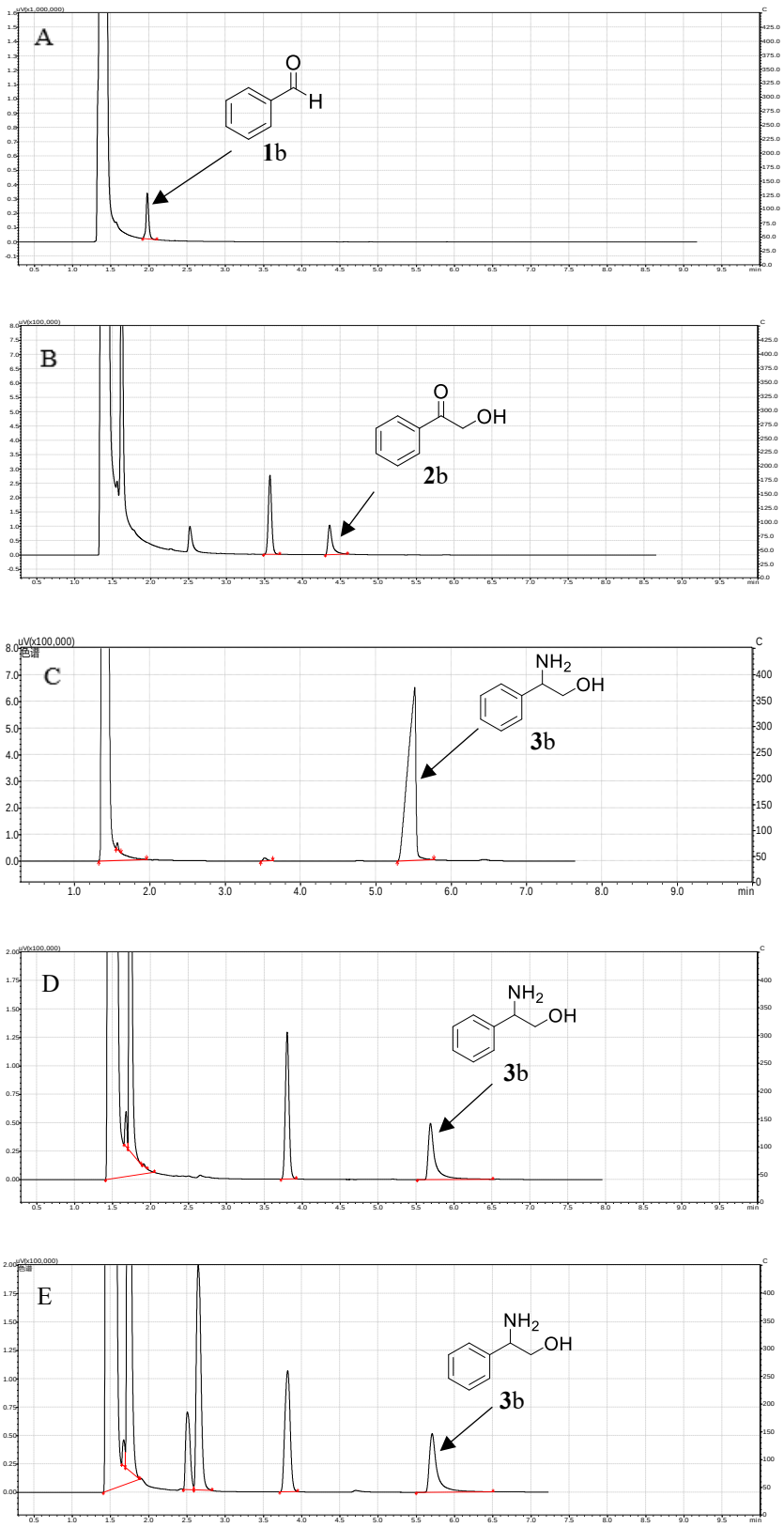


Figure S10. Achiral GC chromatograms of **3b.** A: **1b** standard. B: **2b** produced by conversion of **1b** (10 mM) with resting cells of *E. coli* (BAL) at 4 h. C: **3b** standard. D: One-pot two-stage bioconversion of (10 mM) **1b** to **3b** with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 10 h. E: One-pot two-stage bioconversion of (10 mM) **1b** to **3b** with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 7 h.

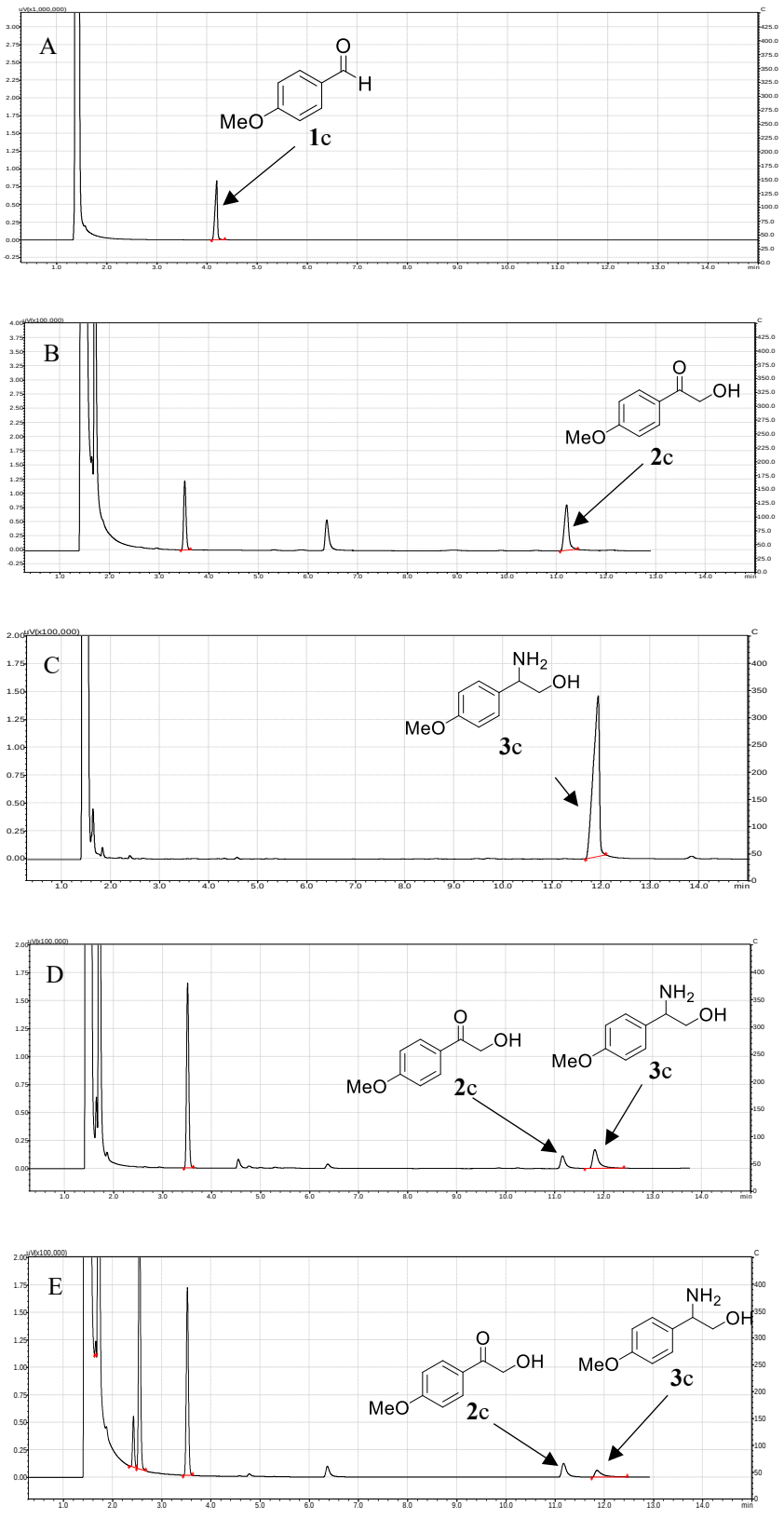


Figure S11. Achiral GC chromatograms of **3**c. A: **1**c standard. B: **2**c produced by conversion of **1**c (10 mM) with resting cells of *E. coli* (BAL) at 4 h. C: **3**c standard. D: One-pot two-stage bioconversion of (10 mM) **1**c to **3**c with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 16 h. E: One-pot two-stage bioconversion of (10 mM) **1**c to **3**c with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 10 h.

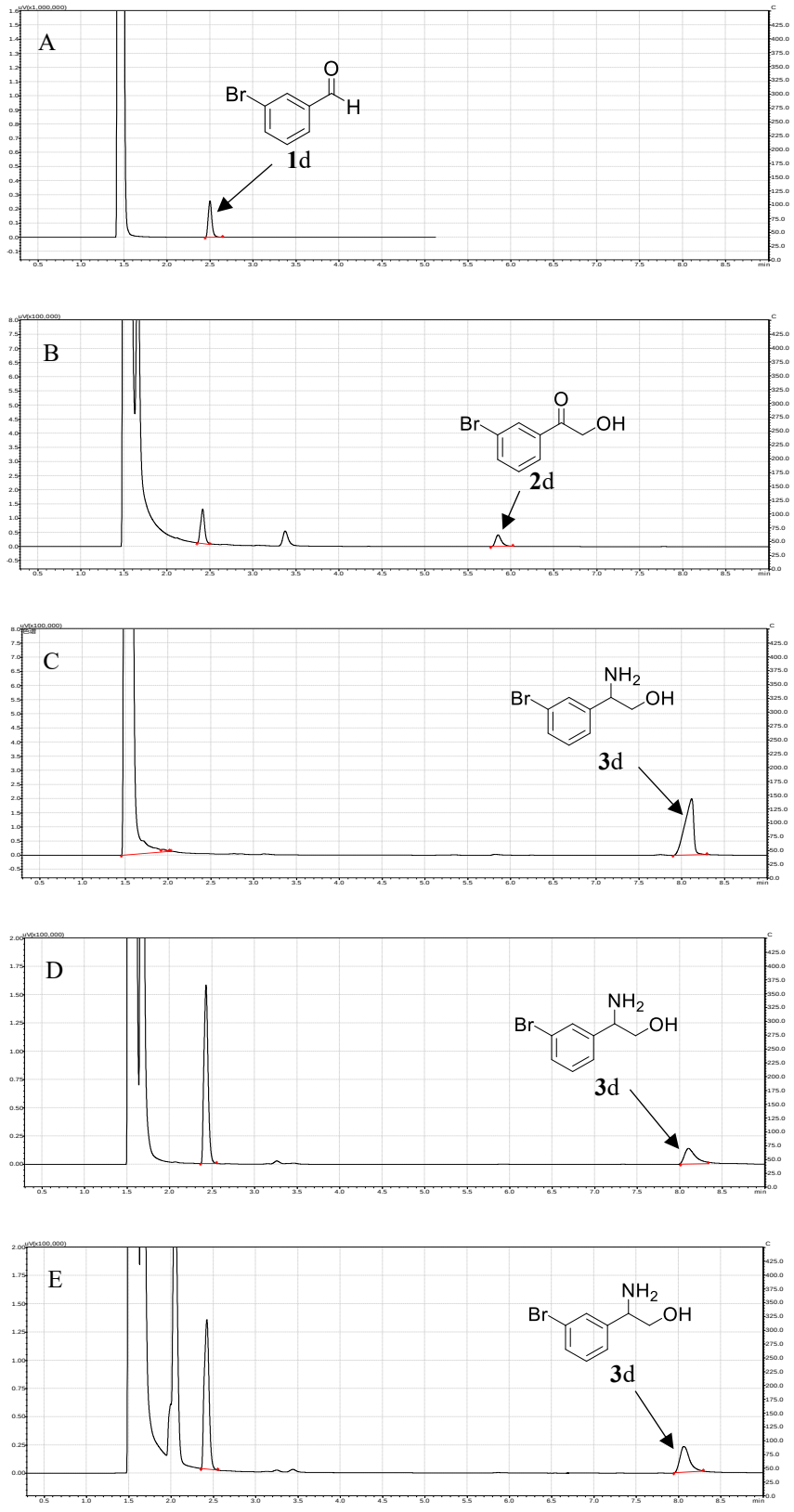


Figure S12. Achiral GC chromatograms of **3**d. A: **1**d standard. B: **2**d produced by conversion of **1**d (10 mM) with resting cells of *E. coli* (BAL) at 4 h. C: **3**d standard. D: One-pot two-stage bioconversion of (10 mM) **1**d to **3**d with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 10 h. E: One-pot two-stage bioconversion of (10 mM) **1**d to **3**d with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 7 h.

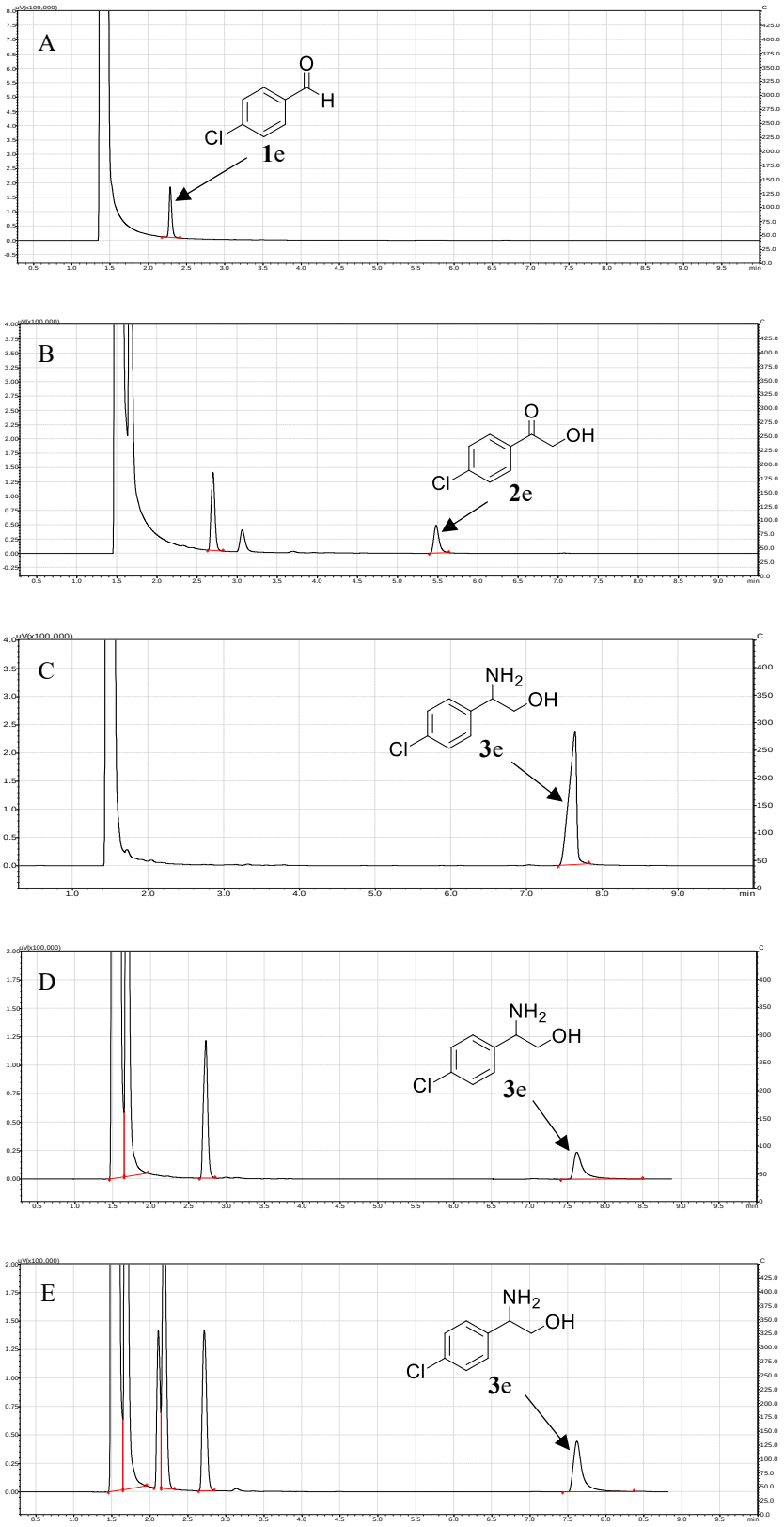


Figure S13. Achiral GC chromatograms of **3e.** A: **1**e standard. B: **2**e produced by conversion of **1**e (10 mM) with resting cells of *E. coli* (BAL) at 4 h. C: **3**e standard. D: One-pot two-stage bioconversion of (10 mM) **1**e to **3**e with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 10 h. E: One-pot two-stage bioconversion of (10 mM) **1**e to **3**e with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 7 h.

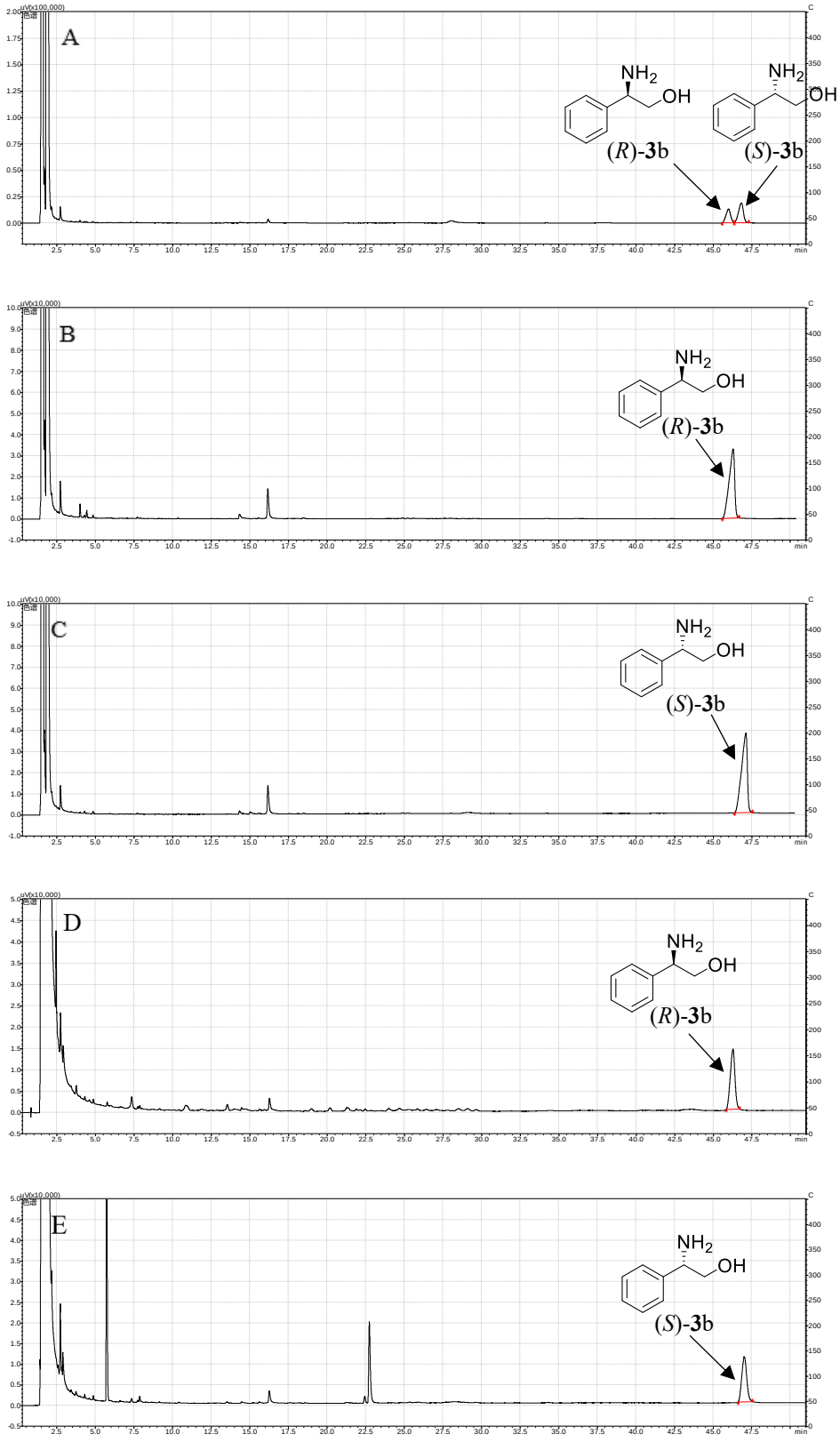


Figure S14. Chiral GC chromatograms of **3b**. **A:** (±)-**3b** standard. **B:** (*R*)-**3b** standard. **C:** (*S*)-**3b** standard. **D:** (*R*)-**3b** produced by conversion of **1b** (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 10 h. **E:** (*S*)-**3b** produced by conversion of **1b** (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 7 h.

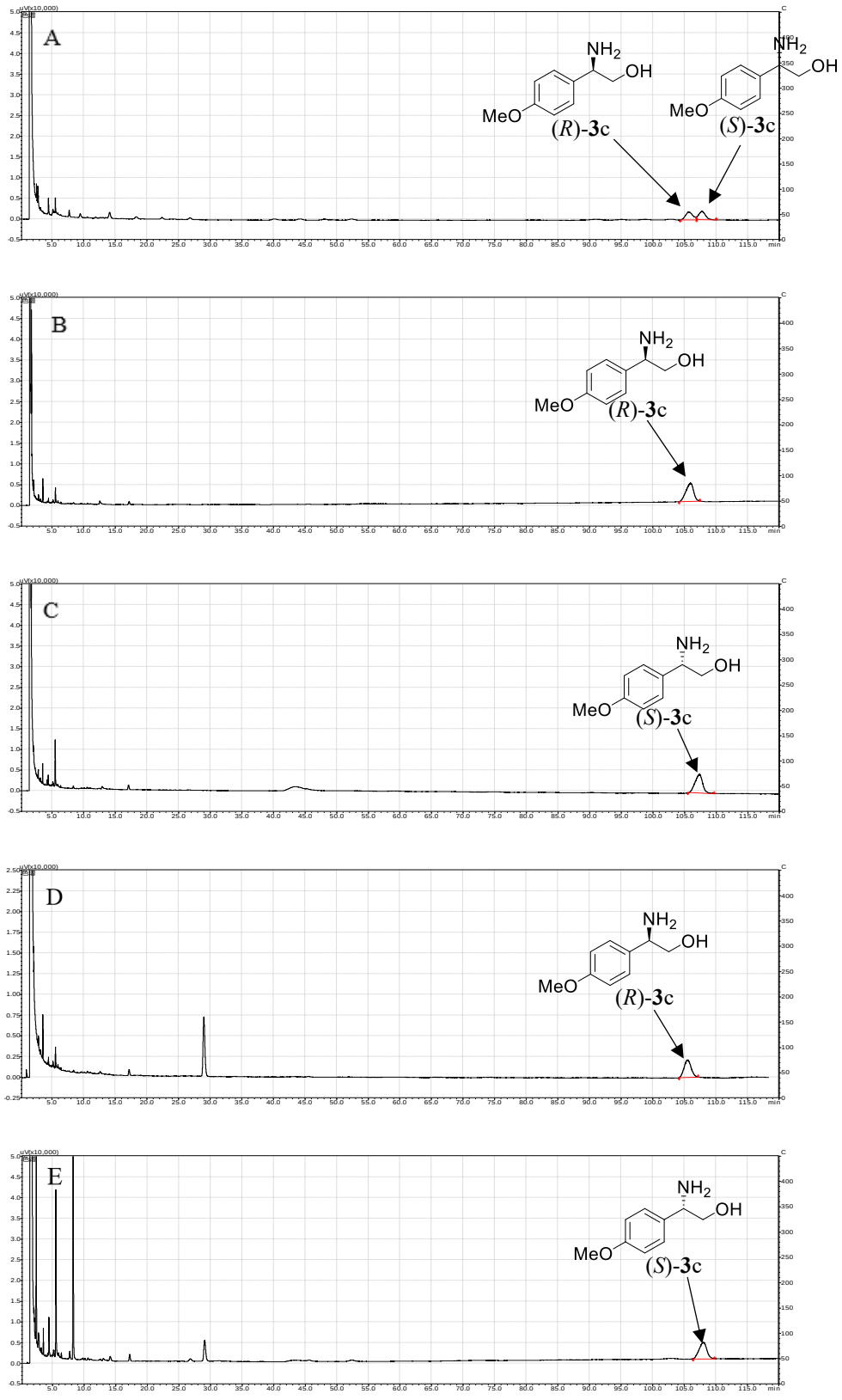


Figure S15. Chiral GC chromatograms of **3c**. **A:** (±)-**3c** standard. **B:** (*R*)-**3c** standard. **C:** (*S*)-**3c** standard. **D:** (*R*)-**3c** produced by conversion of **1c** (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 12 h. **E:** (*S*)-**3c** produced by conversion of **1c** (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 10 h.

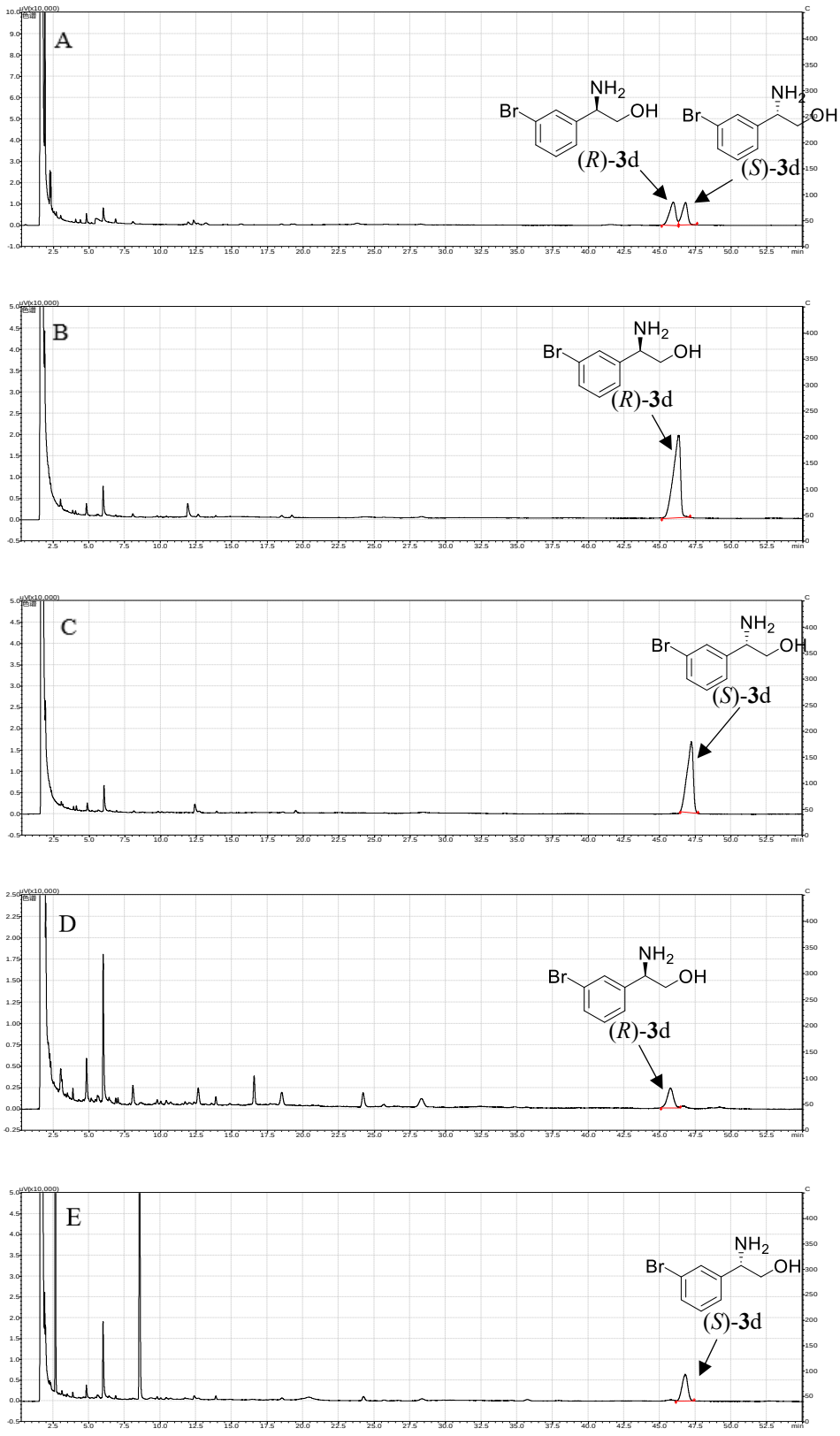


Figure S16. Chiral GC chromatograms of **3d**. **A:** (±)-**3d** standard. **B:** (*R*)-**3d** standard. **C:** (*S*)-**3d** standard. **D:** (*R*)-**3d** produced by conversion of **1d** (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 10 h. **E:** (*S*)-**3d** produced by conversion of **1d** (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 7 h.

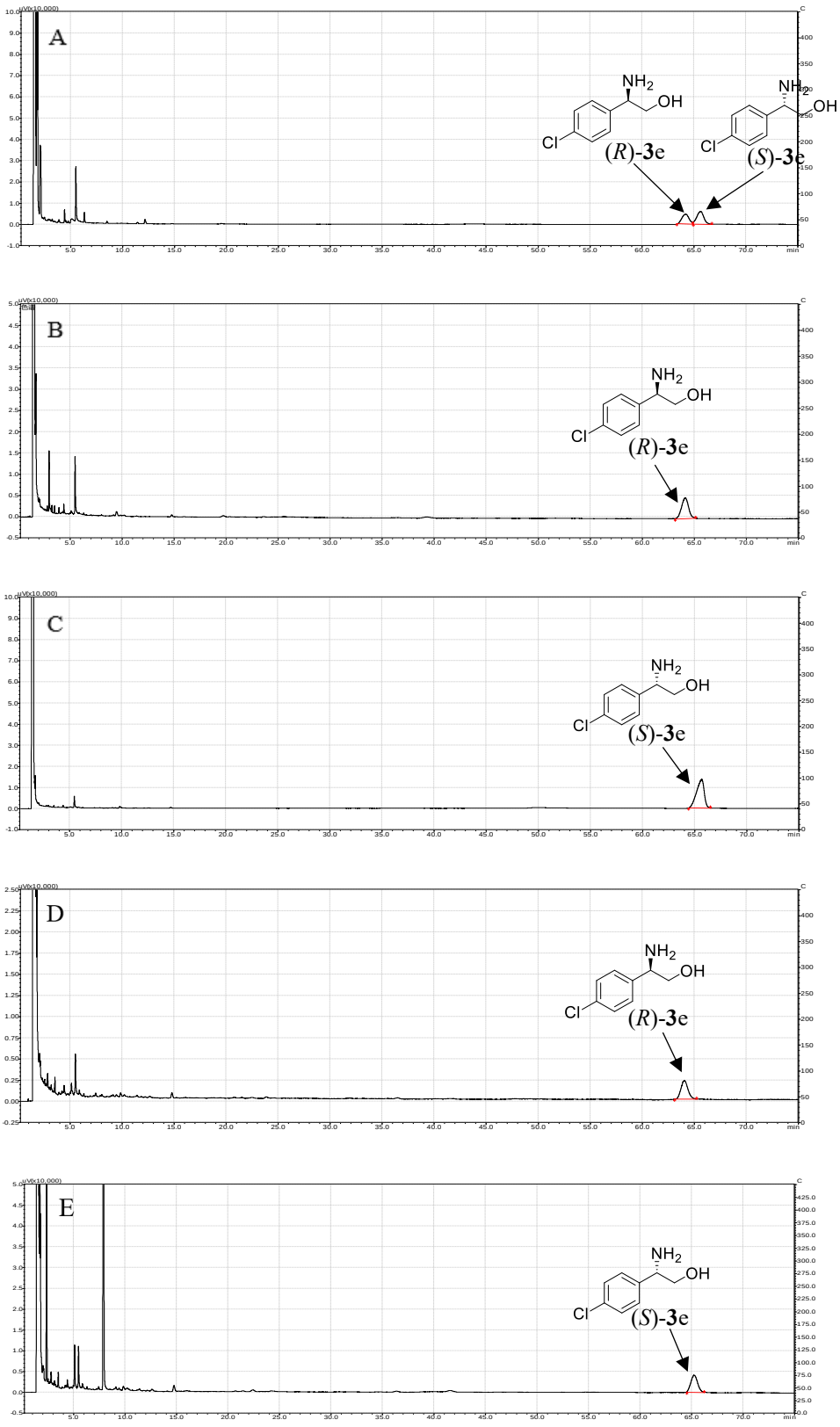


Figure S17. Chiral GC chromatograms of **3**e. **A:** (±)-**3**e standard. **B:** (*R*)-**3**e standard. **C:** (*S*)-**3**e standard. **D:** (*R*)-**3**e produced by conversion of **1**e (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (BMTA) at 10 h. **E:** (*S*)-**3**e produced by conversion of **1**e (10 mM) with resting cells of *E. coli* (BAL) and *E. coli* (MVTA) at 7 h.

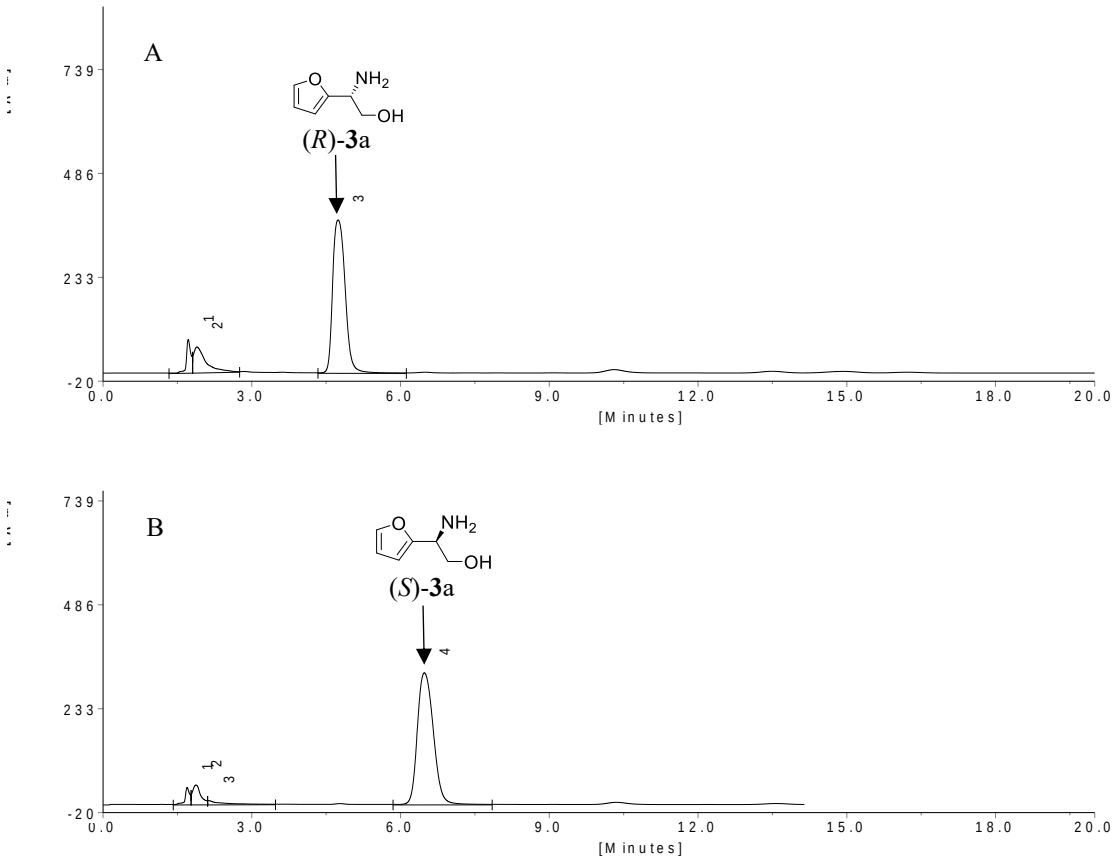


Figure S18. Preparation of (*R*)-**3**a (A) and (*S*)-**3**a (B).

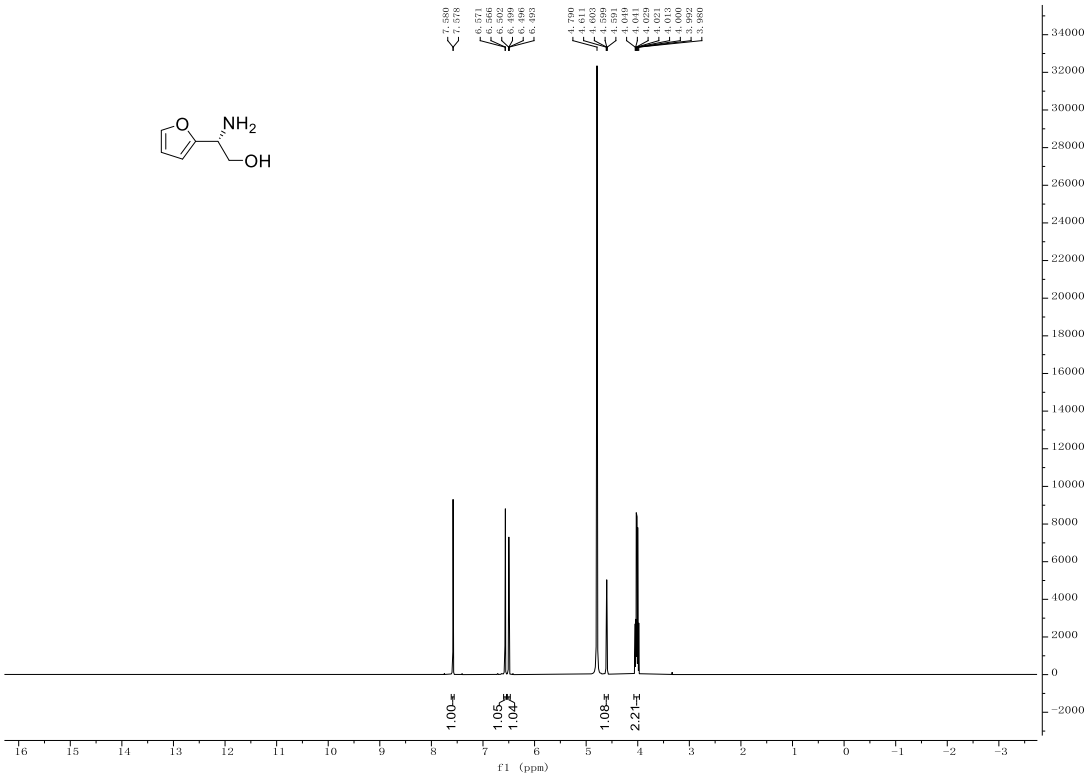


Figure S19. [1]H NMR spectra analysis of prepared (*R*)-**3**a and (*S*)-**3**a.

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