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Development of an engineered thermostable amine dehydrogenase for the synthesis of structurally diverse chiral amines

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Experimental Section

General information

2-Fluorophenylacetone, 2-octanamine, ethylamine, and allylamine were obtained from TCI development Co. Ltd. (Shanghai, China); 2-pentanamine and 3-fluorophenylacetone were obtained from J&K Chemical Co. Ltd. (Beijing, China); 4-methylphenylacetone, 2-heptanamine, (*S*)-2-heptylamine, (*R*)-2-hexanamine, (*S*)-2-hexanamine, and (*R*)-2-octanamine were purchased from Alfa Aesar Chemical Co. Ltd. (Tianjin, China); 4-methoxyphenylacetone and 4-chlorophenylacetone were obtained from Accela ChemBio Co., Ltd (Shanghai, China); 4-chloroamphetamine was obtained from Amatek Scientific Co. Ltd. (Suzhou, China); 4-fluorophenylacetone and 4-bromophenylacetone were obtained from Meryer Chemical Technology Co. Ltd (Shanghai, China); cyclopropylamine, methylamine, and 2-octanone were obtained from Aladdin (Shanghai, China); (*R*)- and (*S*)-4-methoxyamphetamine were obtained from J&W Pharmlab (Shanghai, China); Titanium isopropoxide was obtained from Macklin Chemical Co. Ltd. 4-Methylamphetamine, 3-fluoroamphetamine, and 2-fluoroamphetamine were synthesized by reductive amination of the corresponding ketones with sodium borohydride as previous reported[1]; *N*-methyl-4-fluoroamphetamine, *N*-ethyl-4-fluoroamphetamine, and *N*-cyclopropyl-4-fluoroamphetamine were synthesized by reductive amination of the corresponding ketones with sodium borohydride as previous reported[2]. All the other chemicals and reagents were purchased from local suppliers with >97% purity and used without further purification.

The genes encoding formic acid dehydrogenase *Ct*FDH from *Candida boidinii,* reductive aminase *Asp*RedAm from *Aspergillus oryzae* and glucose dehydrogenase *Bm*GDH from *Bacillus megaterium* were synthesized in Genscript (Nanjing, China), and inserted into expressing vector pET-28a (+). The resultant recombinant plasmid was transformed into *E. coli* BL21 (DE3) for protein expression. The oligonucleotide primers for cloning and mutagenesis were synthesized in Generay Biotech. (Shanghai, China).

Clone of phenylalanine dehydrogenases

pBLAST program was performed in the UniProt database with default parameters by setting *Bb*AmDH as the query sequence. The sequences sharing 30%–70% identity with *Bb*AmDH and noted as phenylalanine dehydrogenases (PheDHs) were selected from the BLAST results. Four genes encoding PheDH from *Geobacillus kaustophilus* (DSM 7263), *Geobacillus thermoglucosidasius* (DSM 2542), *Oceanbacillus iheyensis* (DSM 14371), and *Rhodococcus erythropolis* (DSM 11395) were amplified by PCR using a BIO-RAD T-100 Thermal Cycler with the following program: 98°C for 5 min, (98°C for 10 s, 55°C for 5 s, 72°C for 90 s) × 30 cycles, 72°C for 10 min. PCR reaction mixtures contained 1.5 μL each of forward and reverse primer (10 μM), 25 μL 2 × Prime STAR, 1 μL genome (75 ng/μL), 1 μL DMSO and 20 μL distilled water. The PCR product was analysed by gel electrophoresis, and the target fragment was digested with restriction endonucleases. The fragment was ligated into expressing vector pET-28a (+) that was digested with the same restricted enzymes by T4 DNA Ligase. After transforming into the *E. coli.* BL21 (DE3), the recombinant plasmid was extracted and verified by sequencing.

Gene sequence of *Gk*PheDH

ATGAATGTCATGCTATCGCCAAACATGTCACAAAGATTGGATTTGTTTTTCCAAATGCGTGAACATGAACAGGTGGTGTTTTGTCT
CGATGAAGCGACCGGCCTAAGGGCGATCATCGCCATTCATAGCACGGCTCTTGGGCCGGCGCTCGGCGGCTGTCGGATGCAT
CCGTATGCCACGACGGAAGAGGCGCTCGCCGATGCGCTTCGGCTGTCGAAAGGGATGACGTATAAATGCTTGGCTGCCGATG
TCGATTTTGGCGGCGGCAAGGCGGTGATCATCGGCGATCCGCGCAAAGACAAATCGCCGGAATTGTTTCGCGCTTTCGGCCAG
TTCGTGGAGTCATTAGGCGGCCGGTTTTACACGGGCACGGATATGGGGACGACGCCGGACGATTTTGTGCACGCGCTGAAAG
AGACGAATTGCATCGTCGGCGTTCCGGAAGCGTATGGCGGAAGCGGCGACTCATCCGTGCCGACCGCCGAGGGCGTTGTTTA
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GTCGTGGCGTACGGCAAGCAAATCGGGGCGTCCGTCAAGCCGGTGAACGGAACGGATATTTATCGCGTCGAAGCCGATGTGT
TCGTCCCGTGCGCATTTGGCGGCGTCATCAACGATGAAACGATCGCCGAGCTGCGAGTGAAAGCGGTCGTCGGTTCGGCGAA
CAATCAGCTGGCTGACAAACGCCACGCCCGTATGTTGAAAGAAAAGGGCATCATGTATGCCCCCGATTATATCGTCAACGCCG
GCGGCCTCATTCAAGTGGCCGATGAACTGTACGGAGCGAACAAAGAGCGGGTGCTGGCGAAAACGAAAGCGATTTATGATACG

CTGCTCGCCATTTATGCGCGGGCTGAATCGGAAGGAATAACGACGATCGAGGCAGCCGATCAATTTTGCGAAGAGCGGATCGA
GAAACGCAAACGCCGCAATCATTTTTTCACGCACCAAAAGCGGCCGAAGTGGGATATTCGACGTTAA

Gene sequence of *Gt*PheDH

ATGAATGTCATGCTATCGCCAAACATGTCACAAAGATTGGATTTGTTTTTCCAAATGCGTGAACATGAACAGGTGGTGTTTTGTCT
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CCGTATGCCACGACGGAAGAGGCGCTCGCCGATGCGCTTCGGCTGTCGAAAGGGATGACGTATAGCTGCTTGGCCGCTGATG
TCGATTTTGGCGGCGGCAAGGCGGTGATCATCGGCGATCCGCGCAAAGACAAATCGCCGGAATTGTTTCGCGCTTTCGGCCAG
TTCGTGGAGTCATTAGGCGGCCGGTTTTACACGGGCACGGATATGGGGACGACGCCGGACGATTTTGTGCACGCGCTGAAAG
AGACGAATTGCATCGTCGGCGTTCCGGAAGCGTATGGCGGAAGCGGCGACTCATCCGTGCCGACCGCCGAGGGCGTTGTTTA
CGGCATTCAGGCGACGAACGATGTTGTGTTTGGCAGCAAGCATTTGCATGGCAAAACGTATGCGGTGCAAGGGCTCGGAAAAG
TCGGAAAAAAAGTGGCGCTTCGTTTGCTTGAAGAAGGGGCGGATCTGTATGTGTGCGATTTGAACGAAGCGGCAGTCAAAGAG
GTCGTGGCGTACGGCAAGCAAATCGGGGCGTCCGTCAAGCCGGTGAACGGAACGGATATTTATCGCGTCGAAGCCGATGTGT
TCGTCCCGTGCGCATTTGGCGGCGTCATCAACGATGAAACGATCGCCGAGCTGCGAGTGAAAGCGGTCGTCGGTTCGGCGCT
GAATCAGCTGGCTGACAAACGCCACGCCCGTATGTTGAAAGAAAAGGGCATCATGTATGCCCCCGATTATATCGTCAACGCCG
GCGGCCTCATTCAAGTGGCCGATGAACTGTACGGAGCGAACAAAGAGCGGGTGCTGGCGAAAACGAAAGCGATTTATGATACG
CTGCTCGCCATTTATGCGCGGGCTGAATCGGAAGGAATAACGACGATCGAGGCAGCCGATCAATTTTGCGAAGAGCGGATCGA
GAAACGCAAACGCCGCAATCATTTTTTCACGCACCAAAAGCGGCCGAAGTGGGATATTCGACGTTAA

Gene sequence of *Oi*PheDH

ATGAAACAGCTTGAATTTGTCAAACCGAAGGAGAAAAATACATTTGAAAAAATAGCTAACCATGAACAGGTAGTGTTCTGTAATG
ATCCGGCAACGGGACTTCAAGCAATTATTGCCATTCATGATACTACACTTGGTCCTGCATTGGGCGGAACAAGGATGTATCCAT
ATAAAAGTGTGGATGATGCATTGGAAGATGTCCTGCGACTGTCAGAAGGAATGACCTATAAATGTGCAGCTTCGGGTCTTGATTT
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TCTTTGAATGGACGTTTTTATACAGGTACAGATATGGGAACAACAACCGATGACTTTGTGGAGGCATTTAAAGAAACGAATTTTAT
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CAATGAATACCTATTTAATAATAATGATTTAGGAAACAGAACTTATGCGATTCAAGGGTTAGGAAAAGTCGGTTATAAGGTTGCCG
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TACAAATGGTGAAGTAGAACAGGTAGATAGTGAAGCTATTTACAGTACGGATGCAGATATTTTTATCCCTTGTGCAATGGGGGCG
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GCGAAAATGTTGCAAGAGAAAGGGATTTTATATGCACCAGATTATATTGTCAATGCCGGAGGACTGATACAAGTTGCTGATGAAC
TATATGGCCCGAATACGAAACGAGTACTTGTAAAGACAAAAGCGATATACCGTTCCCTTTTAGATATCTATGTACAAGCTGAATT
GGATGCGATTACAACAGTAGAAGCGGCAAATAGGAAAGTACGCAGTGTATTGGAAGAACAGCGGAATCGAAATAACTTTTATTC
TCGCAAGCGAAGACCGAAATGGAATATCAGAGAGTAA

Gene sequence of *Re*PheDH

ATGAGCATCGACGACGAACTGCGCTGGGACGGTGAACTGACCGTCACCCGACATGATCGGGAGACCGACACGACCTTTGTGAT
CCGAATCGACTCCACCCGTCTGGGACCGGCGTCGGGTGGCACGCGGGCGGCGCATTACCCCTCGATCGGTCACGCGCTGGC
GGACGCCGGGAAGCTGGCCGGCGCAATGACTTTGAAGATGGCTGTCTCCGACCTGCCGATGGGTGGCGGGAAGTCGGTGATA
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GGCGCGTCGTCGTGGACTCGGAACACTCGACGGCCGAAAGGTGCTGGTTCAGGGGCTCGGCGCCGTCGGCGGAGATGTTGT
GCGTCTCGCGGCGCAGGCTGGGGCACGCCTGCTTGTTGTCGATACGGACCCGCAGCGCCTCGAGGCCGCTTCTCTTGCGGG
GCACACGATCGTTCCTGCGGACGAGGTACTTCGAACGTCGTGTGACCTCTTTGCTCCCTGTGCGATGGGAGGCATCATCGATT
CCGCTGCTGCTGCGTCGATTCCGACTCTGGCCGTGGCGGGCGCAGCAAACAACATCTTGACCGATGCCGCTGCGGGCGAGGT
TCTGCGGAGCCGCGGGGTTCTCTGTGCCCCTGATTTCGTCGCGAACGCAGGCGGAGCCTTTCACCTCGTGGGGCGAGAAGTC

CTGGGGTGGAACGAAGACGATGTCGTCGAACGAACCCGAGGGATCGGTCGAACTCTGGACGAGGTGTATTCGTTGAGCGAGG
AACGTGGCATCACTACCGAGGCCGCAGCGCTTCTGCTCGCCCGGACGCGGCTATCCCCCGCATCCACCACCGCCGCCGCCAC
ATCCACCACCGCCGCTGCAGCTGCCGCCTCCCCCGTCTGA

Creation of amine dehydrogenases from PheDHs

The two key mutations K78S and N276L were introduced into aforementioned four PheDHs to create amine dehydrogenases (AmDHs)[3]. The site-directed mutagenesis was conducted using recombinant plasmids containing PheDH genes as templates with the following program: 98°C for 5 min, (98°C for 10 s, 55°C for 5 s, 72°C for 8 min) × 30 cycles, 72°C for 10 min. PCR reaction mixtures contained 0.3 μL each of forward and reverse primer (10 μM), 5 μL 2 × Prime STAR, 1 μL recombinant plasmid, 0.4 μL DMSO and 3 μL distilled water. The PCR products were digested by *Dpn* I and transformed into the *E. coli.* BL21 (DE3). The sequences were verified by sequencing and the reductive amination activity of developed AmDHs was detected via spectrophotometric assay. Primers used in this work are shown in **Table S1**.

Table S1. Primers used for cloning and site-directed mutagenesis.

Primer	Sequence (5'-3')
*Gt*PheDH-FP	CGGGATCCATGAATACCGTTACCAATCAGTGGA
*Gt*PheDH-RP	CCCAAGCTTTTACCGGCGGATATCCCACTTCGGC
*Gt*PheDH-K66S-FP	AAGGGATGACGTATAGCTGCCTTGCGGCAGA
*Gt*PheDH-N274L-FP	TTGTCGGTTCCGCCCTGAATCAATTACTTGA
*Oi*PheDH-FP	CGGGATCCATGAAACAGCTTGAATTTGTCAAAC
*Oi*PheDH-RP	CCCAAGCTTTTACTCTCTGATATTCCATTTCGGT
*Re*PheDH-FP	CGGGATCCATGAGTATCGACGACGAACTGCG
*Re*PheDH-RP	CCCAAGCTTTCAGGCGGGGGAGGCAGCAGCT
*Gk*PheDH-FP	CGGGATCCATGAATGTCATGCTATCGCCAAACA
*Gk*PheDH-RP	CCCAAGCTTTTAACGTCGAATATCCCACTTCGGC
*Gk*PheDH-K78S-FP	AAGGGATGACGTATAGCTGCTTGGCCGCTGA
*Gt*PheDH-N276L-FP	TCGTCGGTTCGGCGCTGAATCAGCTGGCTGA
*Gk*AmDH-K78G-F	CGAAAGGGATGACGTATGGTTGCTTGGCCGCTGATGT
*Gk*AmDH-K78A-F	CGAAAGGGATGACGTATGCGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78H-F	CGAAAGGGATGACGTATCACTGCTTGGCCGCTGATGT
*Gk*AmDH-K78R-F	CGAAAGGGATGACGTATCGTTGCTTGGCCGCTGATGT
*Gk*AmDH-K78F-F	CGAAAGGGATGACGTATTTCTGCTTGGCCGCTGATGT
*Gk*AmDH-K78C-F	CGAAAGGGATGACGTATTGCTGCTTGGCCGCTGATGT
*Gk*AmDH-K78Q-F	CGAAAGGGATGACGTATCAATGCTTGGCCGCTGATGT
*Gk*AmDH-K78D-F	CGAAAGGGATGACGTATGATTGCTTGGCCGCTGATGT
*Gk*AmDH-K78E-F	CGAAAGGGATGACGTATGAGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78M-F	CGAAAGGGATGACGTATATGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78Y-F	CGAAAGGGATGACGTATTATTGCTTGGCCGCTGATGT

*Gk*AmDH-K78T-F	CGAAAGGGATGACGTATACCTGCTTGGCCGCTGATGT
*Gk*AmDH-K78I-F	CGAAAGGGATGACGTATATTTGCTTGGCCGCTGATGT
*Gk*AmDH-K78W-F	CGAAAGGGATGACGTATTGGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78P-F	CGAAAGGGATGACGTATTGGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78V-F	CGAAAGGGATGACGTATGTGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78L-F	CGAAAGGGATGACGTATCTGTGCTTGGCCGCTGATGT
*Gk*AmDH-K78N-F	CGAAAGGGATGACGTATAACTGCTTGGCCGCTGATGT
*Gk*AmDH-N276G-F	CGGTCGTCGGTTCGGCGGGTAATCAGCTGGCTGACAA
*Gk*AmDH-N276A-F	CGGTCGTCGGTTCGGCGCACAATCAGCTGGCTGACAA
*Gk*AmDH-N276H-F	CGGTCGTCGGTTCGGCGCACAATCAGCTGGCTGACAA
*Gk*AmDH-N276R-F	CGGTCGTCGGTTCGGCGCGTAATCAGCTGGCTGACAA
*Gk*AmDH-N276F-F	CGGTCGTCGGTTCGGCGTTCAATCAGCTGGCTGACAA
*Gk*AmDH-N276C-F	CGGTCGTCGGTTCGGCGTGCAATCAGCTGGCTGACAA
*Gk*AmDH-N276Q-F	CGGTCGTCGGTTCGGCGCAAAATCAGCTGGCTGACAA
*Gk*AmDH-N276D-F	CGGTCGTCGGTTCGGCGGATAATCAGCTGGCTGACAA
*Gk*AmDH-N276E-F	CGGTCGTCGGTTCGGCGGAGAATCAGCTGGCTGACAA
*Gk*AmDH-N276M-F	CGGTCGTCGGTTCGGCGATGAATCAGCTGGCTGACAA
*Gk*AmDH-N276Y-F	CGGTCGTCGGTTCGGCGTATAATCAGCTGGCTGACAA
*Gk*AmDH-N276I-F	CGGTCGTCGGTTCGGCGATTAATCAGCTGGCTGACAA
*Gk*AmDH-N276T-F	CGGTCGTCGGTTCGGCGACCAATCAGCTGGCTGACAA
*Gk*AmDH-N276W-F	CGGTCGTCGGTTCGGCGTGGAATCAGCTGGCTGACAA
*Gk*AmDH-N276P-F	CGGTCGTCGGTTCGGCGCCGAATCAGCTGGCTGACAA
*Gk*AmDH-N276V-F	CGGTCGTCGGTTCGGCGGTGAATCAGCTGGCTGACAA
*Gk*AmDH-N276K-F	CGGTCGTCGGTTCGGCGAAGAATCAGCTGGCTGACAA
*Gk*AmDH-N276S-F	CGGTCGTCGGTTCGGCGAGCAATCAGCTGGCTGACAA

Protein expression and purification

The recombinant *E. coli* BL21 (DE3) cells were precultured in 4 mL TB media containing 50 μg/mL kanamycin at 37°C and 220 rpm for 12 h. Then, 1 mL preculture was inoculated into shake flask containing 100 mL TB media with 50 μg/mL kanamycin at 37°C and 220 rpm until the culture's optical density (OD_{600}) reached 0.6-0.8. 0.2 mM IPTG (final concentration) was added for protein expression and the cultures were cultivated at 16°C and 180 rpm for further 24 h. The cells were harvested by centrifugation (13,000 × *g*, 5 min) and resuspended by potassium phosphate buffer (10 mM, pH 7.0). The cells were lysed through ultrasonication with an ultrasonic oscillator (JY92-II, Scientz Biotech Co.) in an ice bath for 25 min, and subsequently centrifuged at 13,000 × *g* for 30 min to remove the cell debris. The supernatant was stocked at −80°C for overnight and then freeze-dried for 72 h to access lyophilized cell-free extract.

AmDHs was purified by using Ni-NTA affinity chromatography. All the collected cells were resuspended in Tris-HCl buffer (50 mM, pH 8.0) and were lysed by ultrasonication in an ice bath. The lysates were centrifuged at 13,000 × *g* for 30 min to remove the cell debris. The supernatant was loaded onto a pre-equilibrated Ni-NTA column (GE, 5 mL) with buffer A containing 50 mM Tris-HCl,

500 mM NaCl, 10 mM imidazole at pH 8.0 and subsequently eluted by 50 mM Tris-HCl buffer (500 mM NaCl, pH 8.0) containing 50 mM, 250 mM and 500 mM imidazole for 8-10 column volumes, respectively. The fractions containing target protein was verified by SDS-PAGE analysis and concentrated by ultrafiltration. The concentrated solution was replaced by storing buffer (50 mM Tris-HCl buffer containing 150 mM NaCl, 1 mM DTT and 10% (v/v) glycerol) for three times and the purified enzyme was stored at −80°C for further use. The protein concentration of purified enzyme was determined by Nanodrop 2000c spectrophotometer (Thermo Scientific) at 280 nm and the extinction coefficients from the Expay protParam Tool was taken into consideration.

Activity assay and kinetic analysis

The activity of AmDHs was measured by monitoring the initial change in the absorbance of NADH at 340 nm using an UV-Vis spectrophotometer (Shimadzu, Japan). The activity assay was carried out in 1 mL reaction mixture containing $NH_3{\cdot}H_2O/NH_4Cl$ or $NH_3{\cdot}H_2O/NH_4COOH$ buffer with different concentrations, 0.2 mM NADH, appropriate concentration of substrate and enzyme at 30°C. One unit activity was defined as the amount of enzyme that catalyzed the conversion of 1 μM substrate during 1 min. To obtain the kinetic parameters (K_M and k_{cat} values), the activity was measured at different substrate concentration and the data was fitting to the Michaelis-Menten equation using Origin 8.6.

Table S2. Amination activity of engineered AmDHs based on different PheDHs towards the model substrate **1**.[a]

Enzyme	Specific activity[a] (U/mg protein)
*Gt*AmDH	5.0
*Oi*AmDH	n.d.[b]
*Re*AmDH	n.d.
*Gk*AmDH	6.0

[a] Activity was measured in 2 M $NH_3{\cdot}H_2O/NH_4Cl$ buffer (pH 9.6) containing 0.2 mM NADH, 10 mM substrate **1** and different enzymes at 30°C. [b] No detectable activity.

Characterization of *Gk*AmDH

The optimal temperature, pH and organic cosolvents of *Gk*AmDH were characterized in 2 M $NH_3{\cdot}H_2O/NH_4Cl$ buffer containing 0.2 mM NADH, 10 mM substrate **1** and appropriate amount of purified *Gk*AmDH. The temperature's effect on activity was investigated at a range of temperatures from 30ºC to 65ºC in pH 9.6 $NH_3{\cdot}H_2O/NH_4Cl$ buffer (**Figure S1A**). The effect of pH on activity was studied in the $NH_3{\cdot}H_2O/NH_4Cl$ buffer with different pH values (pH 7.0–11.5, **Figure S1B**) at 30ºC. The highest activity was set as 100%. For the investigation of the organic solvent tolerance, appropriate amount of purified *Gk*AmDH was preincubated in 10% (v/v) organic solvents for 1 h, and the residual activity was detected in pH 9.6 $NH_3{\cdot}H_2O/NH_4Cl$ buffer. The activity of *Gk*AmDH incubated in water was set as 100% (**Figure S1C**). The activities of *Gk*AmDH at different NH_3/NH_4^+ concentrations were measured in $NH_3{\cdot}H_2O/NH_4Cl$ buffer (pH 9.6) with different concentrations (from 0 M to 5 M), 0.2 mM NADH, 10 mM substrate **1** and appropriate amount of purified *Gk*AmDH. The result was shown in **Figure S2**.

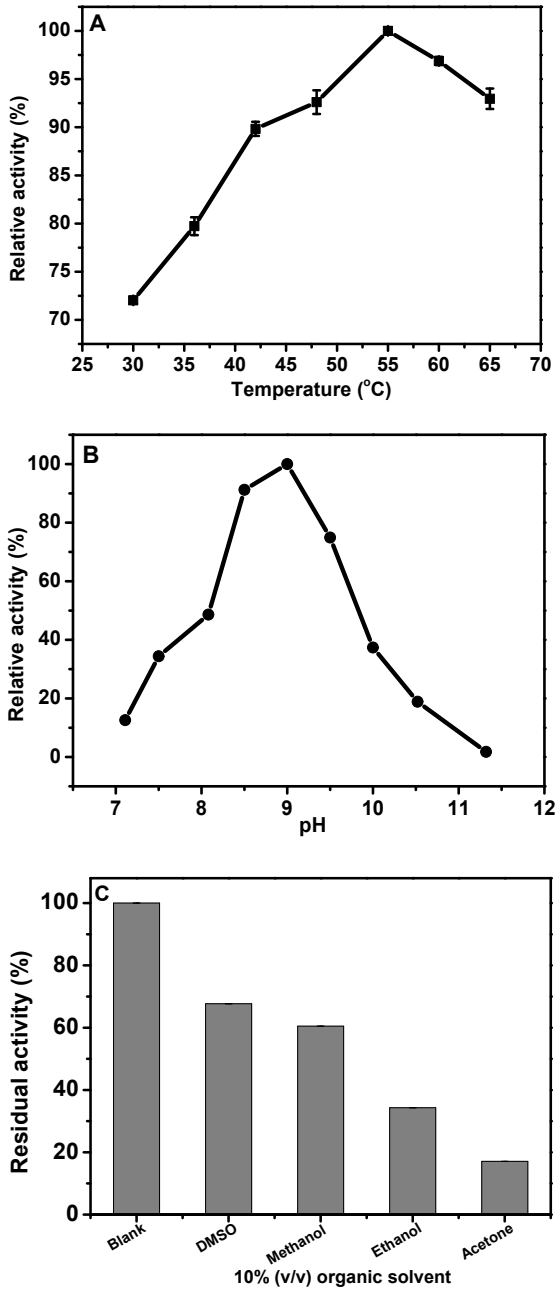

Figure S1. The investigation of optimal temperature (A), pH (B) and organic cosolvents (C) of *Gk*AmDH.

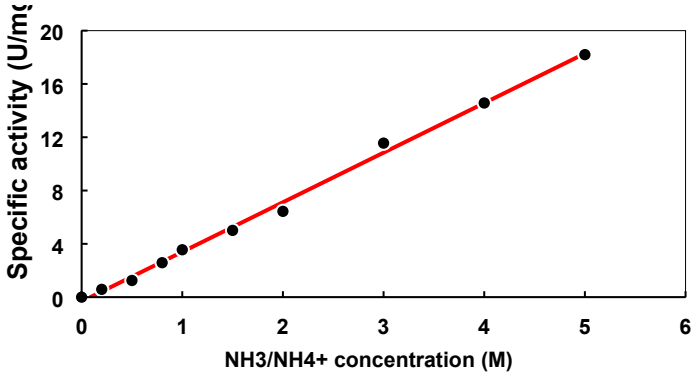

Figure S2. The activity of *Gk*AmDH towards substrate **1** at different concentration of NH_3/NH_4^+.

Melting temperature assay

The melting temperature (T_m) was measured using the circular dichroism (CD) spectroscopy. The purified enzymes were diluted to final concentration of 0.3 mg/mL with 10 mM potassium phosphate buffer (pH 7.0). The CD spectra of the diluted samples was determined in a 0.2 cm-path-length cuvette using a Chirascan circular dichroism spectrograph (Applied Photophysics, UK) at 180 nm–260 nm (1 nm/step) under increasing temperature. Control experiment was performed using the potassium phosphate buffer (10 mM, pH 7.0) without enzyme and scanning the CD spectra at 30°C. The result was set as background signals. The samples with enzymes was determined using the same process but subtracting the background signals before scanning and the temperature varied from 40°C to 95°C with 2°C increments. Then the protein's CD spectra measured as a function of temperature were analysed using Global 3 analysis software (Applied Photophysics, UK) to calculate the T_m values. The melting temperatures of *Gk*PheDH and *Gk*AmDH were shown in **Table S3**.

Table S3. The melting temperatures of *Gk*PheDH and *Gk*AmDH.

Enzymes	T_m (°C)
*Gk*PheDH	71.8 ± 0.2
*Gk*AmDH	71.5 ± 0.3

Activity assay of *Gk*AmDH, *Bm*GDH and *Ct*FDH in different NH_3/NH_4^+ concentrations

The lyophilized cell-free extracts of *Gk*AmDH, *Bm*GDH and *Ct*FDH were incubated in pH 9.0 $NH_3{\cdot}H_2O/NH_4Cl$ or $NH_3{\cdot}H_2O/NH_4COOH$ buffer with different concentrations of NH_3/NH_4^+ (from 2 M to 5 M) at 30°C, and then determined their initial activity and residual activity at 0 h and 16 h, respectively. Relative activity = Activity detected in 2 M (or 3 M, 4M, 5M) buffer/Activity detected in 2 M buffer × 100%, and the activity of enzymes in buffer containing 2 M NH_3/NH_4^+ was normalized as 100%.

Modelling and molecular docking

The complex crystal structure (PDB: 1C1X) of phenylalanine dehydrogenase *Rs*PheDH from *Rhodococcus sp.* M4 (36% sequence identity with *Gk*AmDH) was used as a template for homology modelling. The sequence of *Gk*AmDH was aligned with the chain A of the 1C1X and the result was output into FASTA format. Homology models of *Gk*AmDH was constructed with Modeller 9.11 algorithm. The best model was selected according to the scoring system, and was used for molecular docking. The hydroxy ketone **14** was drawn using ChemDraw 14.0 software and their energy was optimized using Minimize Energy function from ChemDraw 3D. Afterwards, the hydroxy ketone **14** and *Gk*AmDH model were optimized and output into PDBQT format in AutoDock Tools. The substrate **14** was docked into the active site of *Gk*AmDH within 16 × 16 × 16 Grid box using AutoDock Vina.

Biocatalytic synthesis of chiral primary amines

500 μL reaction mixture containing $NH_3{\cdot}H_2O/NH_4COOH$ buffer (5 M NH_4^+, pH 9.0), 10–50 mM ketone, 1 mM NAD^+, 1–20 mg/mL *Gk*AmDH (0.37 U/mg for **1,** lyophilized cell-free extract) and 5 mg/mL FDH (3.7 U/mg, lyophilized cell-free extract) were shaken at 40°C and 1000 rpm for 24 h, and subsequently terminated by adding 100 μL 10 M NaOH. The mixture was extracted with 500 μL tert-butyl methyl ether (MTBE). The half extracts were analysed by gas chromatography for conversion. Subsequently, another half sample was derivatized by adding 50 μL acetic anhydride and 10 μL pyridine. The mixture was shaken at 1000 rpm and room temperature for 2 h and washed by saturated brine for twice. The organic layer was dried with anhydrous Na_2SO_4, and used for analysis of ee by gas chromatography.

Biocatalytic synthesis of chiral secondary amines

500 μL reaction mixture containing amino donors/HCOOH buffer (2.5 M organic amino donors adjusting pH to 9.0 by adding HCOOH), 10 mM ketone **1**, 1 mM NAD^+, 20 mg/mL *Gk*AmDH (lyophilized cell-free extract) and 10 mg/mL FDH (lyophilized cell-free

extract) were shaken at 1000 rpm and 40°C for 24 h and subsequently terminated by adding 200 μL 10 M NaOH. The mixture was extracted with 500 μL tert-butyl methyl ether (MTBE), and the extracts were analysed by gas chromatography for conversions. Subsequently, 200 μL samples were derivatized by adding 50 μL trifluoroacetic anhydride and 10 μL pyridine. The mixture was shaken at room temperature and 1000 rpm for 2 h, and washed by saturated brine for twice. The organic layer was dried with anhydrous Na_2SO_4, and used for analysis of ee by gas chromatography or volatilized and resolved by mobile phase before analyzing the ee values by liquid chromatography.

Preparative biosynthesis

Preparation of **7a**

100 mL reaction system containing 5 M $NH_3{\cdot}H_2O/NH_4COOH$ buffer (pH 9.0), 20 mg/mL *Gk*AmDH cell-free extract, 10 mg/mL *Ct*FDH cell-free extract, 1 mM NAD^+, 200 mM 4-methoxyphenylacetone and 5% DMSO (v/v) were stirred at 200 rpm and 40ºC. The reaction process was monitored by gas chromatography (see **Figure S3**). After totally conversion of **7**, the reaction mixtures were alkalinized to pH > 12 with 10 M NaOH and extracted with MTBE (2 × 100 mL). The organic layer was combined and washed with 3 M HCl (2 × 100 mL). The aqueous layer was alkalinized to pH 12 with 10 M NaOH and was extracted with MTBE (3 × 50 mL). The organic layers were combined and dried with anhydrous Na_2SO_4. The final organic layer was evaporated with reduced pressure to afford the **7a**.

4-methoxyamphetamine **7a**: yield 43.0%, ee >99%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13-7.08 (d, 2H), 6.87-6.82 (d, 2H), 3.79 (s, 3H), 3.19-3.07 (m, 1H), 2.65 (dd, *J* = 13.4, 5.3 Hz, 1H), 2.52-2.40 (dd, 1H), 1.33-1.20 (br, 2H), 1.10 (d, *J* = 6.3 Hz, 3H).

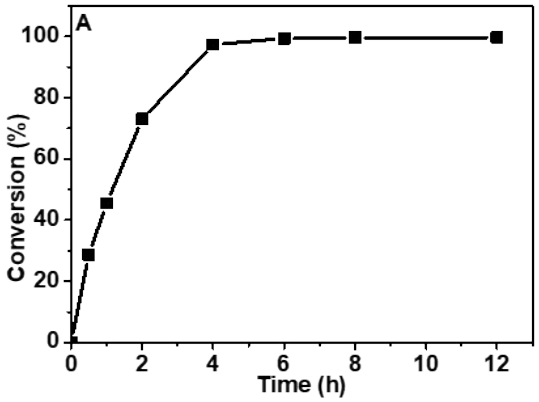

Figure S3. Reaction time-courses of preparative-scale reaction for synthesizing *(R)*-4-methoxy- amphetamine

Preparation of **1b**

50 mL reaction systems containing 2.5 M Methylamine /HCOOH buffer (pH 9.0), 20 mg/mL *Gk*AmDH cell-free extract, 10 mg/mL *Ct*FDH cell-free extract, 1 mM NAD^+ and 10 mM **1** were stirred with 200 rpm at 40ºC for 24 h. The reaction process was monitored by gas chromatography. The reaction mixture was alkalinized to pH > 12 with 10 M NaOH and extracted with MTBE (2 × 50 mL). The organic layer was combined and washed with 3 M HCl (2 × 30 mL). The aqueous layer was alkalinized to pH 12 with 10 M NaOH and extracted with MTBE (3 × 50 mL). The organic layers were combined and dried with anhydrous Na_2SO_4. The final organic layer was evaporated with reduced pressure to afford **1b**.

N-methyl-4-fluoroamphetamine **1b**: 45.0% yield and 76% ee. ^{1}H NMR (400 MHz, $CDCl^3$): δ 7.53-7.31 (m, 2H), 7.20 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.00 (t, *J* = 8.6 Hz, 2H), 3.41 (d, *J* = 13.0 Hz, 1H), 3.30 (s, 1H), 2.87-2.77 (m, 1H), 2.71 (s, 3H), 1.31 (d, *J* = 6.4 Hz, 3H).

Preparation of **14a**

10 mL reaction system containing 5 M $NH_3{\cdot}H_2O/NH_4COOH$ buffer (pH 9.0), 10 mg/mL *Gk*AmDH$_{K78S/N276T}$ cell-free extract, 1 mg/mL *Ct*FDH cell-free extract, 2 mM NAD^+, 35 mM **14** and 4% DMSO (v/v) were stirred at 1000 rpm and 30ºC for 24 h. For HPLC analysis,

100 μL reaction solution was taken timely and mixed with 500 μL of acetonitrile. After filtration, 20 μL of the filtrate was taken and mixed with 20 μL of 14 mM Marfey reagent in acetonitrile, 36 μL of 1 M carbonate and 100 μL of DMSO uniformly. The mixture was incubated at 40°C for 2 h and then quenched with 40 μL of 1 M HCl solution for HPLC analysis. The 10 mL reaction mixture was then quenched by addition of 2 mL 10 M NaOH and extracted with dichloromethane (2 × 10 mL). The organic layer was combined and washed with 1 M HCl (2 × 10 mL). The aqueous layer was washed with dichloromethane (3 × 10 mL) and then alkalinized to pH 12 with 10 M NaOH and extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with brine and dried with anhydrous $MgSO_4$. The final organic layer was evaporated with reduced pressure to afford the **14a**.

(*S*)-2-amino-3-phenylpropanol **14a**: yield 40.6%, ee >99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08 (3H, s), 2.55 (1H, dd, *J* = 8.7, 13.5 Hz), 2.80 (1H, dd, *J* = 5.3, 13.5 Hz), 3.10−3.20 (1H, m), 3.40 (1H, dd, *J* = 7.2, 10.6 Hz), 3.65 (1H, dd, *J* = 3.7, 10.7 Hz), 7.16−7.34 (5H, m). The data is in agreement with the reference.[5]

GC and HPLC assay

Gas chromatography analysis: The conversions and enantiomeric excess (ee) values were partly determined on a GC Shimadzu-2014 with a flame ionization detector (FID) using nitrogen as carrier gas. An Agilent J&W DB-1701 capillary column (30 m × 0.25 mm, 0.25 μm) and an Agilent J&W CP-Chiralsil-DEX CB capillary column (25 m × 0.25 mm, 0.25 μm) were used for determining the conversion and ee, respectively. The ee of several products was determined by high performance liquid chromatography on a Shimadzu LC-2010A Liquid Chromatograph equipped with a UV detector and a Chiralcel OD-H (0.46 mm × 25 cm) or Chiralcel IA (0.46 mm × 25 cm) column. The absolute configurations of the amine products were assigned by comparing with standard compounds or by comparison of elution order of products analysed with the same column and derivatization method ((trifluoro)acetic anhydride as the derivatization reagent). The detailed GC and HPLC analytic programs are listed below:

Program 1: Column DB1701-30 m: constant pressure 100 kPa, T injector 250°C, split ratio 30:1, T detector 280°C, T initial 90°C, hold for 2 min; gradient 10 °C/min up to 120°C, hold for 4 min, gradient 20°C/min up to 200°C, hold for 5 min.

Program 2: Column DB1701-30 m: constant pressure 100 kPa, T injector 280°C, split ratio 30:1, T detector 280°C, T initial 50°C, hold for 2 min; gradient 10°C/min up to 90°C, hold for 0 min, gradient 20°C/min up to 200°C, hold for 1 min.

Program 3: Column Rxi®-5Sil MS-30 m: constant pressure 100 kPa, T injector 280°C, split ratio 30:1, T detector 280°C, T initial 90°C, hold for 2 min; gradient 10°C/min up to120°C, hold for 4 min, gradient 20°C/min up to 250°C, hold for 5 min.

Program 4: CP Chirasil-DEX-CB-25 m: constant pressure 100 kPa, T injector 280°C, split ratio 30:1, T detector 280°C, T initial 70°C, hold for 0 min; gradient 20°C/min up to 120°C, hold for 2 min, gradient 10°C/min up to 180°C, hold for 2 min.

Program 5: CP Chirasil-DEX-CB-25 m: constant pressure 100 kPa, T injector 280°C, split ratio 30:1, T detector 280°C, T initial 70°C, hold for 2 min; gradient 10°C/min up to 160°C, hold for 2 min, gradient 10°C/min up to 180°C, hold for 10 min.

Program 6: CP Chirasil-DEX-CB-25 m: constant pressure 100 kPa, T injector 280°C, split ratio 30:1, T detector 280°C, T initial 90°C, hold for 0 min; gradient 5°C/min up to 140°C, hold for 0 min, gradient 1°C/min up to 160°C, hold for 5 min, gradient 5°C/min up to 200°C, hold for 4 min.

Program 7: The ee value of the products was analyzed by normal phase chiral HPLC equipped with Daicel CHIRALPAK®OD-H 250 mm × 4.6 mm and n-hexane/isopropanol (99:1) was used as eluent phase. The flow rate was maintained at 0.5 mL min^{-1} and elutes were detected by the UV detector at a wavelength of 265 nm.

Program 8: The ee value of the product was analyzed by normal phase chiral HPLC equipped with Daicel CHIRALPAK®IA 250 mm × 4.6 mm using n-hexane/isopropanol (99:1) as eluent phase. The flow rate was maintained at 0.5 mL min^{-1} and elutes were detected by the UV detector at a wavelength of 265 nm.

Program 9: The ee value of **14** was analyzed by reverse phase HPLC equipped with Elite Hypersil ODS 250 mm × 4.6 mm × 2 5 μm column using water (0.1% trifluoroacetic acid/methanol (0.1% trifluoroacetic acid) as elute phase: initial ratio 60%A/40%B, hold for 20 min; decrease the percentage of B to 30% in 10 min, hold for 10 min. The flow rate was maintained at 0.6 mL min^{-1} and elutes were detected by the UV detector at a wavelength of 340 nm.

Methods and retention times of carbonyl substrates and amine products were shown in Table S4. The absolute configurations of the amine products were assigned by comparison of retention times with derivatized authentic reference materials or by comparison

of elution orders with those in the reported article.

Table S4. Retention times of ketones and the corresponding amines.

Ketone	Amino donor	Analysis for conversion Retention time (min)			Analysis for ee value[a] Amine Retention time (min)		
		Method	Ketone	Amine	Method	T^1	T^2
1	**a**	program 1	10.37	9.13	program 5	14.81 (*S*)	14.97 (*R*)
1	**b**	program 3	6.69	8.06	program 8	18.73[c] (*S*)	19.63[c] (*R*)[2]
1	**c**	program 3	6.69	9.50	program 6	13.13[b] (*S*)	13.34[b] (*R*)
1	**d**	program 3	6.69	11.31	program 6	14.62[b](*R*)	14.83[b] (*S*)[4]
1	**e**	program 3	6.69	11.81	program 7	11.01[b] (*S*)	11.37[b] (*R*)
2	**a**	program 1	10.31	9.16	program 5	14.58 (*S*)	14.74 (*R*)
3	**a**	program 1	9.82	8.70	program 5	13.90 (*S*)	14.24 (*R*)
4	**a**	program 1	13.42	12.71	program 5	19.31 (*S*)	19.55 (*R*)
5	**a**	program 1	14.78	14.36	program 5	20.26 (*S*)	20.54 (*R*)
6	**a**	program 1	11.70	11.18	program 5	15.72 (*S*)	15.87 (*R*)
7	**a**	program 1	14.19	13.49	program 5	20.36 (*S*)	20.51 (*R*)
8	**a**	program 1	11.96	11.25	program 5	16.77 (*S*)	16.95 (*R*)
9	**a**	program 2	3.782	3.21	program 4	3.99 (*S*)	4.27 (*R*)
10	**a**	program 2	5.40	4.58	program 4	5.21 (*S*)	5.53 (*R*)
11	**a**	program 2	7.14	6.58	program 4	6.59 (*S*)	6.82 (*R*)
12	**a**	program 2	8.59	8.06	program 4	7.92 (*S*)	8.12 (*R*)
14	**a**	program 9	-	-	program 9	13.28 (*S*)	25.82 (*R*)

[a] The amine product was derivatized by acetic anhydride with pyridine.
[b] The amine product was derivatized by trifluoroacetic anhydride with pyridine
[c] The amine product was directly analyzed without treatment.

GC and HPLC Chromatograms

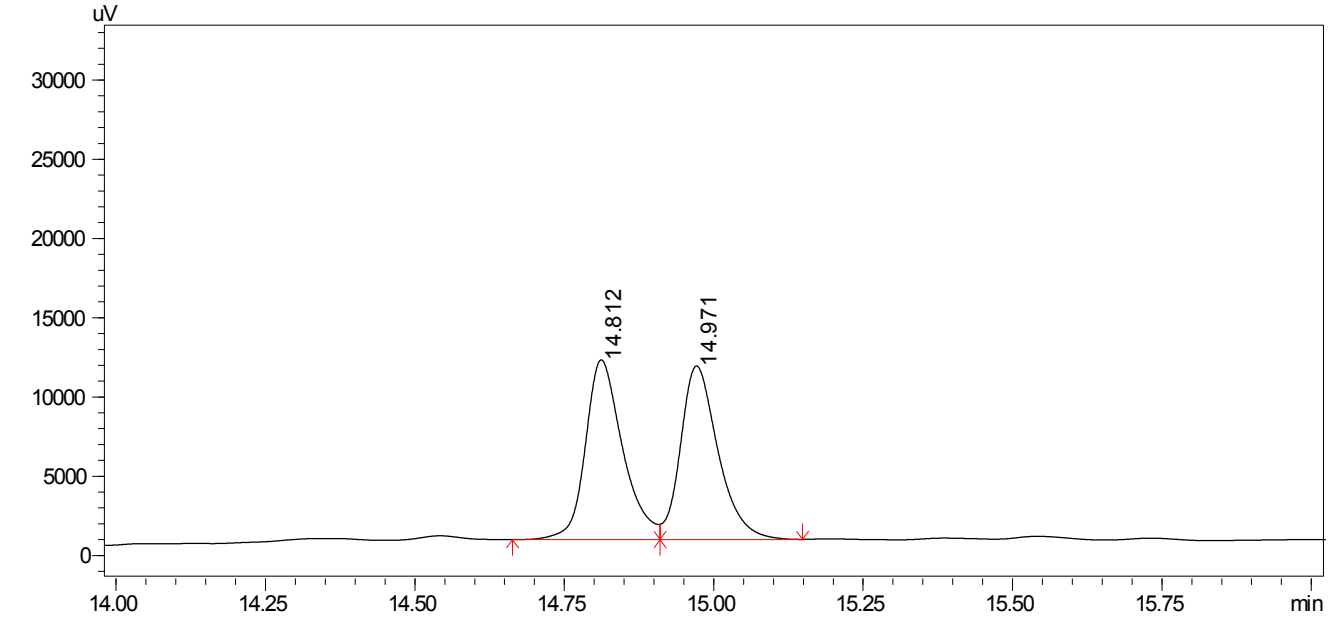

Figure S4. GC chromatogram of racemic **1a**.

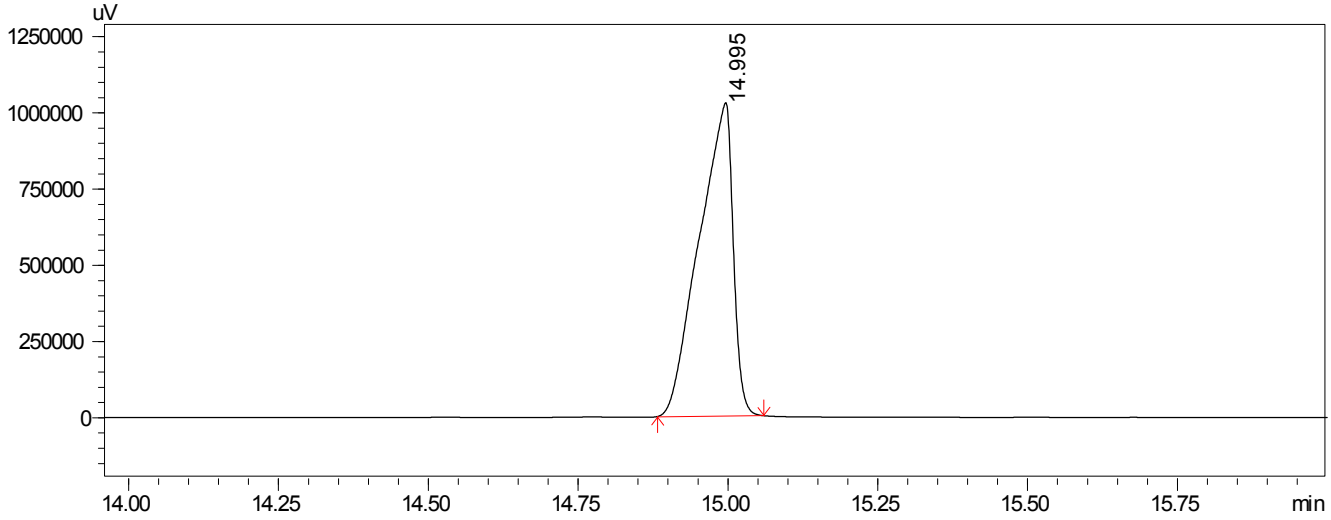

Figure S5. GC chromatogram of (*R*)-**1a** yielded by *Gk*AmDH.

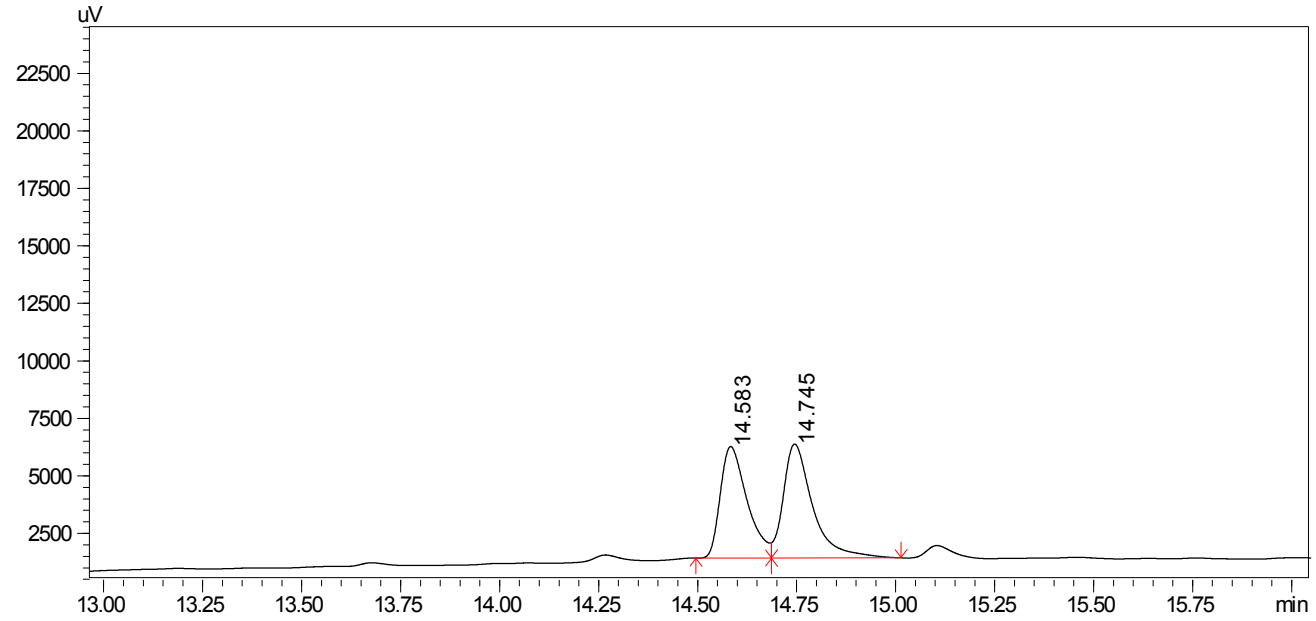

Figure S6. GC chromatogram of racemic **2a**.

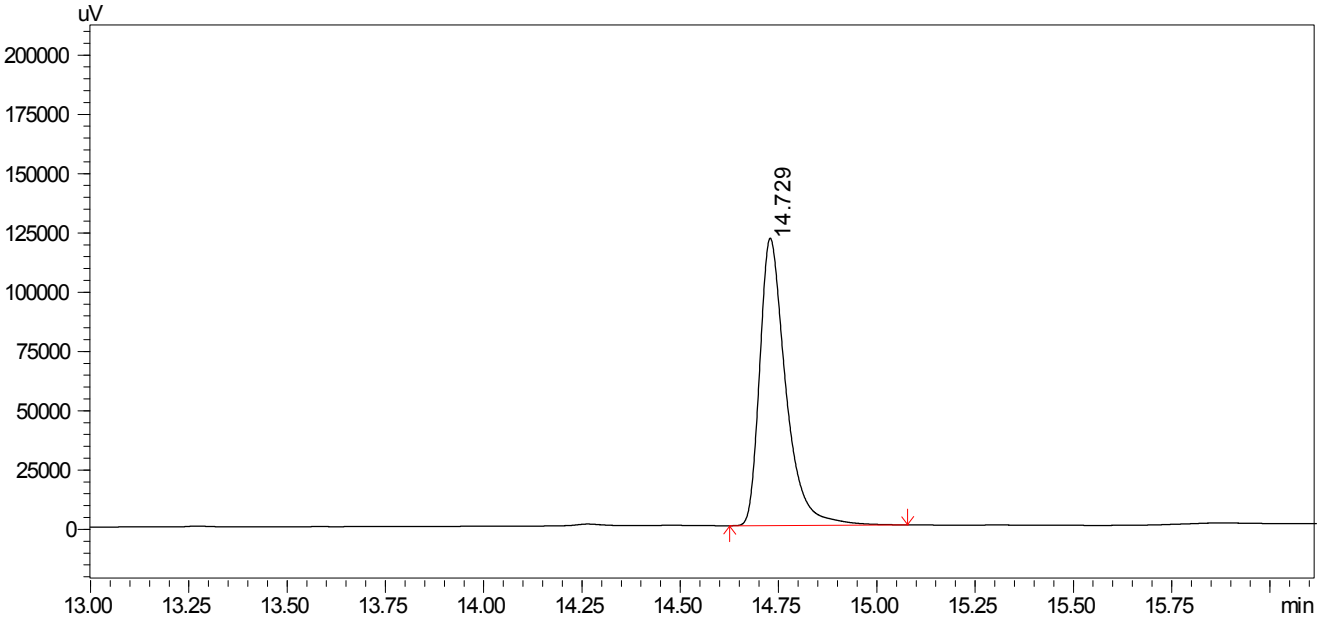

Figure S7. GC chromatogram of product (*R*)-2a yielded by *Gk*AmDH.

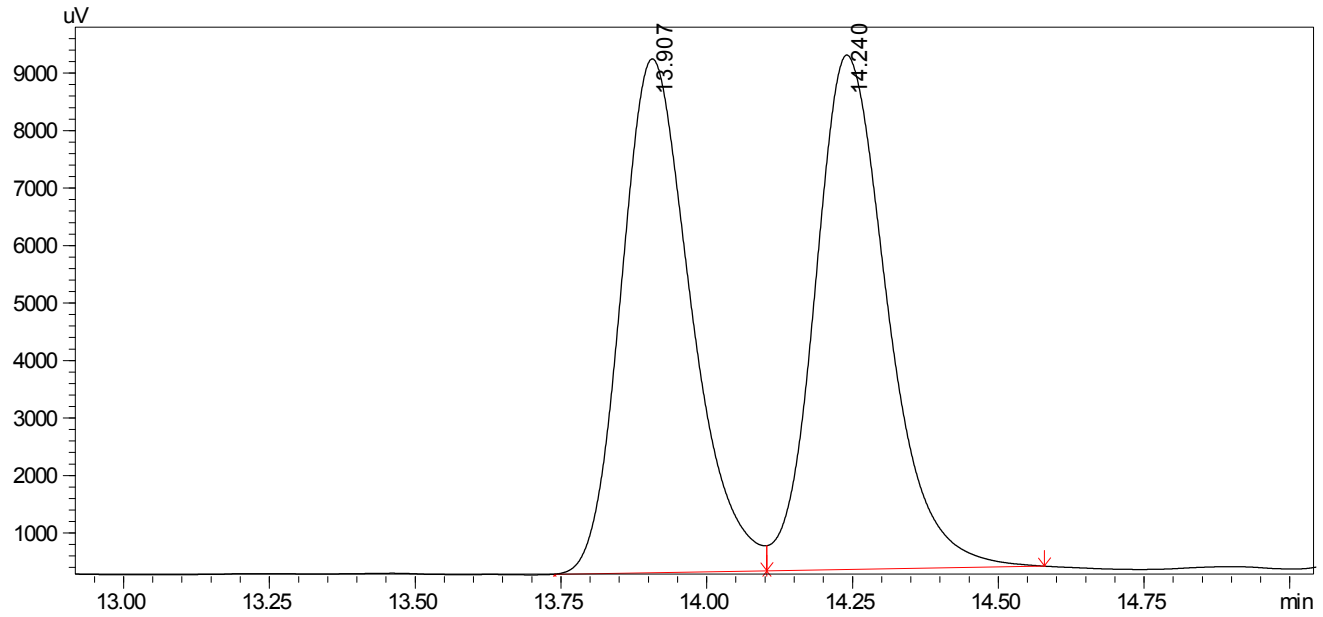

Figure S8. GC chromatogram of racemic **3a**.

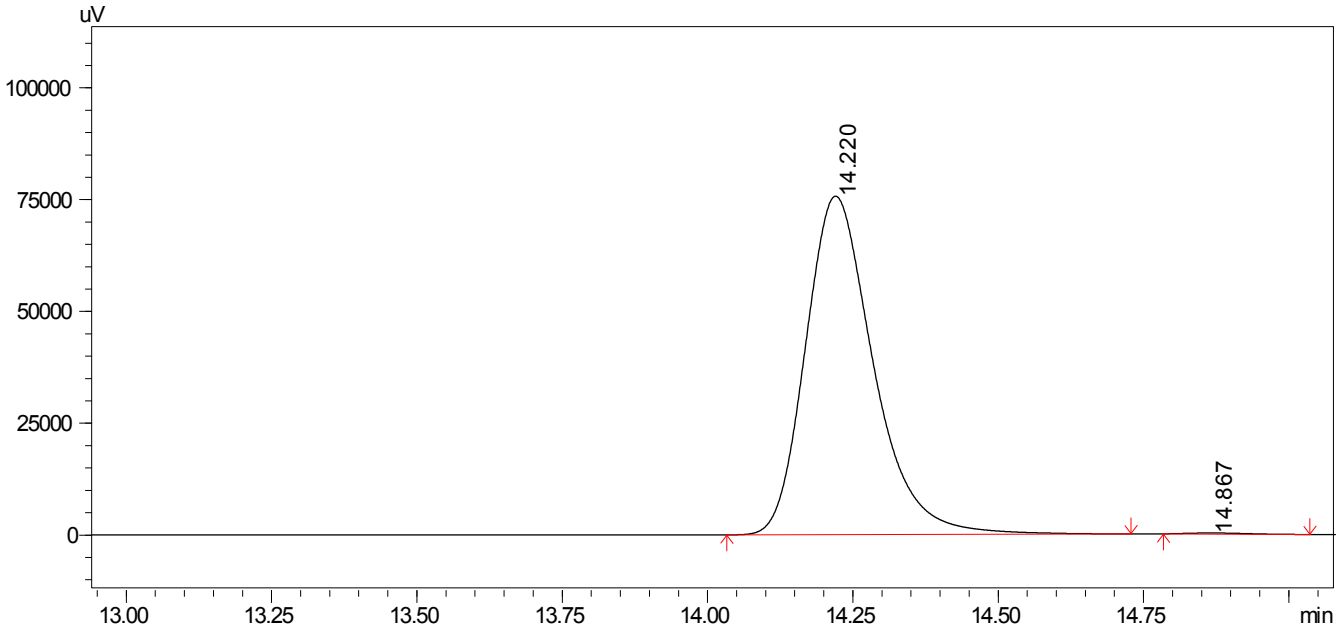

Figure S9. GC chromatogram of product (*R*)-**3a** yielded by *Gk*AmDH.

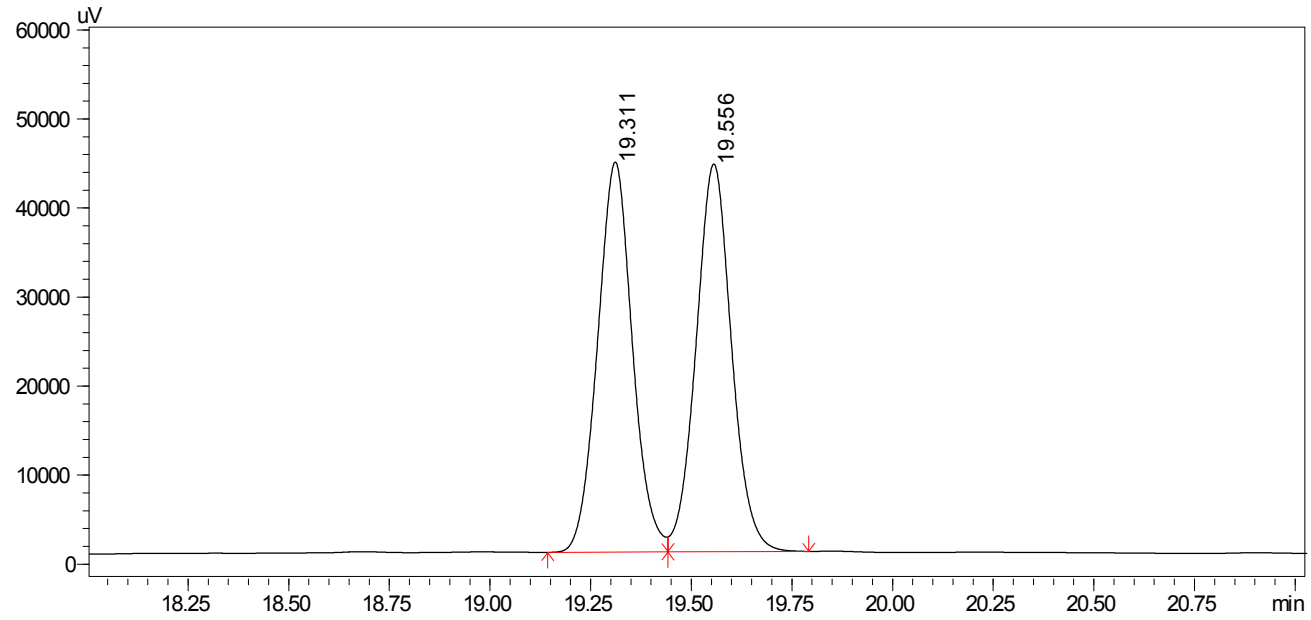

Figure S10. GC chromatogram of racemic **4a**.

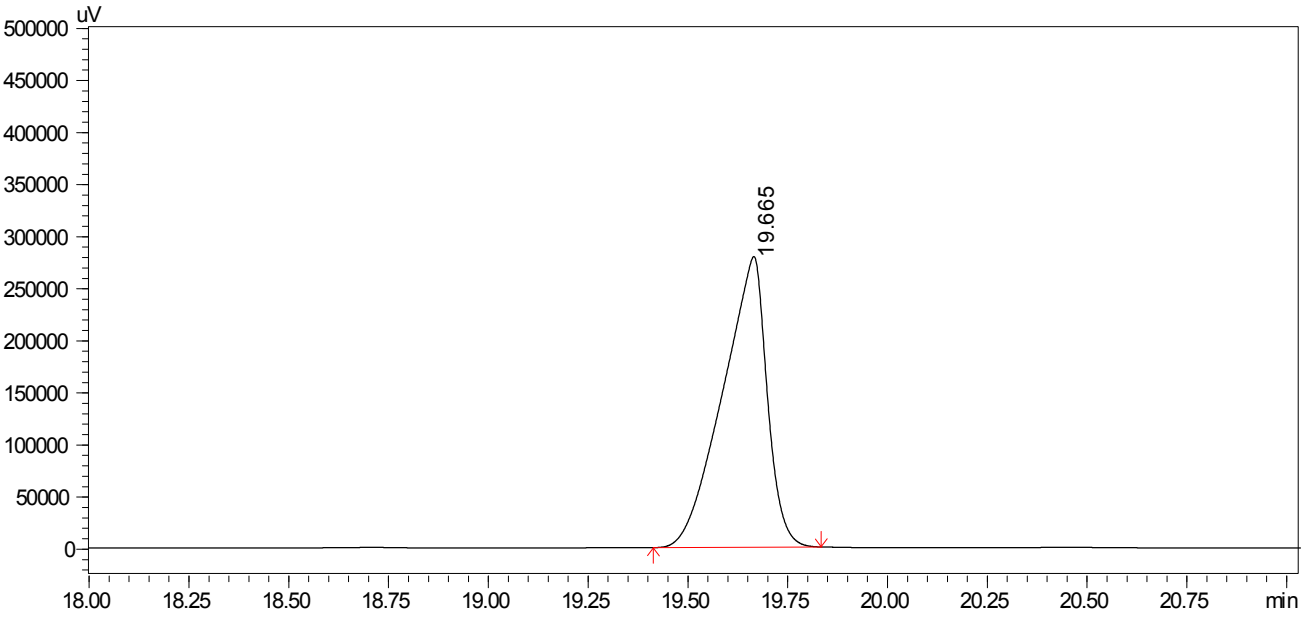

Figure S11. GC chromatogram of product (*R*)-**4a** yielded by *Gk*AmDH.

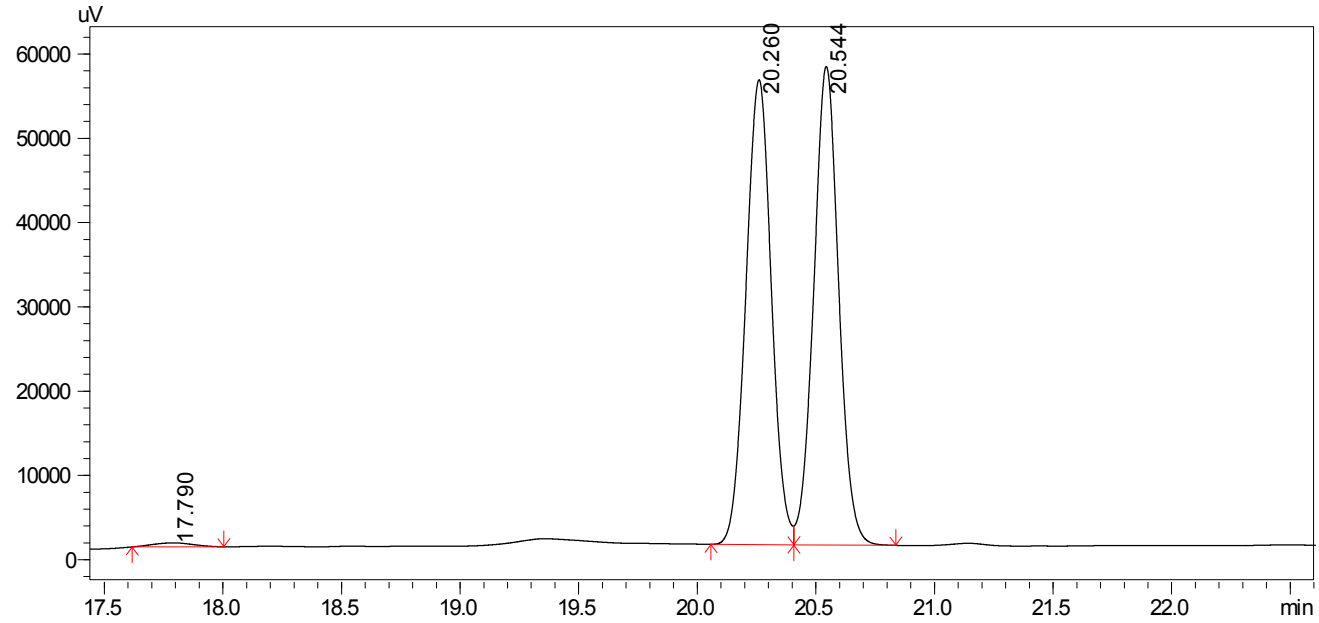

Figure S12. GC chromatogram of racemic **5a**.

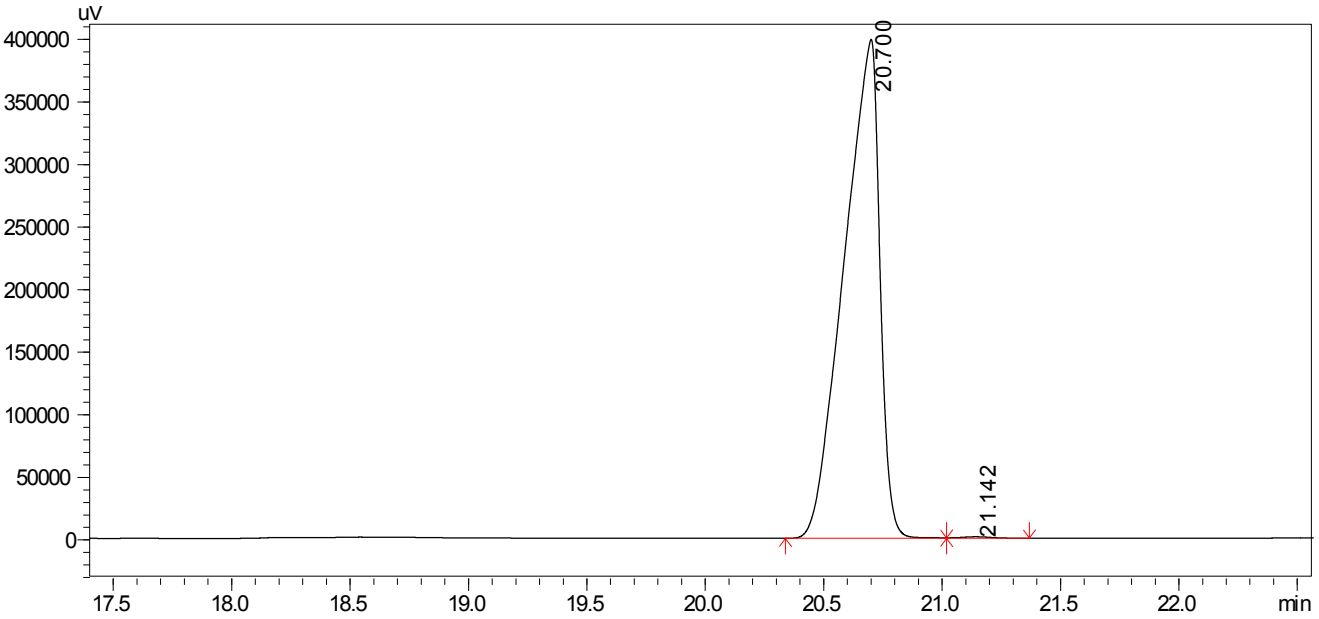

Figure S13. GC chromatogram of product (*R*)-**5a** yielded by *Gk*AmDH.

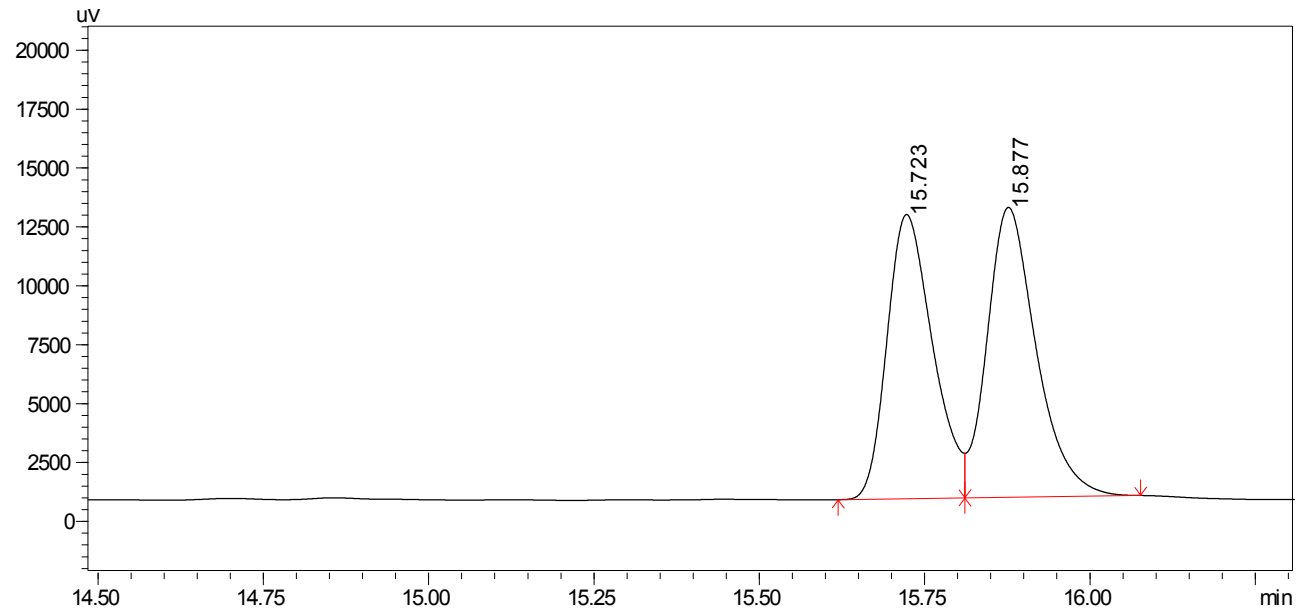

Figure S14. GC chromatogram of racemic **6a**.

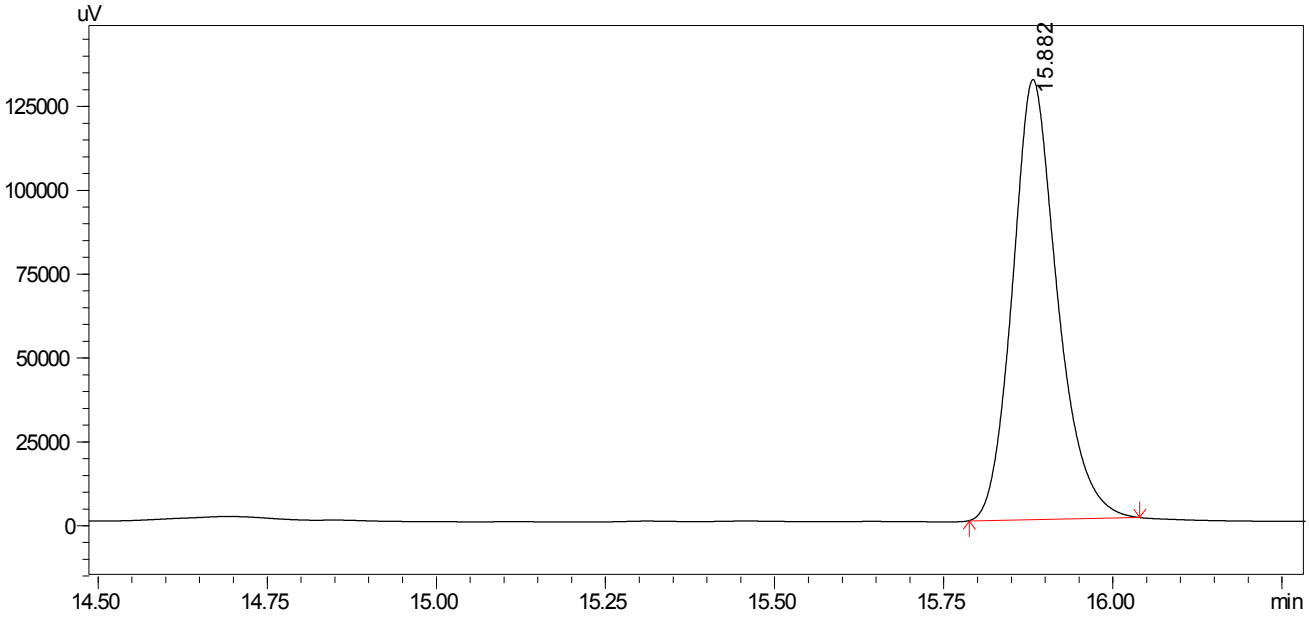

Figure S15. GC chromatogram of product (*R*)-**6a** yielded by *Gk*AmDH.

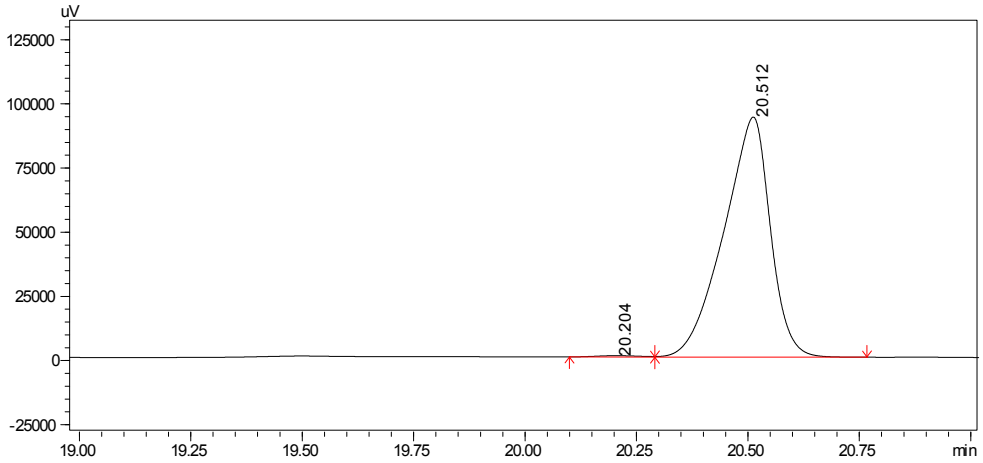

Figure S16. GC chromatogram of standard (*R*)-**7a**.

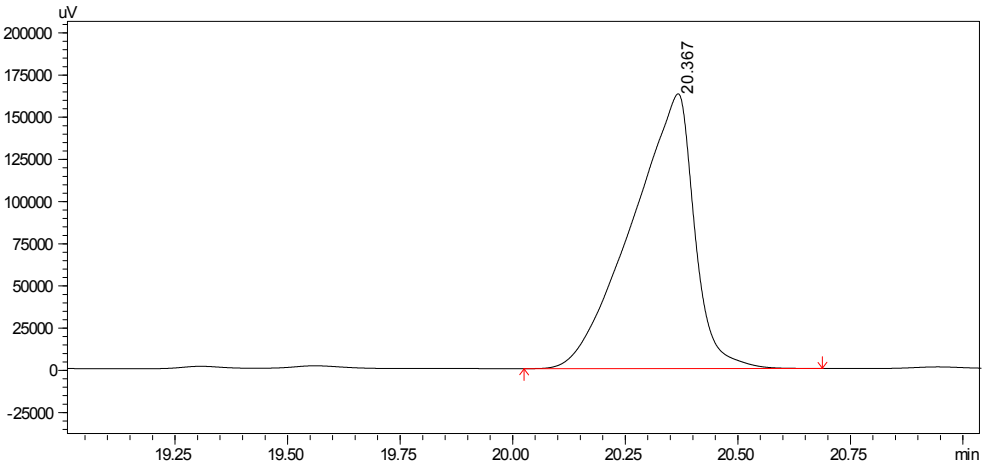

Figure S17. GC chromatogram of standard (*S*)-**7a**.

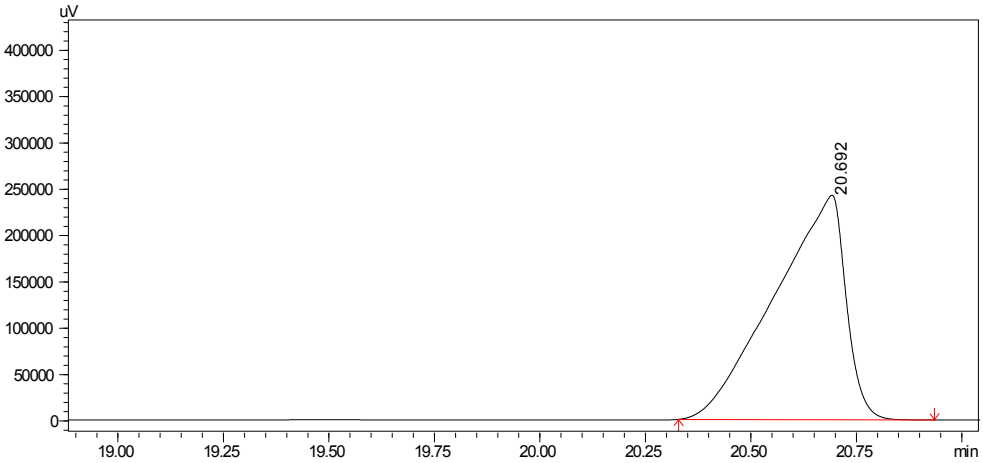

Figure S18. GC chromatogram of product (*R*)-**7a** yielded by *Gk*AmDH.

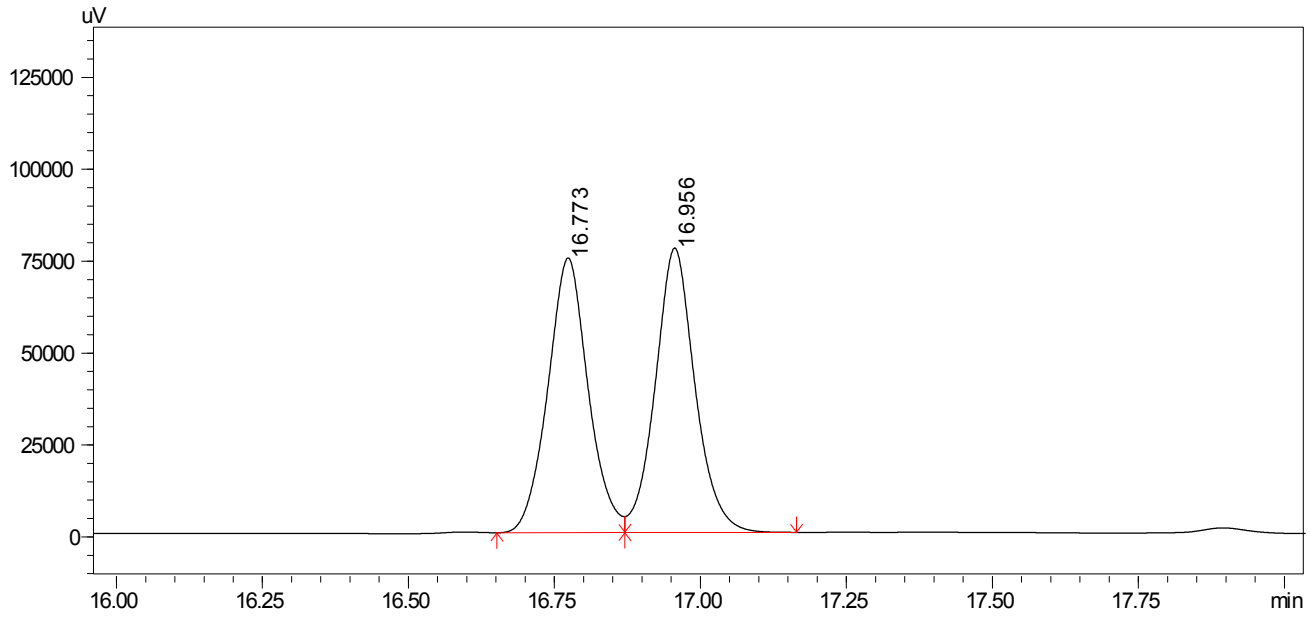

Figure S19. GC chromatogram of racemic **8a**.

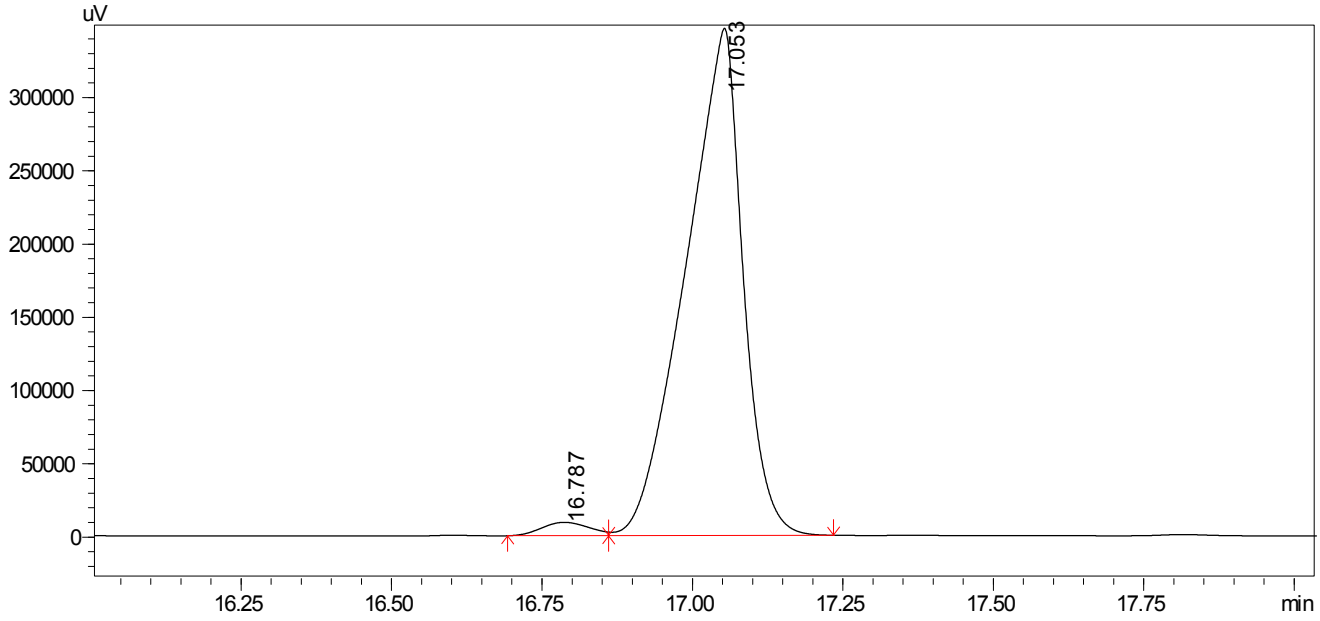

Figure S20. GC chromatogram of product (*R*)-**8a** yielded by *Gk*AmDH.

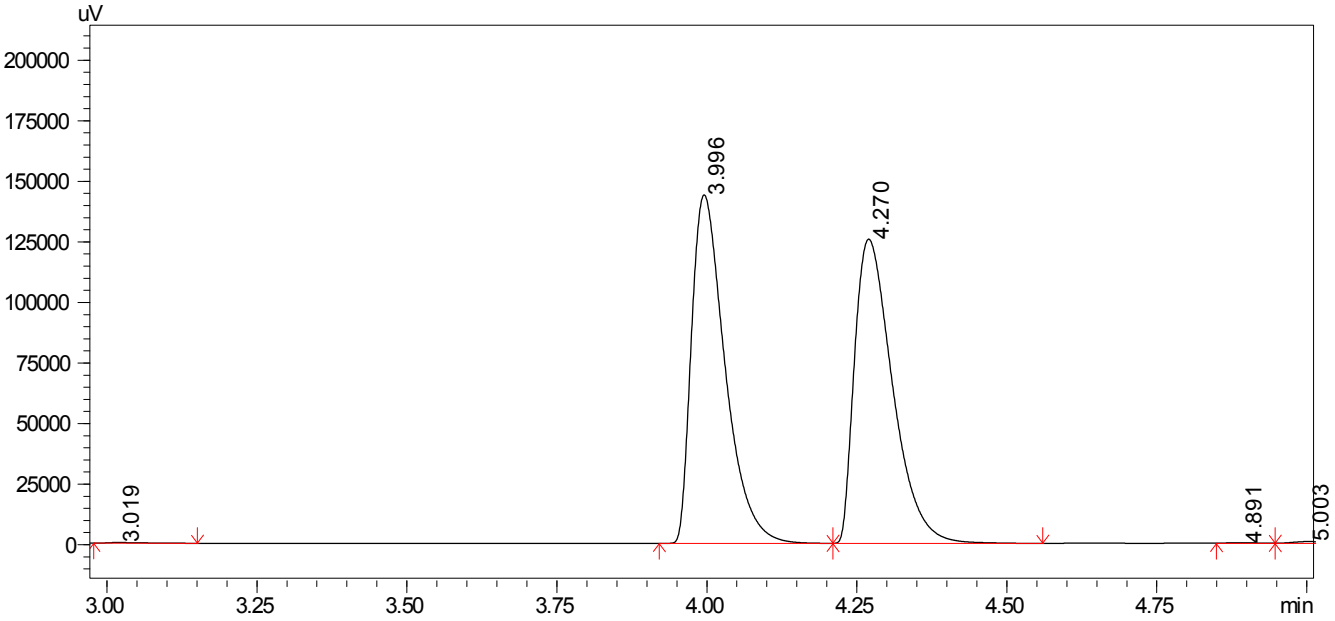

Figure S21. GC chromatogram of racemic **9a**.

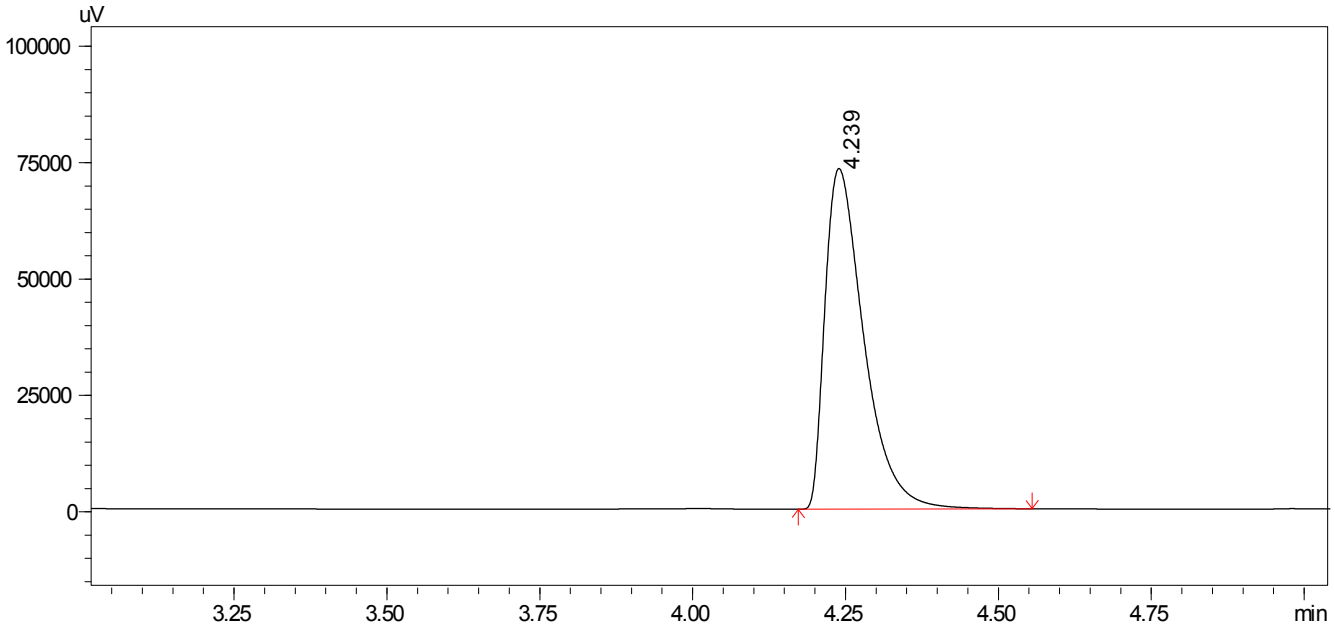

Figure S22. GC chromatogram of product (*R*)-**9a** yielded by *Gk*AmDH.

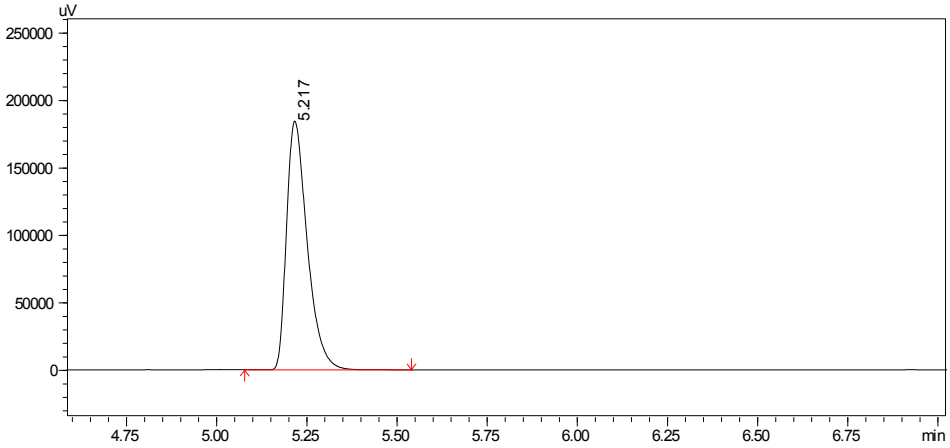

Figure S23. GC chromatogram of standard (*S*)-**10a**.

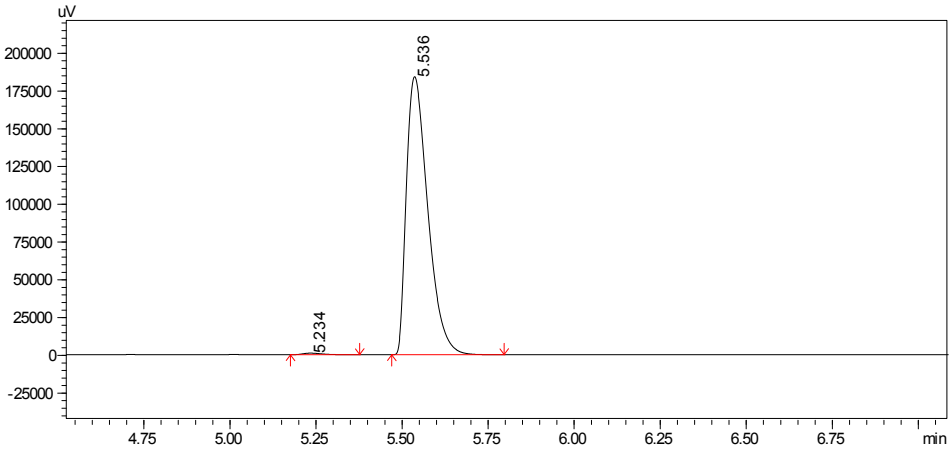

Figure S24. GC chromatogram of standard (*R*)-**10a**.

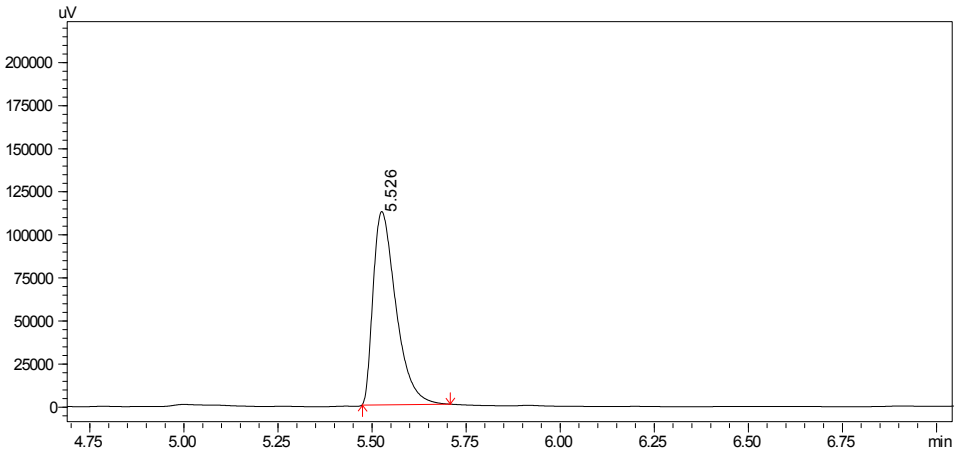

Figure S25. GC chromatogram of product (*R*)-**10a** yielded by *Gk*AmDH.

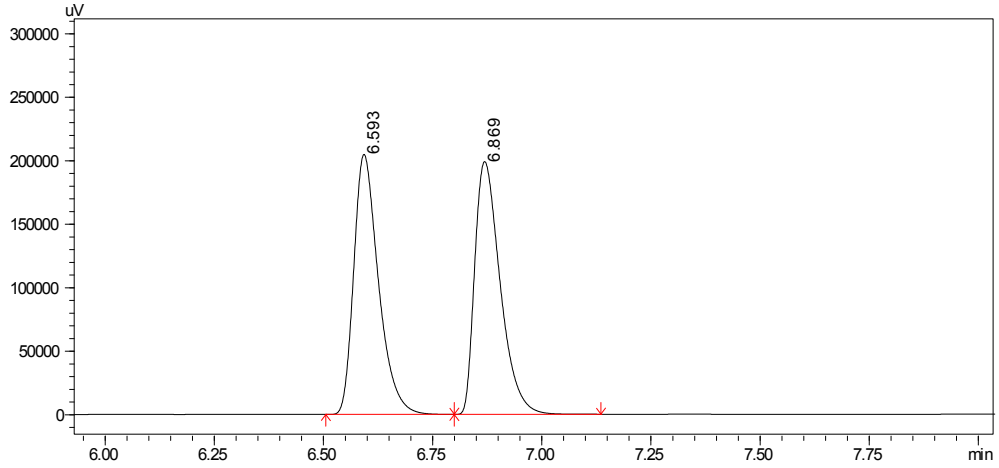

Figure S26. GC chromatogram of standard racemic **11a**.

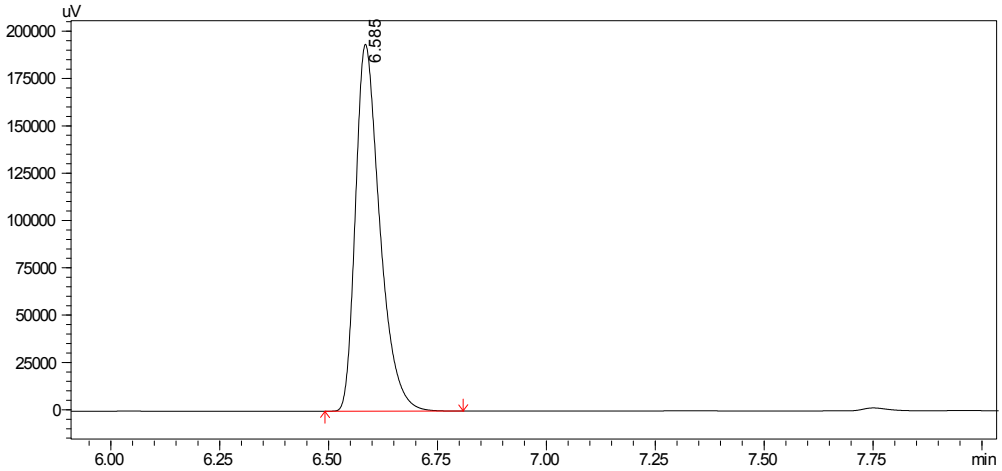

Figure S27.GC chromatogram of standard (*S*)-**11a**.

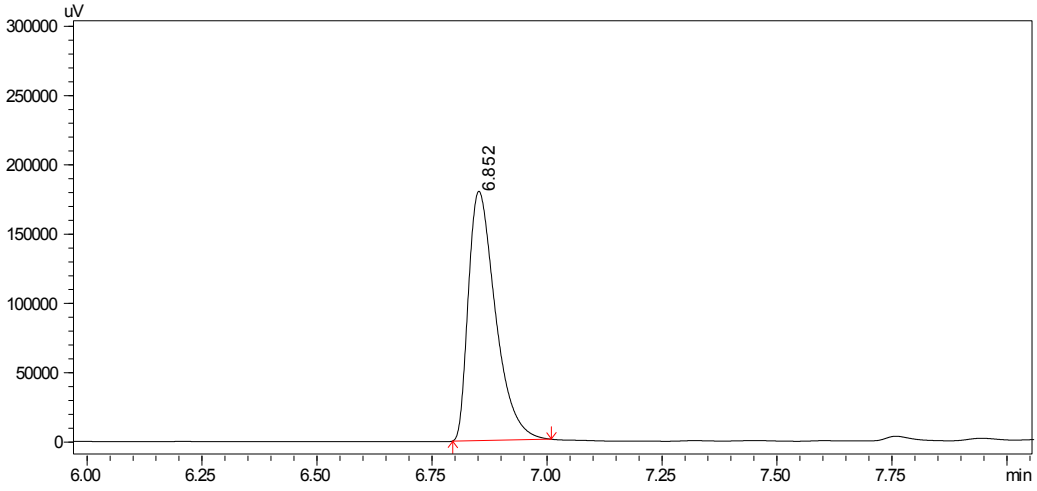

Figure S28. GC chromatogram of product (*R*)-**11a** yielded by *Gk*AmDH.

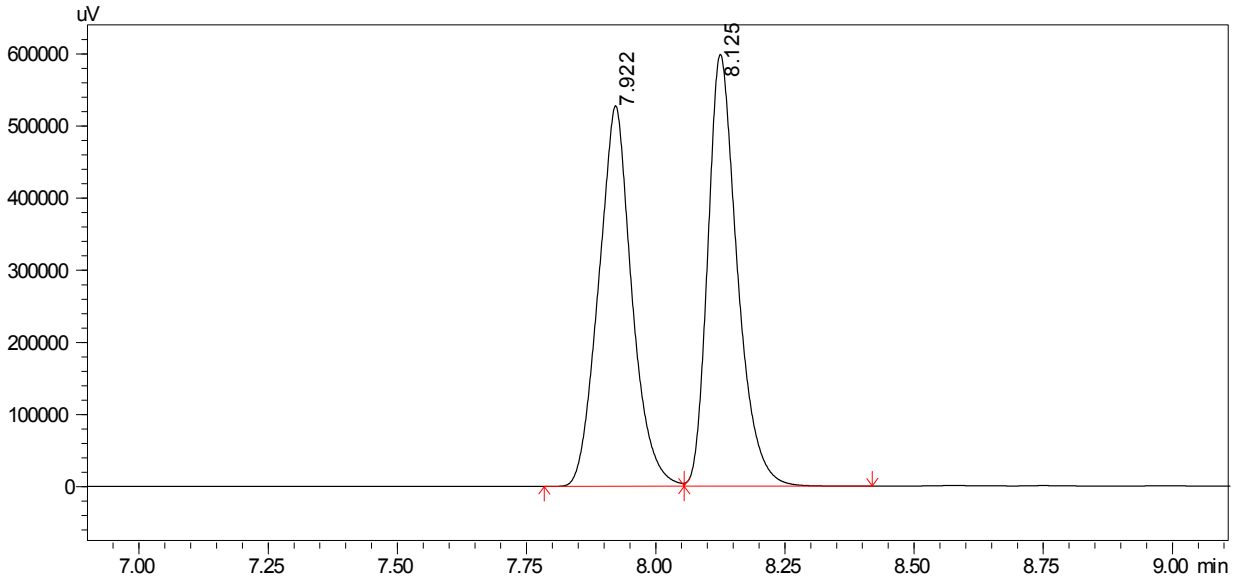

Figure S29. GC chromatogram of standard racemic **12a**.

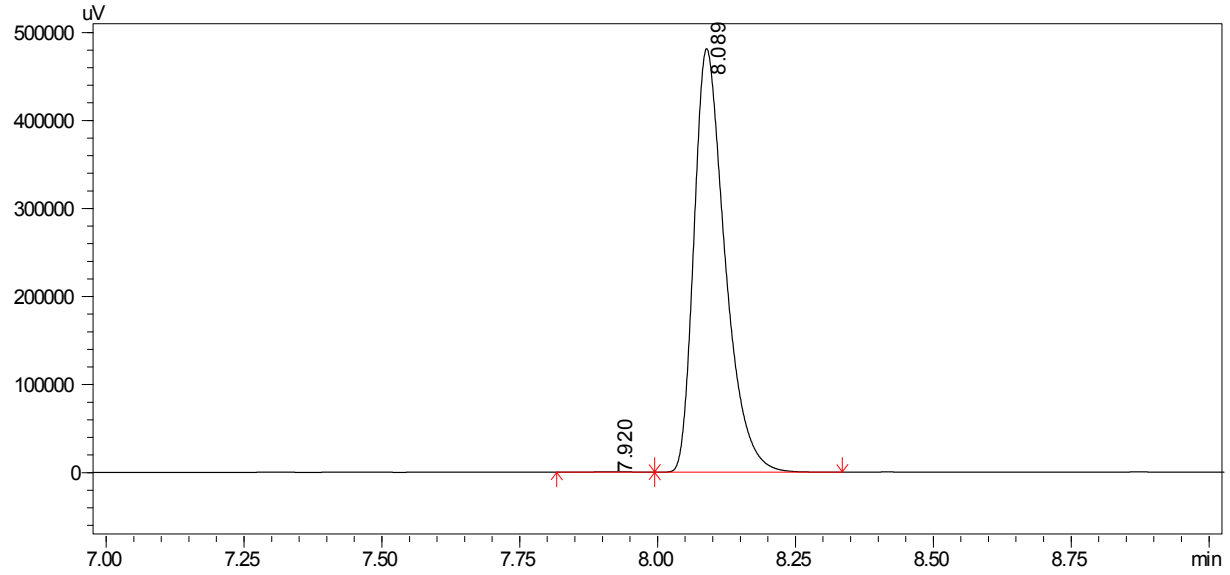

Figure S30. GC chromatogram of product (*R*)-**12a** yielded by *Gk*AmDH.

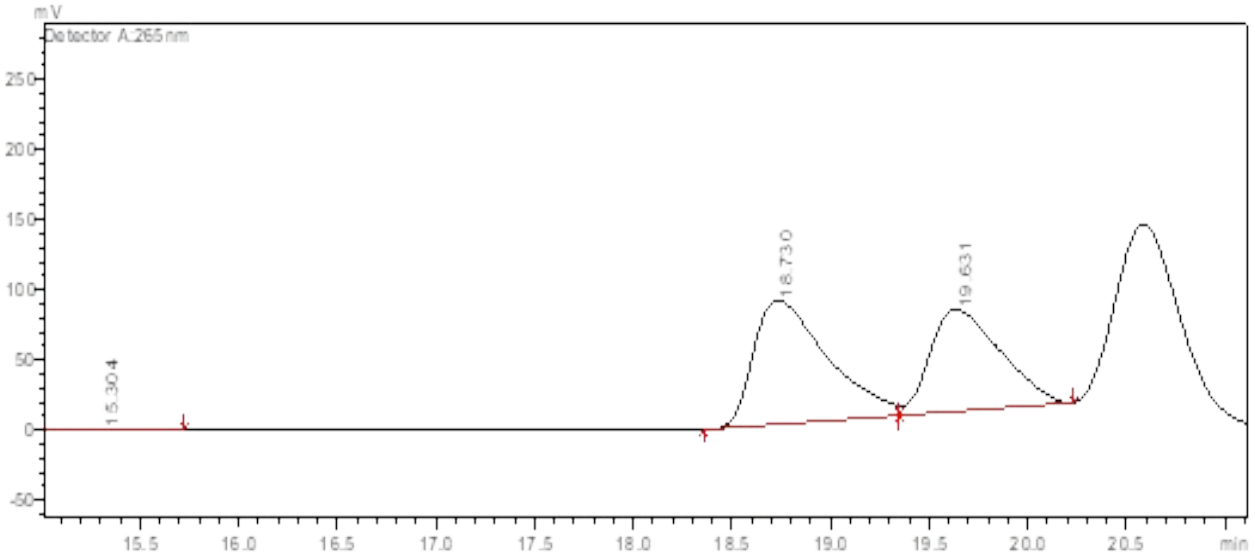

Figure S31. HPLC chromatogram of standard racemic **1b**.

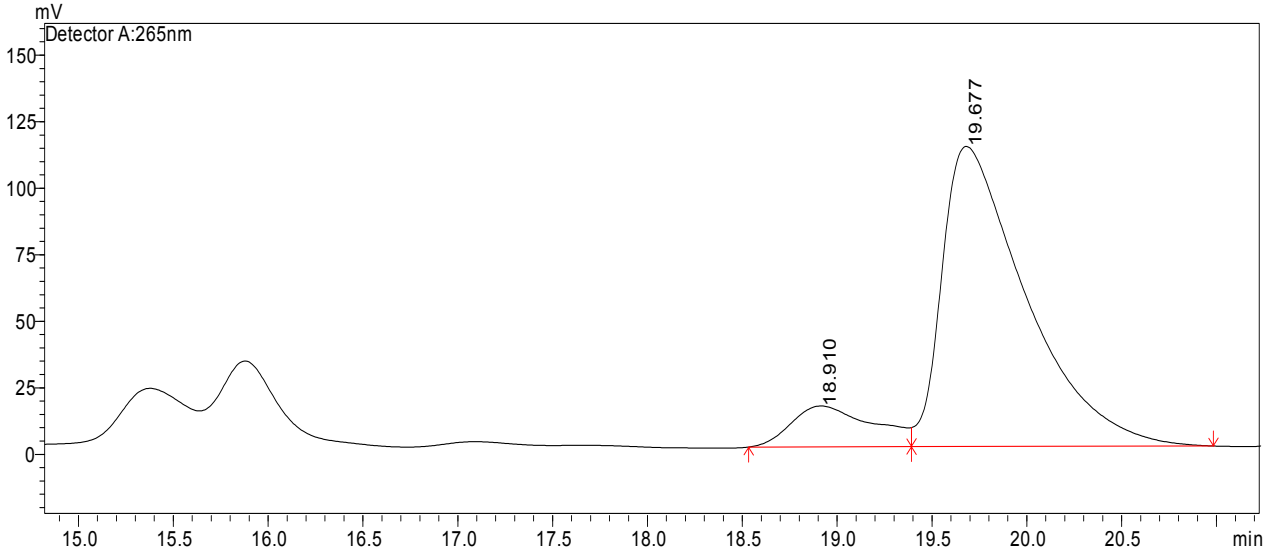

Figure S32. HPLC chromatogram of product **1b** yielded by *Gk*AmDH.

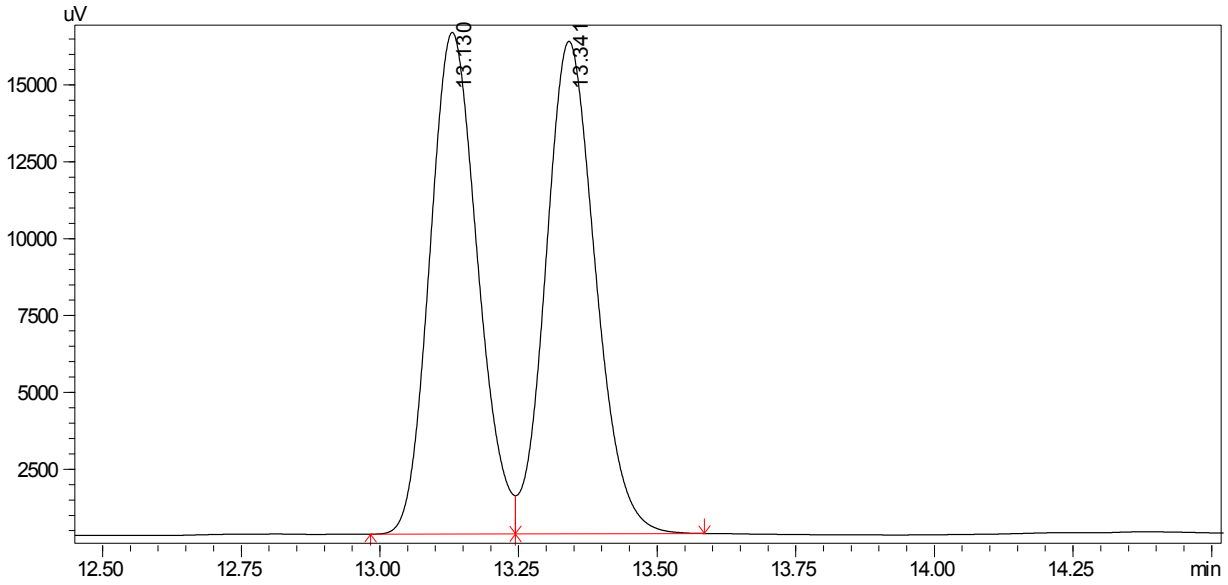

Figure S33. GC chromatogram of standard racemic **1c**.

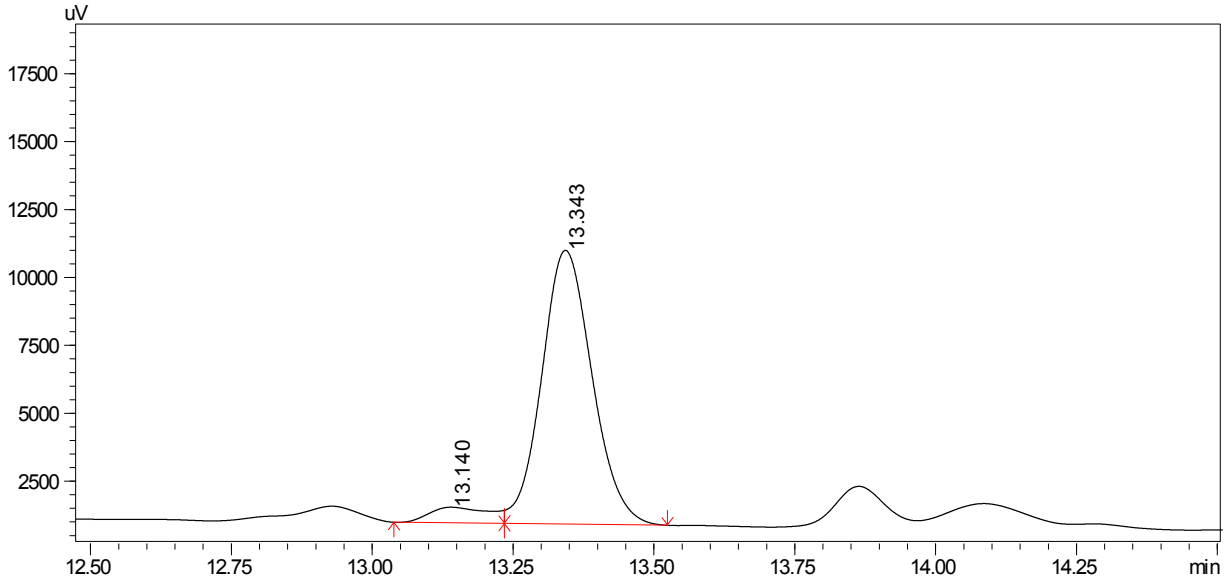

Figure S34. GC chromatogram of product **1c** yielded by *Gk*AmDH.

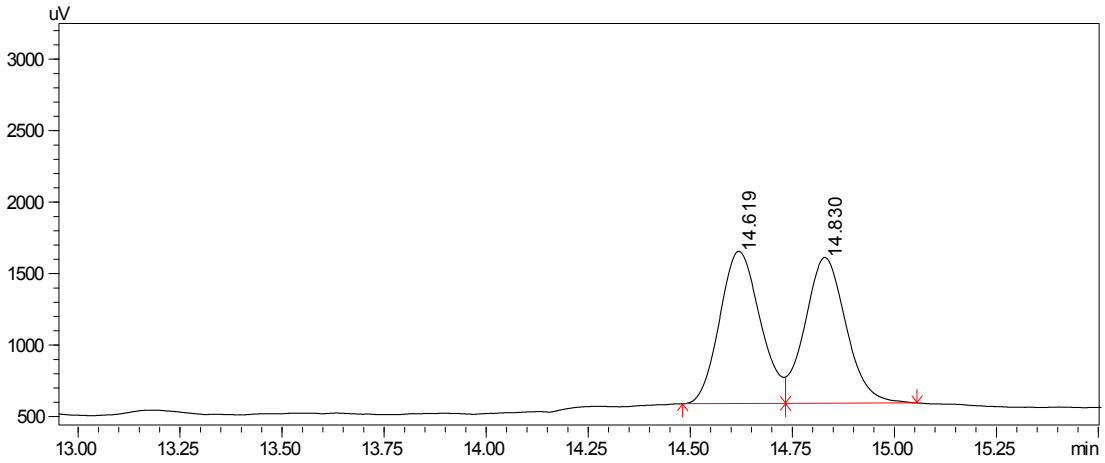

Figure S35. GC chromatogram of standard racemic **1d**.

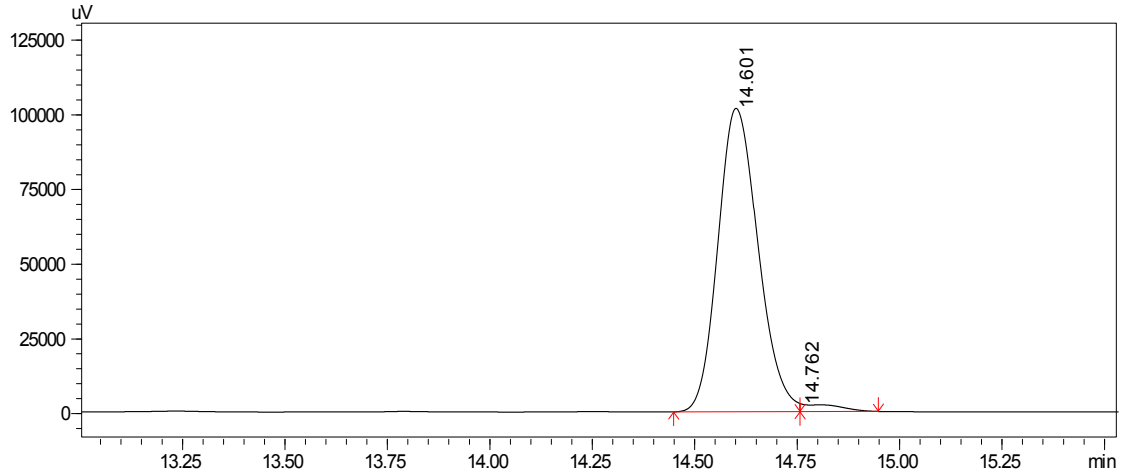

Figure S36. GC chromatogram of product **1d** yielded by *Asp*RedAm.

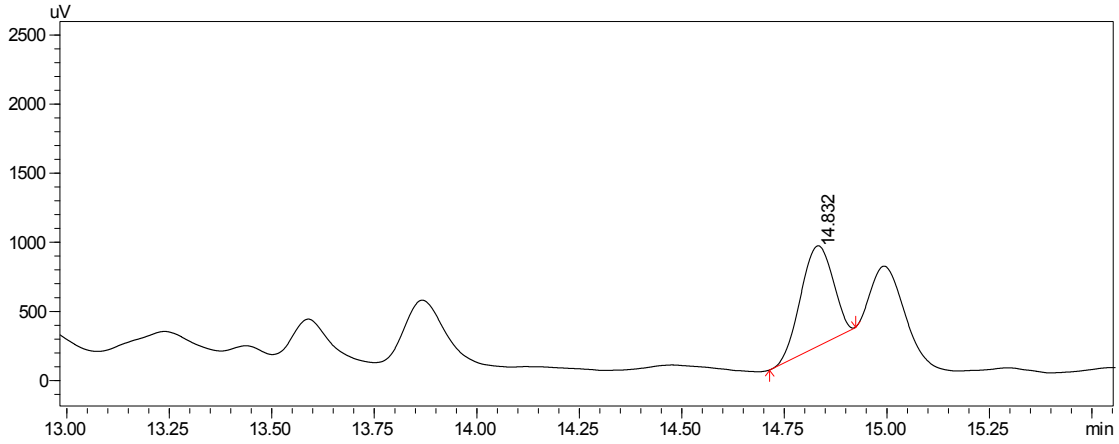

Figure S37. GC chromatogram of product **1d** yielded by *Gk*AmDH.

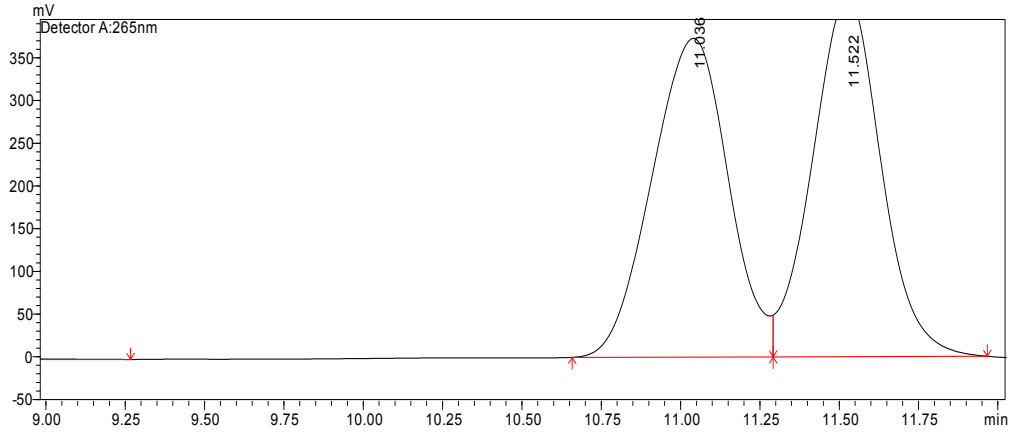

Figure S38. HPLC chromatogram of standard racemic **1e**.

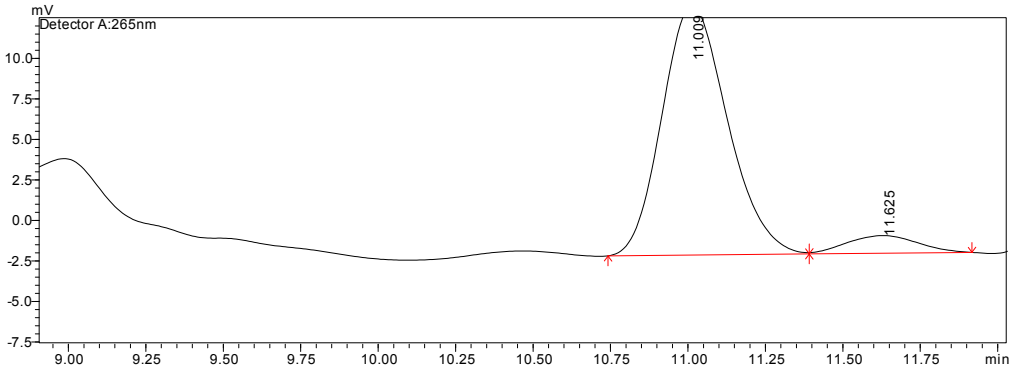

Figure S39. HPLC chromatogram of product **1e** yielded by *Gk*AmDH.

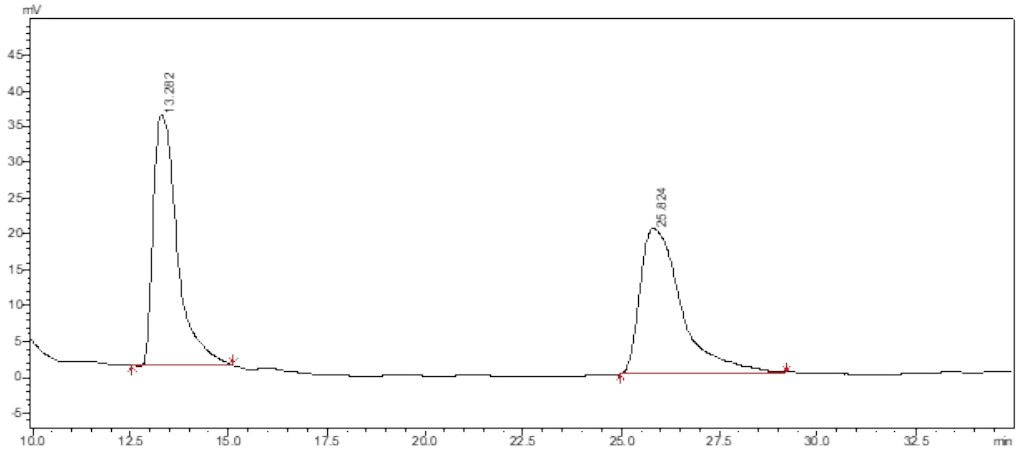

Figure S40. HPLC chromatogram of standard racemic **14a**

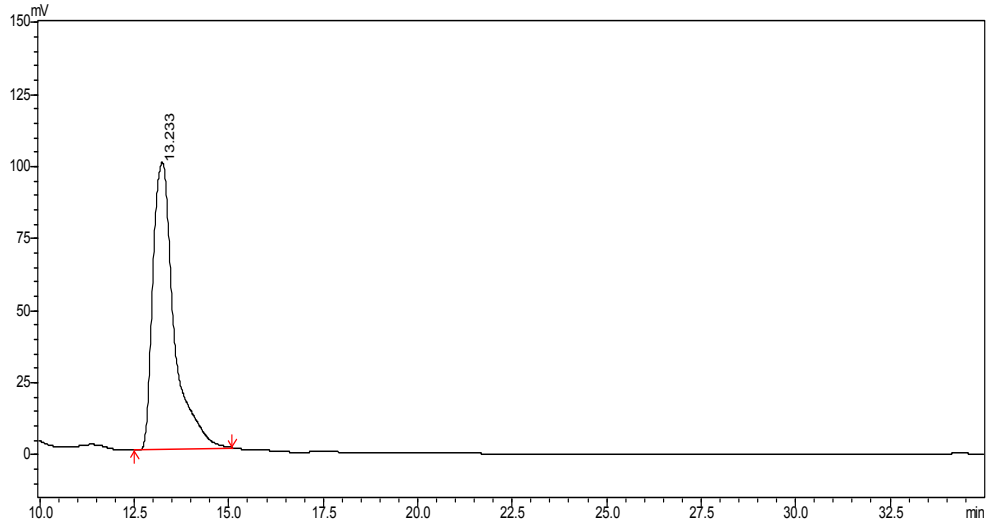

Figure S41. HPLC chromatogram of standard (*S*)-**14a**

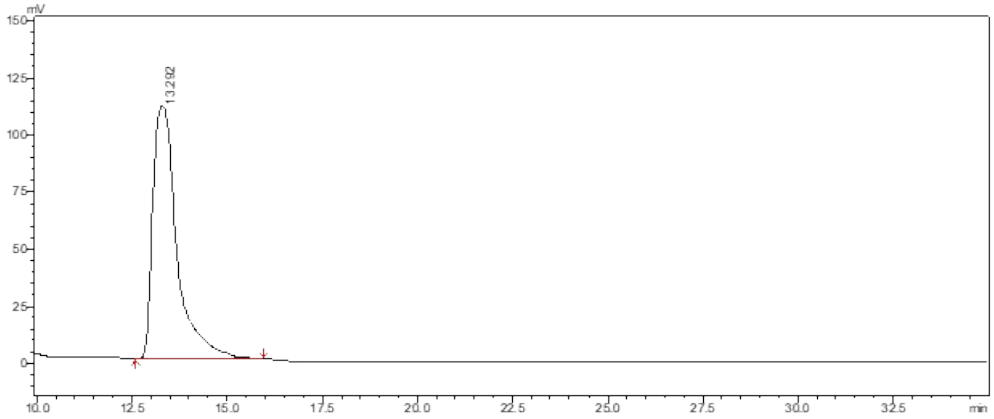

Figure S42. HPLC chromatogram of product (*S*)-**14a** yielded by *Gk*AmDH.

NMR Spectra

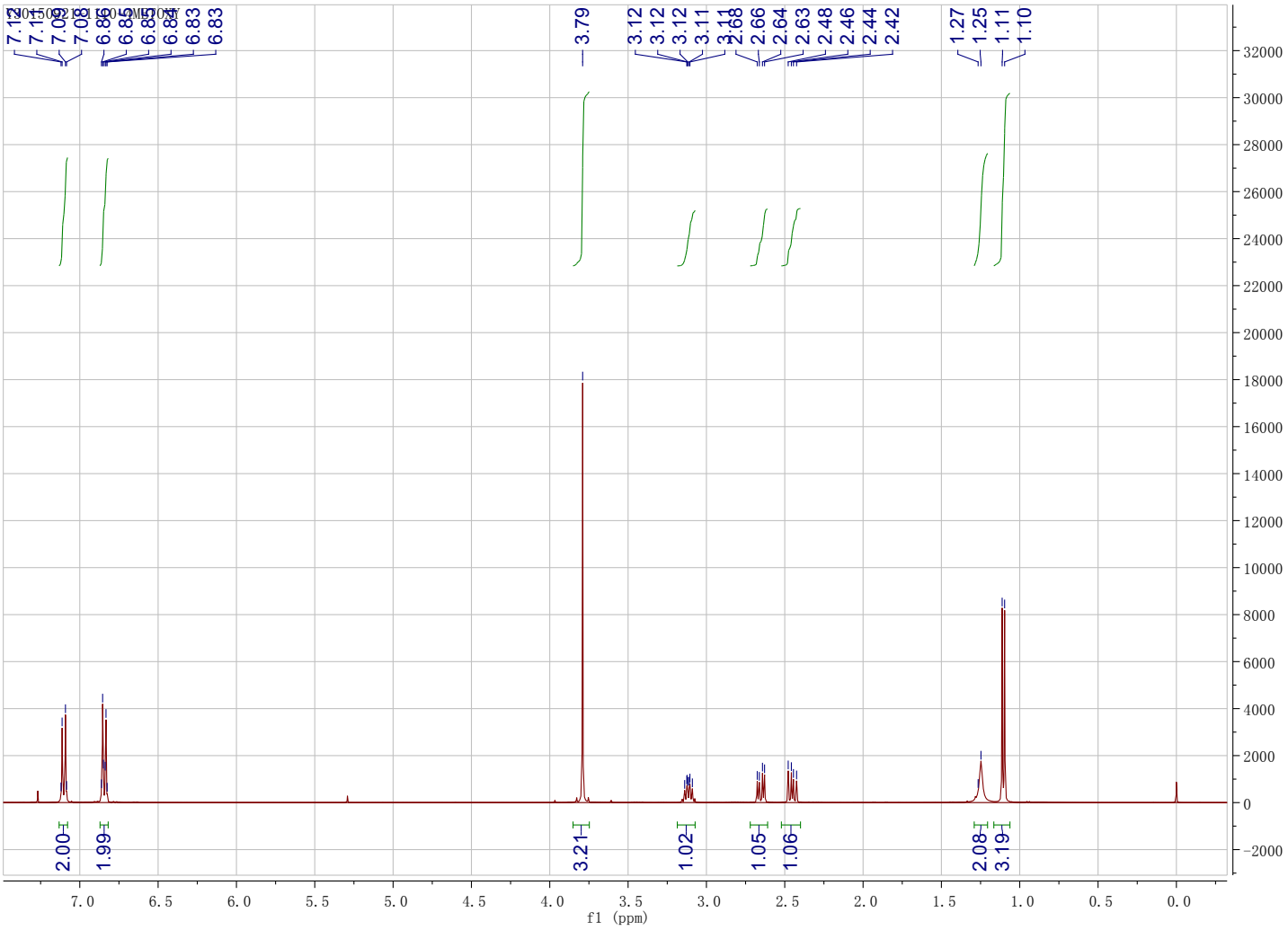

Figure S43. ^{1}H NMR spectrum of (*R*)-**7a** yielded by *Gk*AmDH.

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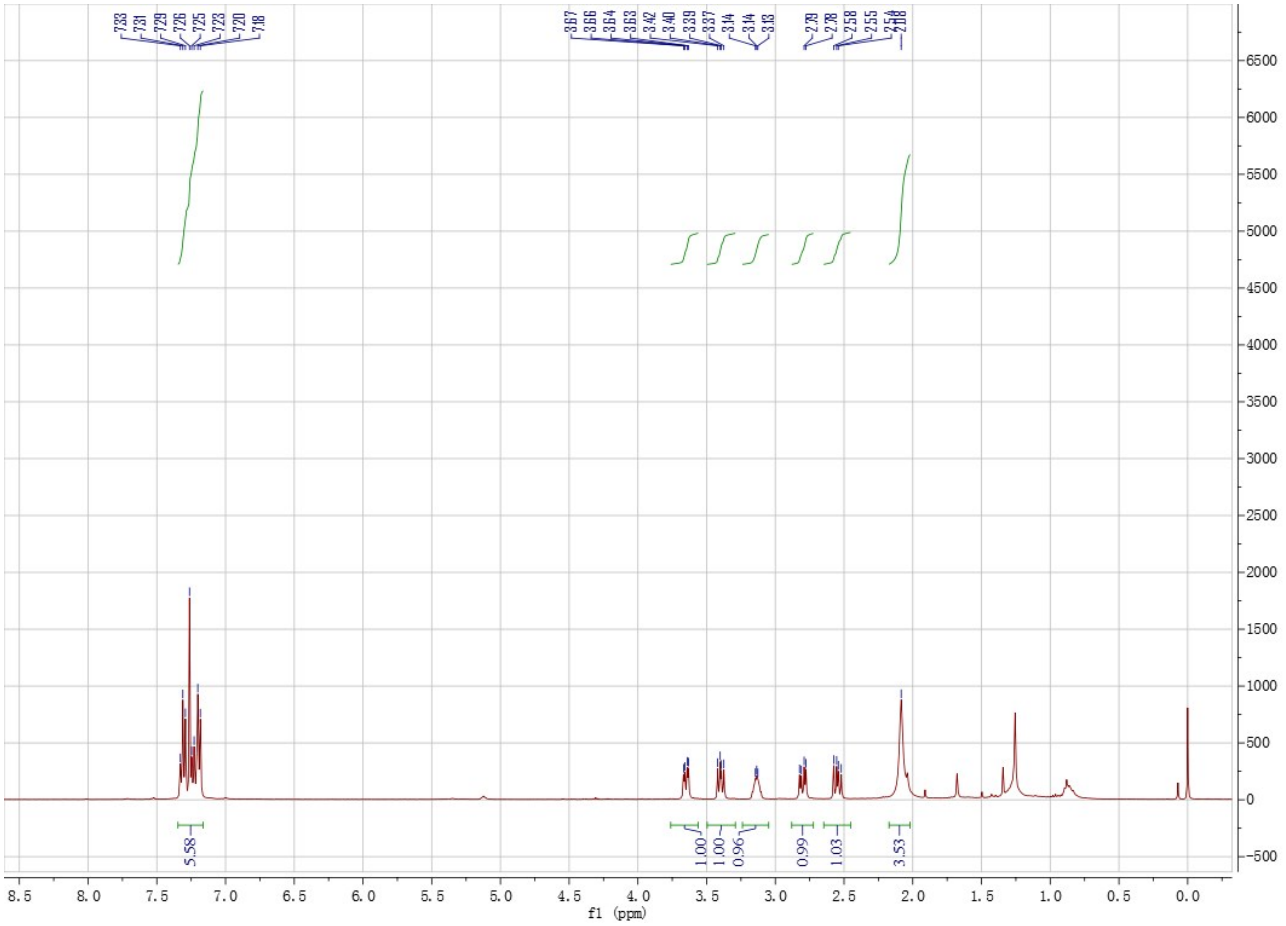

Figure S44. ^{1}H NMR spectrum of (*S*)-**14a** yielded by *Gk*AmDH.

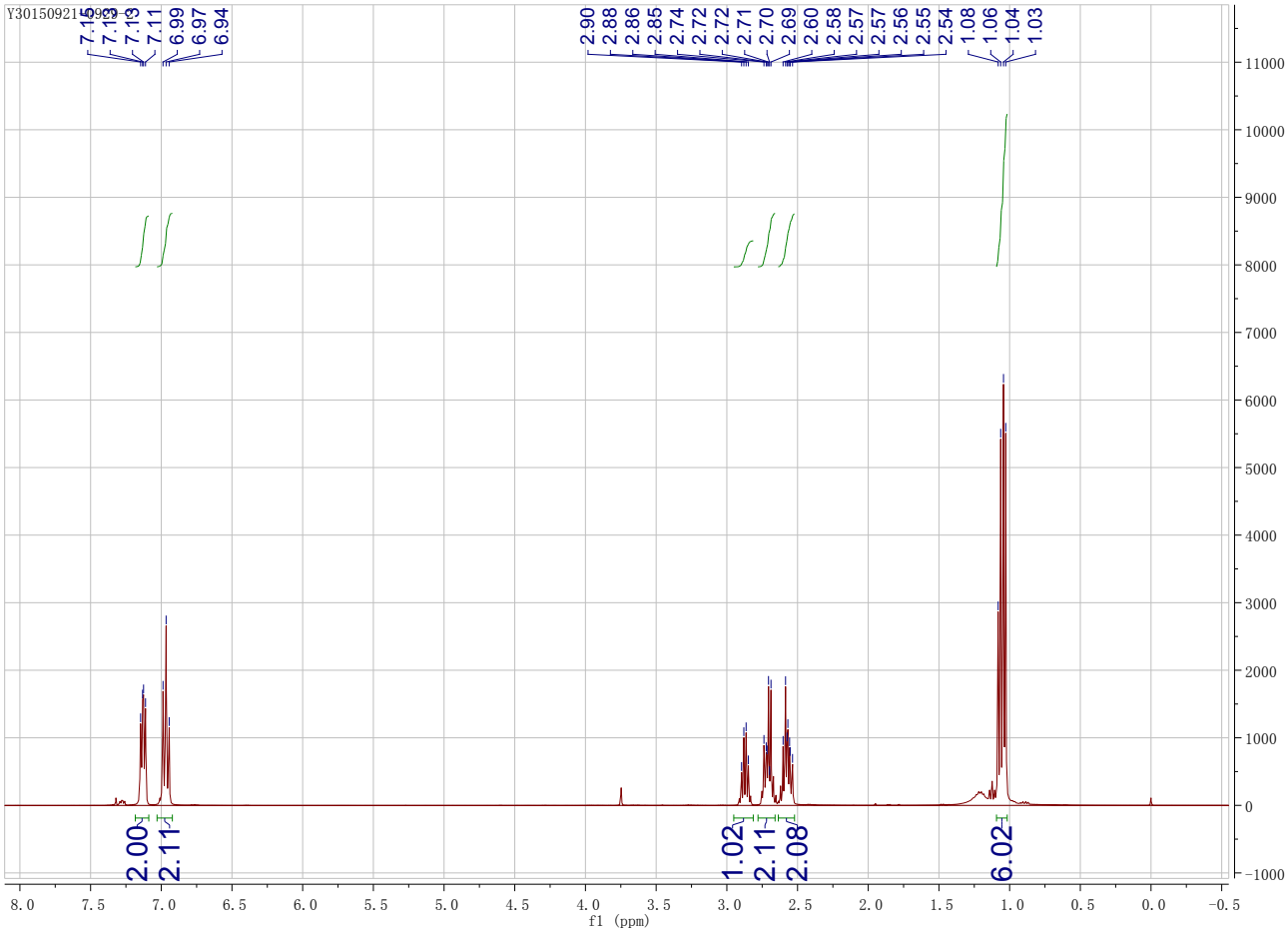

Figure S45. ^{1}H NMR spectrum of **1b** yielded by *Gk*AmDH.

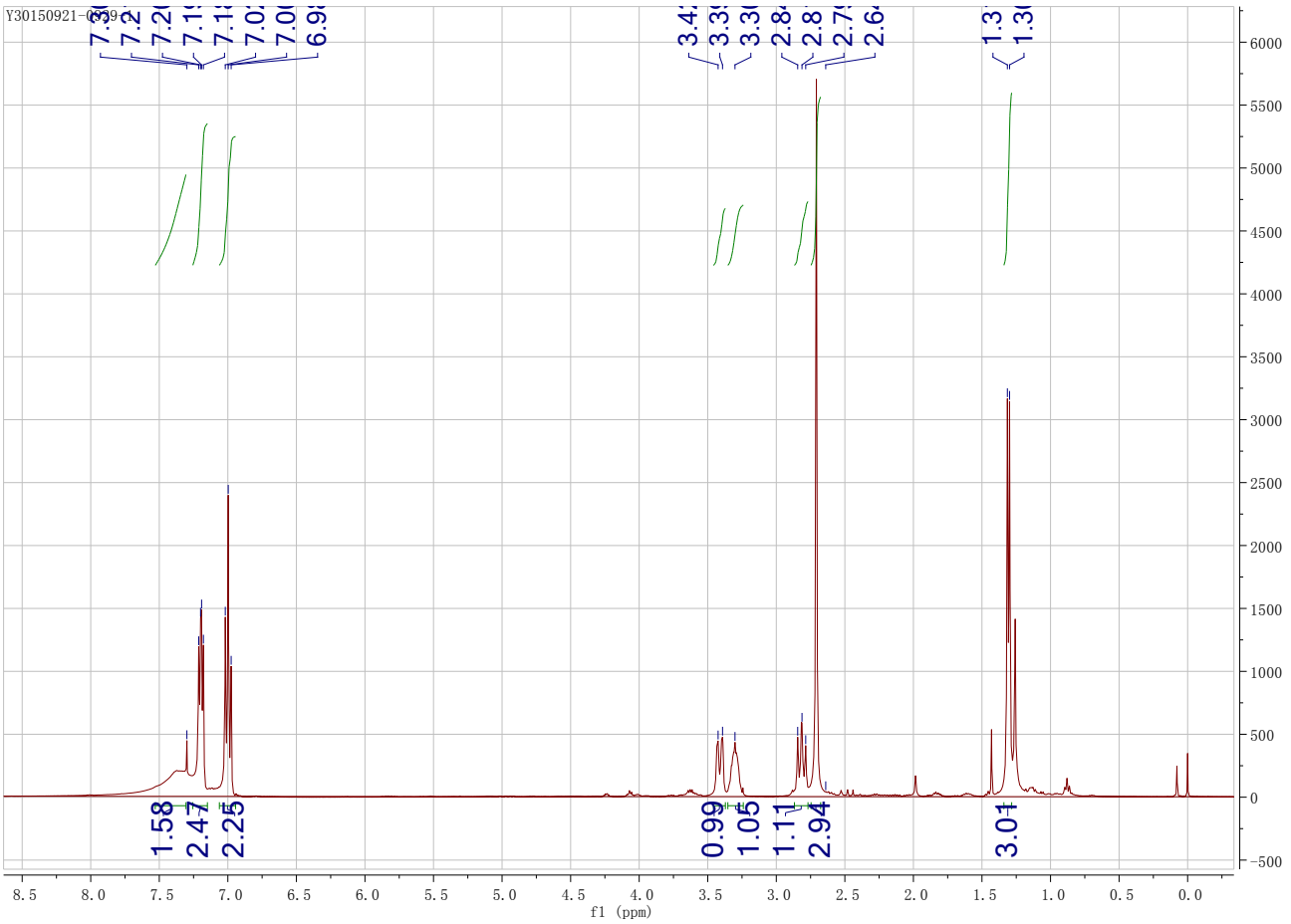

Figure S46. ^{1}H NMR spectrum of **1c** yielded by *Gk*AmDH.

N-ethyl-4-fluoroamphetamine (**1c**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18–7.09 (m, 2H), 6.97 (dd, *J* = 12.1, 5.3 Hz, 2H), 2.87 (dd, *J* = 12.9, 6.4 Hz, 1H), 2.78 – 2.66 (m, 2H), 2.64 – 2.52 (m, 2H), 1.05 (dd, *J* = 14.8, 6.8 Hz, 6H).

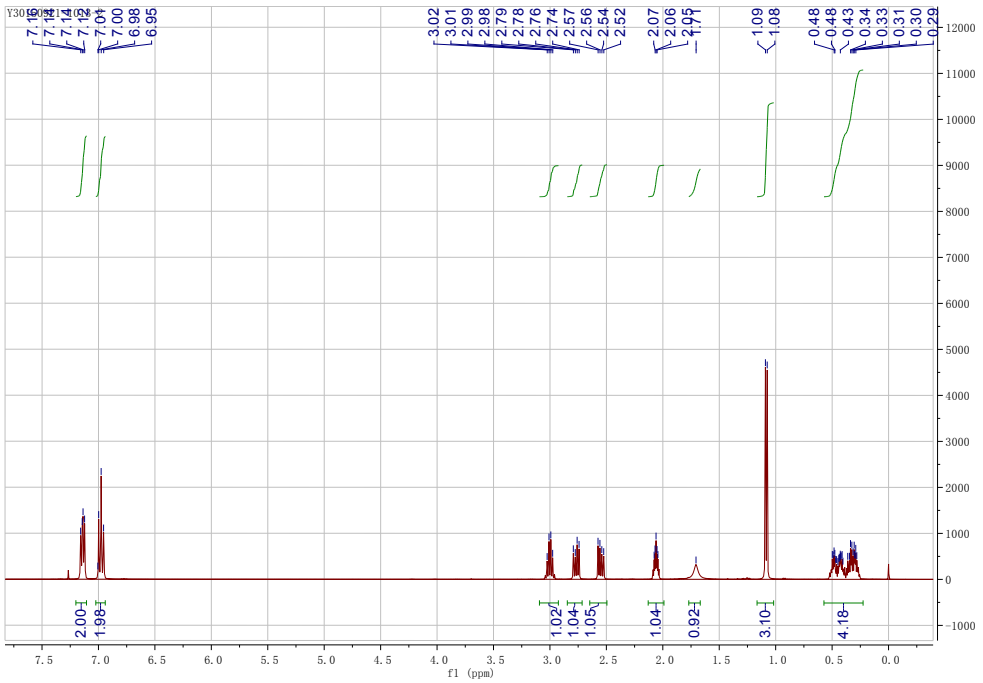

Figure S47. ^{1}H NMR spectrum of **1e** yielded by *Gk*AmDH.

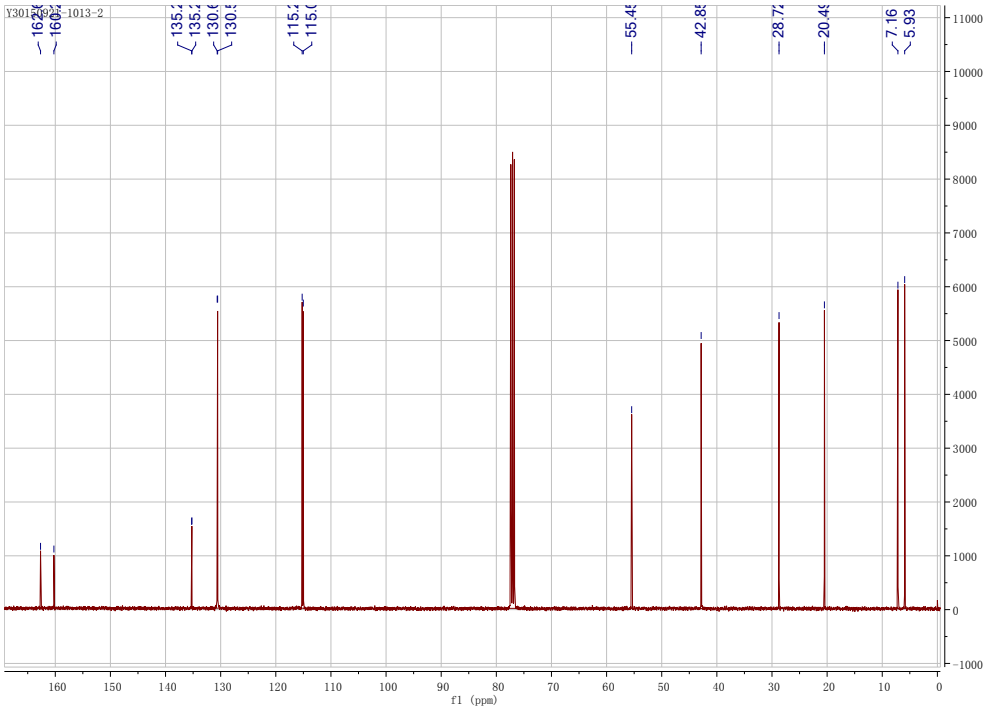

Figure S48. ^{13}C NMR spectrum of **1e** yielded by *Gk*AmDH.

N-cyclopropyl-4-fluoroamphetamine (**1e**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.02–6.94 (m, 2H), 3.00 (h, *J* = 6.5 Hz, 1H), 2.77 (dd, *J* = 13.5, 6.8 Hz, 1H), 2.55 (dd, *J* = 13.5, 6.8 Hz, 1H), 2.13–1.99 (m, 1H), 1.74 (d, *J* = 28.1 Hz, 1H), 1.08 (d, *J* = 6.3 Hz, 3H), 0.57–0.23 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.67, 160.25, 135.26, 135.22, 130.63, 130.55, 115.22, 115.01, 55.45, 42.85, 28.72, 20.49, 7.16, 5.93. MS: m/z 194 [M^+], HRMS calculated. for $C_{12}H_{17}FN^+$ [M+H]$^+$: 194.1341, found 194.1346.

Reference

1 L. Munoz, A. M. Rodriguez, G. Rosell, M. P. Bosch, A Guerrero, *Organ. Biomol. Chem.*, 2011, **9**, 8171.

2 G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan, N. J. Turner, *Nat. Chem.* 2017, **9**, 961.

3 M. Abrahamson, J. E. Vázquez-Figureueroa, N. B. Woodall, J. C. Moore, A. S. Bommarius, *Angew. Chem. Int. Ed.,* 2012, **51**, 3969; *Angew. Chem.* 2012, **124**, 4036.

4 N. J. Turner, G. Aleku, J. Mangas-Sanchez, J. Citoler, S. Frances, S. Montgomery, M. Thompson, R. Health, *ChemCatChem,* 2017, **10**, 515.

5 J. C.-H. Yim, J. A. Bexrud, R. O. Ayinla, D. C. Leitch, L. L. Schafer, *J. Org. Chem.* 2014, **79**, 2015.