Supporting Information for

Development of β-Amino Acid Dehydrogenase for the Synthesis of β-Amino Acids via Reductive Amination of β-Keto Acids

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Experimental Section

Construction of pET21b-DAHDHcca. The codon-optimized 3,5-DAHDHcca gene from *Candidatus Cloacamonas acidaminovorans* was synthesized with a 6×His-tag coding sequence at the N-terminus, subcloned into the pET-21b(+) vector between the *Nde* I and *Hin*d III restriction sites, and expressed in *E. coli* BL21(DE3) host cells.

Mutant library preparation. All mutant libraries were created with the template plasmid pET21b containing the 3,5-DAHDHcca gene and pairwise primers in Table S1 (Chart S1 in Supporting Information). A primer pair (F-com / UP-Rev primer or DOWN-For primer / R-com) used to create a pool of DNA fragments with diversity at the corresponding sites was added in a 50 μL reaction mixture containing 500 ng of templates, 0.2 mM each dNTPs, 5 μL of KOD buffer and 2.5 U KOD-plus DNA polymerase. After denaturation for 3 min at 94 °C, the PCRs were run for 30 cycles of 94 °C, 30 s; 55 °C,30 s; 68 °C, 30 s; with a final extension step at 68 °C for 5 min. PCR products were purified with magnetic beads method kit (GenMagBio, Beijing). A mutagenesis library was created with mega primer PCR of whole plasmids as follows: 0.5 μg of mega primer (purified DNA fragments), 50 ng of template plasmids, 0.5 mM each dNTP and 2.5 U of KOD DNA polymerase. After denaturation for 5 min at 94 °C, the PCRs was run for 18cycles (94°C,30 s; 55°C, 30 s; 68°C, 7 min); with a final extension step at 68 °C for 10 min. Finally, The PCR products were digested with *Dpn*I restriction enzyme at 37 °C for 2 hours. After isolation with magnetic beads method kit, *E. coli* BL21(DE3) competent cells were transformed with the purified plasmids through electroporation.

Screening of mutant libraries. Randomly selected 180 colonies of each library along with 8 parent colonies were grown in 150 μL of Luria-Bertani (LB) medium containing 100 μg·mL^{-1} ampicillin at 37 °C for 12 h in two 96-well microplates. All cultures were duplicated into the new 96-well microplates carrying 300 μL of LB medium with 100 μg·mL^{-1} ampicillin and incubated at 37°C with shaking at 200 rpm for 6 hours. The original plates were stored. After induction with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.1 mM) and incubation at 25°C for 12 hours, the cultures were centrifuged at 4000 rpm and 4 °C for 30 min and the supernatants were discarded. Each cell pellets was resuspended in 50 μL of potassium phosphate buffer (100 mM, pH 8.0) appended 4mg·mL^{-1} Lysozyme. The cells were disrupted by freezing at −80°C for 2 hour and by heat shocking at 45°C for 3 min, and then incubated for 30 min at 37 °C. The cell lysates were precipitated by centrifugation at 4000 rpm and 4 °C for 30 min. Five μL of each supernatant were transferred into 96-well microplates for the activity assays by spectrophotometrically monitoring the absorbance increase of NADPH (0.5 mM) at 340 nm with a mixture of β-amino acid substrates containing each 5 mM of (*S*)-β-aminobutyric acid ((*S*)-β-AB), β-Ala, (*R*)-β-homoSer, (*R*)-β-homoMet, (*S*)-β-homoGln, (*R*)-β-Phe and (*S*)-β-homoLys dissolved in potassium phosphate buffer (100 mM, pH 8.0). The colonies which showed activity higher than the parent enzyme were selected and confirmed by further studies (Chart S1).

Site-directed mutation. Site-directed mutagenesis was performed with the primers in Table S2 in Supporting Information by utilizing the method as described in mutant library preparation.

Expression and purification of enzymes. The *E. coli* BL21 (DE3) carrying pET21b-DAHDHcca or mutants were cultured in 600-mL LB broth supplemented with ampicillin (100 μg•mL^{-1}) at 37 °C until the

optical density reached 2.4-3.0 at 600 nm. The culture was induced by addition of IPTG (0.5 mM) and incubated at 25 °C for another 12 hours. The cell pellets were harvested by centrifugation at 4000 rpm at 4°C for 30 min, and washed once with phosphate buffer (50mM) containing 5% glycerin and 200 mM NaCl (pH 8.0). The cell lysate of 3,5-DAHDHcca and mutants, which were obtained via disruption with a High Pressure Homogenizer and centrifugation, were bound to the Ni-NTA resins, and the purification was performed on an AKTA purifier 10 system (GE Healthcare). The protein-bound resin was eluted with phosphate buffer (50 mM, pH 8.0) containing 5% of glycerin, 200 mM of NaCl and 30 mM of imidazole, and then with the same buffer but containing 500 mM of imidazole. After imidazole was removed through tubular ultrafiltration modules, the target proteins were concentrated with a Ultra Centrifugal Filter (MERCK Millipore) in phosphate buffer (50 mM, pH 8.0) containing 5% of glycerin and 200 mM of NaCl, and stored at -4°C. The protein concentration was determined with BCA protein assay kit (CWBIO, China).

The small quantity of His-tagged proteins were purified manually on a Ni-NTA column. Cell pellets were re-suspended in 50 mM phosphate buffer (pH 8) containing 5% glycerin, 200 mM NaCl and 20 mm imidazole, and sonicated. The cell debris was removed by centrifugation. The lysate was bound to the Ni-NTA resins for 30 min, and then washed twice with 50 mM phosphate buffers (pH 8) containing 5% glycerine, 200 mM NaCl and 30mM imidazole. The target protein was eluted with the same buffer solution but containing 120 mM imidazole, and collected.

Measurement of deamination activity and kinetic parameters. Oxidative deamination reaction was initiated by adding 3 μL of pure enzymes (the protein concentration was showed in Table S3 of Supporting Information) into Na_2CO_3-$NaHCO_3$ buffer (100 mM, pH 9.5) containing 1 mM of $NADP^+$ and 20 mM of β-amino acid within 96-well transparent plates in a final reaction volume of 200 μL at 30°C. For wild-type enzyme toward (3*S*,5*S*)-DAH, protein with 1 : 50 dilution was used. The reaction rate was measured by spectrophotometrically monitoring the generation of NADPH at 340 nm on a Spectramax M2e instrument (Molecular Devices, USA). One unit was defined as the amount of enzyme producing 1 μmol NADPH per minute with a molar absorption coefficient of 6.22 $mM^{-1}\cdot cm^{-1}$.

Kinetic constant measurement for oxidative deamination was performed at 30°C with Na_2CO_3-$NaHCO_3$ buffer (100 mM, pH 9.5) containing final concentration 0.5 mM of $NADP^+$ in a reaction volume of 200 μL within 96-well plates. For (3*S*,5*S*)-DAH, the substrate concentration was in the range of 0. 625 to 40 mM, and 300 μg of E310G, E310G/A314Y, E310S or E310S/A314N or 5 μg of A314Y, A314N or wild-type enzyme was used. The concentration of (*R*)-β-homoMet varied from 0.625 to 26 mM and 300 μg of β-AADH enzymes were used. One mg of enzyme were used for (*S*)-β-AB with substrate concentration from 0.625 to 26mM and (*R*)-β-Phe with substrate concentration in the range of 0.625 to 40 mM. All the experiments were conducted with three replicates. The K_m and V_{max} values were calculated by nonlinear fitting.

Biotransformation of β-keto acids to β-amino acids. β-keto acids were prepared by hydrolyzing the corresponding β-keto esters (100 mM) by using lipase Novozyme 435 or the corresponding β-keto nitriles (100 mM) with nitrilase NIT6402 in 1 mL phosphate buffer (50 mM, pH 8.0) at 37°C for 8 hours. The resulting β-keto acid solution (100 μL) and 30 μL of pure enzymes (the protein concentration was showed in Table S3 of Supporting Information) were mixed in 870 μL of Na_2CO_3-$NaHCO_3$ buffer (100 mM, pH 9.5) containing 200 mM NH_4Cl, 0.5 mg of NADPH, 12.5 mg of glucose and 1 mg of freeze-dried

GDH. The reaction mixture was incubated at 37°C and 200 rpm for 12 hours. The reaction mixture (100 μL) was mixed with 100 μL of 1 M Na_2CO_3, 1 mL of 0.5 M $NaHCO_3$ and 400 μL of 2,4-dinitrofluorobenzene (DNFB) solution (5 mM) in ethanol, and the resulting mixture was kept at 65°C for 1 hour. After centrifugation and filtration, the resulting solution was subjected to HPLC analysis on an Agilent 1200 series system with an Eclipse XDB-C18 column (4.6 × 150 mm, Agilent, USA) using isocratic elution of aqueous acetic acid solution (50 mM) / methanol (45/55, v/v) at 1 mL/min. The product derivatives were characterized by LC-MS analysis. Chiral analysis of the products was conducted as follows: The reaction mixture (200 μL) was mixed with 80 μL of 1 M $NaHCO_3$ and 400 μL of N-α-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide (FDAA) solution (4 mg/mL) in acetone. The resulting mixture was heated at 40 °C for 1 hour, then 40 μL of 2 N HCl solution was added to quench the reaction. The obtained solution was subjected to HPLC analysis on an Agilent 1200 series system with an Eclipse XDB-C18 column (4.6 × 150 mm, Agilent, USA) at 340 nm using isocratic elution of aqueous phosphoric acid-triethylamine solution (50 mM, pH 3.0) / acetonitrile [70/30 (v/v) for β-homoMet and β-Phe, and 75/25 (v/v) for β-AB] at 0.6 mL/min.

Preparation of β-amino acids by mutant β-AADHs. A solution of 1.5 M 5-(methylthio)-3-oxopentanenitrile dissolved in 2 mL DMSO was added to 23 mL phosphate buffer (50 mM, pH 8.0) containing 50 mg freeze-dried nitrilase NIT6402 and shaken at 30°C. After 8 hours, the reaction mixture was adjusted to pH 9.5 with Na_2CO_3, and then 400 mg β-AADH mutant E310G/A314Y, 260 mg NH_4Cl, 10 mg $NADP^+$, 990 mg of glucose and 25 mg freeze-dried GDH were added. The reaction mixture was continually shaken at 30°C and 200 rpm for 48 hours. The pH of the reaction mixture was adjusted to about 3.0 and the product was isolated with strong acidic cation exchange resin chromatography. (*R*)-β-homoMet was obtained as pale yellow solid (74.8 mg, 16.7% yield). $[\alpha]_D$ = + 13.5 (*c* 0.41, H_2O), standard sample (R)-β-homoMet $[\alpha]_D$ = + 14.7 (*c* 0.38, H_2O). The structure was confirmed by ^1H NMR (Figure S12).

A solution of 0.75 M ethyl benzoylacetate or ethyl 3-ketobutanoate dissolved in 4 mL DMSO were added to 21 mL phosphate buffer (50 mM, pH 8.0) containing 50 mg immobilized lipase Novozyme435 and shaken at 30°C. After 8 hours, the same procedure as described above was followed, except enzyme E310G was used for ethyl benzoylacetate. (*R*)-β-Phe (60.8 mg, 12.3% yield) and (*S*)-β-AB (69.2 mg, 22.1% yield) were obtained as white solid and the structures were confirmed by ^1H NMR (Figure S13, S14).

Preparation of 5-(methylthio)-3-oxopentanenitrile. To a solution of 1.6 M n-butyl lithium (56 mmol, 35 mL) in anhydrous THF(30 mL) was added anhydrous acetonitrile(2.38 g, 58 mmol) under a nitrogen atmosphere at -78℃. The reaction was stirred for 30 minutes at the same temperature. And ethyl 3-(methylthio)propanoate (5.51 g, 37.2 mmol) in anhydrous THF(30 mL) was slowly added dropwise over 30 minutes maintaining the temperature at -60℃. The reaction mixture was allowed to warm to -20℃, and stirred for 2 hours. The reaction mixture was quenched with 2 N hydrochloric acid (100 mL) and extracted with ethyl acetate (100 mL) twice. The combined organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by a silica gel column with ethyl acetate/petroleum ether (10:90) to give 5-(methylthio)-3-oxo pentane nitrile (2.80 g, yield 53%).[1]

Table S1. Primers used in the construction of mutagenesis libraries.

Up-Rev primers	
40/44-rev:	AGCGGAA TCAATAHNTA AACGAATAHN ATCAATCAGC A
41/45-rev:	GAA TCAATATTAHNACGAATAAC AHNAATCAGC ATTTCA
42/46-rev:	CAATATTTA AAHNAATAAC ATCAHNCAGC ATTTCATTG
43/47-rev:	TATTTA AACG AHNAAC ATCAAT AHNC ATTTCATTGT
48/52-rev:	GC TTGTTTTTAA TAHNATGAAA GGAAHNGGAA TCAATATTTA
49/53-rev:	GTTTTTAA TCTGAHNAAA GGAAGCAHNA TCAATATTT
50/54-rev:	TAA TCTGATGAHN GGAAGCGGAAHNAATATTTA A
51/55-rev:	TCTGATGAAA AHNAGCGGAA TCAHNATTTA AACGA
56/60-rev:	TCCT GATGTCCAHN GGCAATCAGAHNGTTTTTAA TCT
57/61-rev:	T GATGTCCTTG AHNAATCAGC TTAHNTTTAA TCTGAT
58/62-rev:	TGTCCTTG GGCAHNCAGC TTGTTAHNAA TCTGATGA
59/63-rev:	CTTG GGCAATAHNC TTGTTTTTAHNCTGATGAAA GGA
64/68-rev:	ATGCTCCGC AAAAHNTTTT TCCAGAHNCT GATGTCCTTG
65/69-rev:	TCCGC AAATGCAHNT TCCAGATCAHNATGTCCTTG GGCA
66/70-rev:	GC AAATGCTTTAHNCAGATCCT GAHNTCCTTG GGCAATC
67/71-rev:	ATGCTTTT TCAHNATCCT GATGAHNTTG GGCAATCAG
72/76-rev:	GT TCTGTTGGTAHNTTCAATAG CAHNCTCCGC AAATGCT
73/77-rev:	TCTGTTGGTC AGAHNAATAG CATGAHNCGC AAATGCTT
74/78-rev:	GTTGGTC AGTTCAHNAG CATGCTCAHN AAATGCTTTT TC
75/79-rev:	GTC AGTTCAATAHNATGCTCCGC AHNTGCTTTT TCCAG
80/84-rev:	AGTATCT TCATTAHNAT GTTTGCCAHN TCTGTTGGTC AG
81/85-rev:	ATCT TCATTTTTAHNTTTGCCGGT AHNGTTGGTC AGTTC
82/86-rev:	T TCATTTTTAT GAHNGCCGGT TCTAHNGGTC AGTTCAAT
83/87-rev:	CATTTTTAT GTTTAHNGGT TCTGTTAHNC AGTTCAATAG C
88/92-rev:	CCGATAAACA TAHNGCCAGA ACCAHNATCT TCATTTTTA

89/93-rev:	GATAAACA TTCCAHNAGA ACCAGTAHNT TCATTTTTAT G
90/94-rev:	AACA TTCCGCCAHN ACCAGTATCAHNATTTTTAT GT
91/95-rev:	CA TTCCGCCAGA AHNAGTATCT TCAHNTTTAT GTTTGC
96/100-rev:	TTAT CACCTATAHN GGCAACACGAHNGATAAACA TTC
97/101-rev:	AT CACCTATAGC AHNAACACGG CCAHNAAACA TTCCGCCA
98/102-rev:	CTATAGC GGCAHNACGG CCGATAHNCA TTCCGCCAG
99/103-rev:	TAGC GGCAACAHNG CCGATAAAAHNTCCGCCAGA ACC
104/108-rev:	A CTTTCACTTC TTCAHNCATC TCAAAAHNAT CACCTATAG
105/109-rev:	ACTTC TTCCTTAHNC TCAAATTTAHNACCTATAGC G
106/110-rev:	C TTCCTTCATAHNAAATTTAT CAHNTATAGC GGCAAC
107/111-rev:	TCCTTCATC TCAHNTTTAT CACCAHNAGC GGCAACAC
112/116-rev:	CAG AGAAGCAATAHNATCCCCCA CAHNCACTTC TTCCT
113/117-rev:	GAAGCAATT TTAHNCCCCA CTTTAHNTTC TTCCTTCAT
114/118-rev:	AGCAATT TTATCAHNCA CTTTCACAHN TTCCTTCATC TC
115/119-rev:	ATT TTATCCCCAHNTTTCACTTC AHNCTTCATC TCAAA
120/124-rev:	TAGGGGA GTCAGAHNCA GCGAGACAHN AGAAGCAATT TTA
121/125-rev:	GGA GTCAGTGAAHNCGAGACCAG AAHNGCAATT TTATC
122/126-rev:	A GTCAGTGACA GAHNGACCAG AGAAHNAATT TTATCCCCCA
123/127-rev:	AGTGACA GCGAAHNCAG AGAAGCAHNT TTATCCCCCA C
128/132-rev:	C ACTTTTTTCA CAHNATTTATTTTAHNGGGA GTCAGTGACA
129/133-rev:	ACTTTTTTCA CTTTAHNTATTTTTAGAHNA GTCAGTGACA G
130/134-rev:	TTCA CTTTATTAHNTTTTAGGGGAHNCAGTGACA GCGAGAC
131/135-rev:	CA CTTTATTTATAHNTAGGGGA GTAHNTGACA GCGAGAC
136/140-rev:	ATCCTT GTCTAAAHNC ACTTTTTTAHNTTTATTTAT TTTTAG
Up-For primer	
F-com:	ATGAAAAGTA ATGGATGCAG ATATGGAACG
Down-For primers	
144/148-forw:	TG TTAGACAAG NDTCAAATGGA G NDTGAAGGG CAAGCA

145/149-forw:	TAGACAAGG AT NDTATGGA GATT NDTGGG CAAGCAAT
146/150-forw:	AGACAAGG ATCAANDTGA GATTGAANDT CAAGCAATTC T
147/151-forw:	AGG ATCAAATGNDTATTGAAGGG NDTGCAATTC TTTTCA
152/156-forw:	ATTGAAGGG CAANDTATTC TTTTCNDTAG TGGCGTTTAT
153/157-forw:	TGAAGGG CAAGCANDTC TTTTCAGCNDTGGCGTTTAT G
154/158-forw:	AGGG CAAGCAATTNDTTTCAGCAG TNDTGTTTAT GCCA
155/159-forw:	CAAGCAATTC TTNDTAGCAG TGGCNDTTAT GCCAAGTTAC
160/164-forw:	AGCAG TGGCGTTNDT GCCAAGTTANDTGATGATCT GGA
161/165-forw:	G TGGCGTTTAT NDTAAGTTAC CGNDTGATCT GGATGA
162/166-forw:	TGGCGTTTAT GCCNDTTTAC CGGATNDTCT GGATGAAAAT
163/167-forw:	GTTTAT GCCAAGNDTC CGGATGATNDTGATGAAAAT TTAG
168/172-forw:	GGATGATCT GNDTGAAAAT TTANDTCTTT CCGTTCT
169/173-forw:	ATGATCT GGATNDTAAT TTAGCGNDTT CCGTTCTGGA
170/174-forw:	ATCT GGATGAANDT TTAGCGCTTNDTGTTCTGGA TGT
171/175-forw:	T GGATGAAAAT NDTGCGCTTT CCNDTCTGGA TGTTGC
176/180-forw:	GCGCTTT CCGTTNDTGA TGTTGCCNDT GCTCCTGCAC A
293/297-forw:	GT GGTATG NDTT ATTTTTTC NDTATGGCTACT TC
294/298-forw:	GT GGTATGGTCNDTTTTTTCTC TNDTGCTACT TCTT
295/299-forw:	GGTATGGTCT ATNDTTTCTC TATGNDTACT TCTTTTAC
296/300-forw:	TATGGTCT ATTTTNDTTC TATGGCTNDT TCTTTTACAA
301/305-forw :	TC TATGGCTACT NDTTTTACAA AANDTGCTCT GGGCGCA
302/306-forw:	ATGGCTACT TCTNDTACAA AAGCANDTCT GGGCGCAGA
303/307-forw:	TACT TCTTTTNDTA AAGCAGCTNDTGGCGCAGAA GGA
304/308-forw:	T TCTTTTACANDTGCAGCTCT GNDTGCAGAA GGAAT
309/313- forw :	CAGCTCT GGGCNDTGAA GGAATTNDTG CCGATGTA
310/314- forw :	CTCT GGGCGCANDT GGAATTGGTNDTGATGTAGA TAT
311/315- forw :	T GGGCGCAGAA NDTATTGGTG CCNDTGTAGA TATGATG
312/316- forw :	GCGCAGAA GGANDTGGTG CCGATNDTGA TATGATGATA
317/321- forw :	GGTG CCGATGTA NDTATGATGATA NDTAATGGTT ATG

318/322- forw :	GATGTAGA T NDTATGATA GGT NDTGGTT ATGCCCA
319/323- forw :	TGTAGA TATG NDTATA GGTAATGGT NDTGCCCATCA TCA
320/324- forw :	AGA TATGATG NDTGGTAATGGTT AT NDTCATCA TCATT
325-forw :	GGTAATGGTT AT NDTCATCA TCATTCCGAA
Down-Rev primer	
R-com:	TTCCGCATAG CGTTCTTTAA ATATCT

Table S2. Primers used in the site-directed mutagenesis of sites 310 and 314

GCTCTGGGCGCAggaGGAATTGGT	16252E310G-F
GCTCTGGGCGCAttcGGAATTGGT	16252E310F-F
GCTCTGGGCGCAttaGGAATTGGT	16252E310L-F
GCTCTGGGCGCAtcaGGAATTGGT	16252E310S-F
GCTCTGGGCGCAtacGGAATTGGT	16252E310Y-F
GCTCTGGGCGCAtgcGGAATTGGT	16252E310C-F
GCTCTGGGCGCAtggGGAATTGGT	16252E310W-F
GCTCTGGGCGCAccaGGAATTGGT	16252E310P-F
GCTCTGGGCGCAcacGGAATTGGT	16252E310H-F
GCTCTGGGCGCAcaaGGAATTGGT	16252E310Q-F
GCTCTGGGCGCAcgaGGAATTGGT	16252E310R-F
GCTCTGGGCGCAataGGAATTGGT	16252E310I-F
GCTCTGGGCGCAatgGGAATTGGT	16252E310M-F
GCTCTGGGCGCAacaGGAATTGGT	16252E310T-F
GCTCTGGGCGCAaacGGAATTGGT	16252E310N-F
GCTCTGGGCGCAaaaGGAATTGGT	16252E310K-F
GCTCTGGGCGCAgtaGGAATTGGT	16252E310V-F
GCTCTGGGCGCAgcaGGAATTGGT	16252E310A-F

GCTCTGGGCGCAgacGGAATTGGT	16252E310D-F
GGAATTGGTcgcGATGTAGATATGAT	16252A314R-F
GGAATTGGTgtcGATGTAGATATGAT	16252A314V-F
GGAATTGGTgacGATGTAGATATGAT	16252A314D-F
GGAATTGGTgaaGATGTAGATATGAT	16252A314E-F
GGAATTGGTggcGATGTAGATATGAT	16252A314G-F
GGAATTGGTttcGATGTAGATATGAT	16252A314F-F
GGAATTGGTtccGATGTAGATATGAT	16252A314S-F
GGAATTGGTctcGATGTAGATATGAT	16252A314L-F
GGAATTGGTtacGATGTAGATATGAT	16252A314Y-F
GGAATTGGTtgcGATGTAGATATGAT	16252A314C-F
GGAATTGGTtggGATGTAGATATGAT	16252A314W-F
GGAATTGGTcccGATGTAGATATGAT	16252A314P-F
GGAATTGGTcacGATGTAGATATGAT	16252A314H-F
GGAATTGGTcaaGATGTAGATATGAT	16252A314Q-F
GGAATTGGTatcGATGTAGATATGAT	16252A314I-F
GGAATTGGTatgGATGTAGATATGAT	16252A314M-F
GGAATTGGTaccGATGTAGATATGAT	16252A314T-F
GGAATTGGTaacGATGTAGATATGAT	16252A314N-F
GGAATTGGTaaaGATGTAGATATGAT	16252A314K-F
CAGCCAACTGAGCTCCCTTTCGGGCTTTGCTAGCAGCCGGAT	pETter-R

Table S3. Protein concentration of β-AADH and mutants in the experimentation

Enzymes	WT	E310G	E310S	E310G/ A314Y	E310S/ A314N	A314Y	A314N
Protein concentration (mg•mL-1)	56.4	110.8	77.2	73.1	136.8	111.1	73.6

Table S4. The kinetic parameters of β-AADHs toward (3S,5S)-3,5-DHA, (*R*)-β-homoMet, (*S*)-β-AB and (*R*)-β-Phe.

	(3S,5S)- DHA		(R)-β-homoMet		(S)-β-AB		(R)-β-Phe	
	K_m (mM)	k_{cat} (min^{-1})	K_m (mM)	k_{cat} (min^{-1})	K_m (mM)	k_{cat} (min^{-1})	K_m (mM)	k_{cat} (min^{-1})
E310G	$(8.6 \pm 0.3) \times 10^{-1}$	$(1.5 \pm 0.1) \times 10^{3}$	$(1.2 \pm 0.1) \times 10^{1}$	$(2.0 \pm 0.3) \times 10^{3}$	$(1.7 \pm 0.2) \times 10^{1}$	2.8 ± 0.3	$(3.0 \pm 0.2) \times 10^{1}$	$(3.5 \pm 0.2) \times 10^{2}$
E310G/A314Y	$(8.0 \pm 0.2) \times 10^{-1}$	$(1.6 \pm 0.1) \times 10^{3}$	8.2 ± 0.3	$(4.1 \pm 0.3) \times 10^{3}$	$(4.5 \pm 0.2) \times 10^{1}$	$(2.1 \pm 0.1) \times 10^{1}$	$(3.9 \pm 0.1) \times 10^{1}$	$(4.1 \pm 0.2) \times 10^{1}$
A314Y	1.5 ± 0.2	$(4.0 \pm 0.2) \times 10^{5}$	$(1.5 \pm 0.2) \times 10^{1}$	$(2.6 \pm 0.2) \times 10^{1}$				
E310S	$(2.2 \pm 0.2) \times 10^{1}$	$(6.9 \pm 0.3) \times 10^{2}$	8.1 ± 0.4	$(1.6 \pm 0.1) \times 10^{3}$	$(2.0 \pm 0.1) \times 10^{1}$	7.9 ± 0.1	$(1.0 \pm 0.1) \times 10^{1}$	$(1.0 \pm 0.1) \times 10^{1}$
E310S/A314N	$(2.4 \pm 0.1) \times 10^{1}$	$(1.1 \pm 0.0) \times 10^{3}$	9.6 ± 0.4	$(3.8 \pm 0.2) \times 10^{3}$	6.2 ± 0.5	$(1.1 \pm 0.2) \times 10^{1}$	$(2.4 \pm 0.1) \times 10^{1}$	$(3.1 \pm 0.2) \times 10^{1}$
A314N	1.6 ± 0.1	$(4.8 \pm 0.4) \times 10^{5}$	$(1.1 \pm 0.1) \times 10^{1}$	5.3 ± 0.3				
WT	1.8 ± 0.2	$(4.8 \pm 0.4) \times 10^{5}$	9.1 ± 0.2	5.2 ± 0.2				

A314Y, A314N and wild-type enzyme did not show measurable activity toward (*S*)-β-AB and (*R*)-β-Phe.

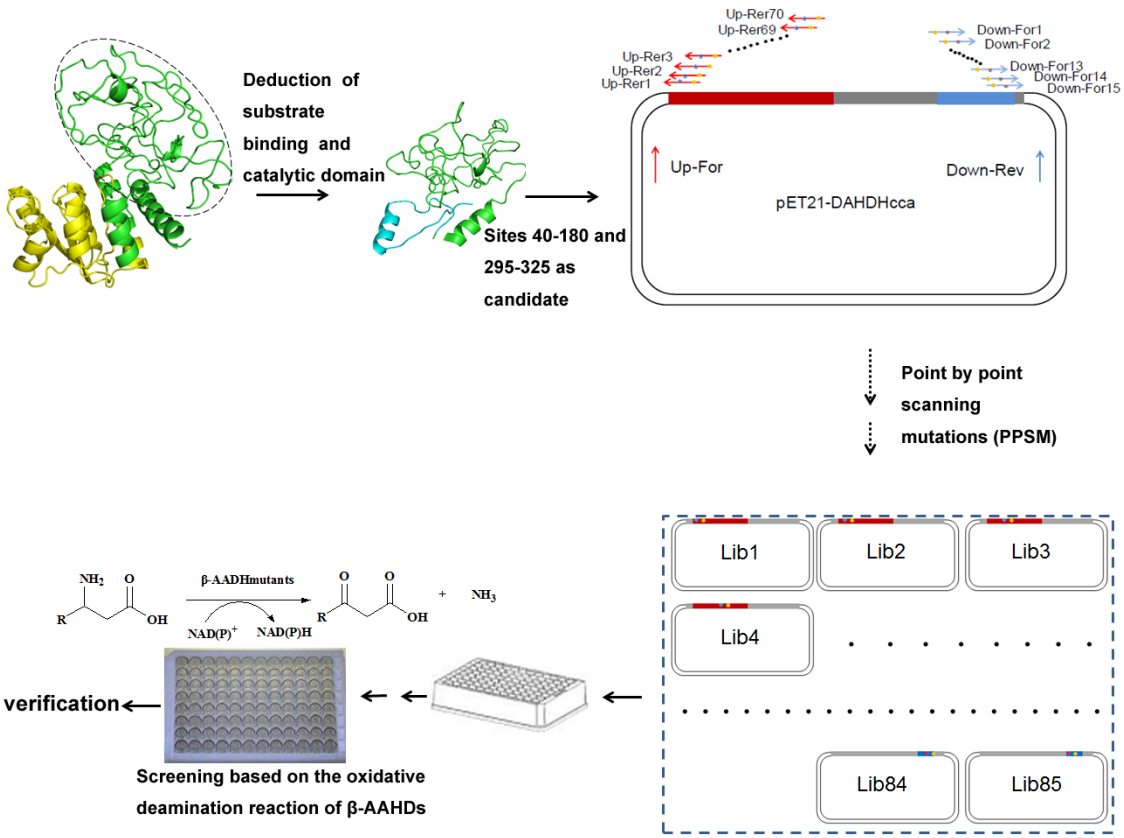


Chart S1. The procedure of engineering 3,5-DAHDHcca.

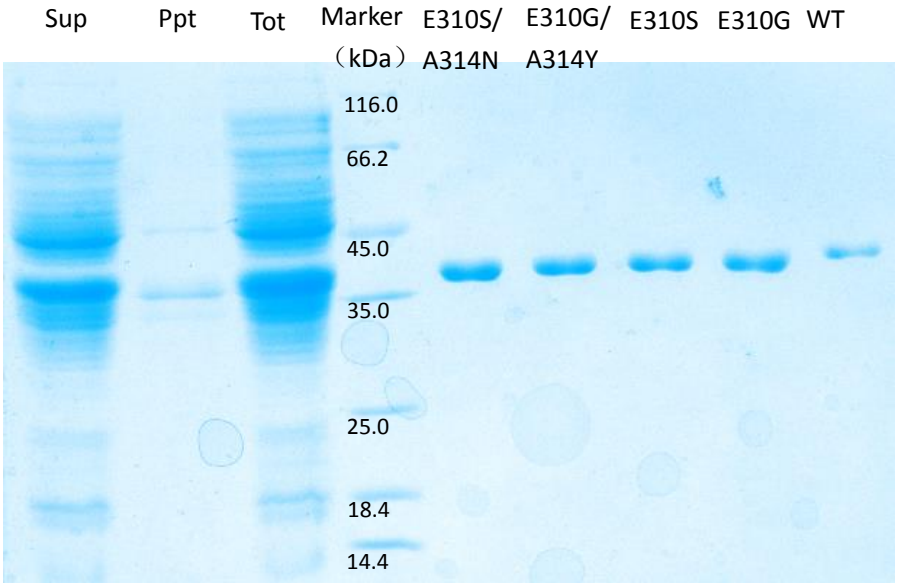


Figure S1. SDS-PAGE analysis of heterologous expression of 3,5-DAHDHcca from *Candidatus Cloacamonas acidaminovorans* in *E. coli* BL21(DE3) host cells and purified enzymes. Tot, total cell; ppt, precipitant fraction; sup, supernatant fraction. Purified wild-type 3,5-DAHDHcca and mutants E310G, E310S, E310G/A314Y and E310S/A314N with Ni-resin affinity chromatography.

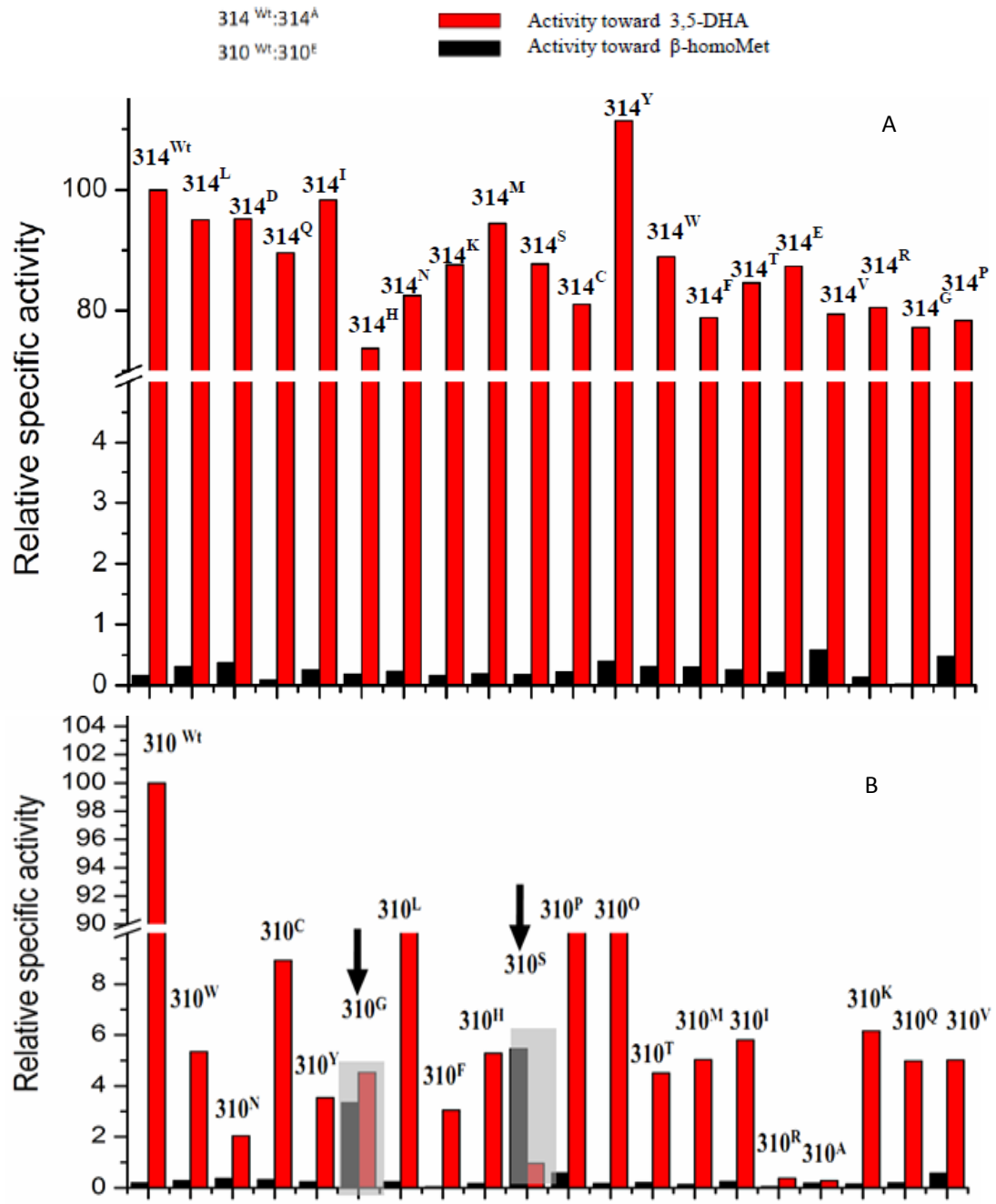


Figure S2. Influence of the substitutions of residues 314 (A) and 310 (B) to other 19 amino acids on the oxidative deamination activity of 3,5-DAHDHcca toward (3*S*,5*S*)-DAH and (*R*)-β-homoMet. The activities of 314^{Wt} and 310^{Wt} toward (3*S*,5*S*)-DAH were referred to 100%. Mutants were purified as described in the method for small quantity of His-tagged protein purification in the experimental section, and the reaction was performed as described by Annett Kreimeyer[2] and Barker H. A.[3]

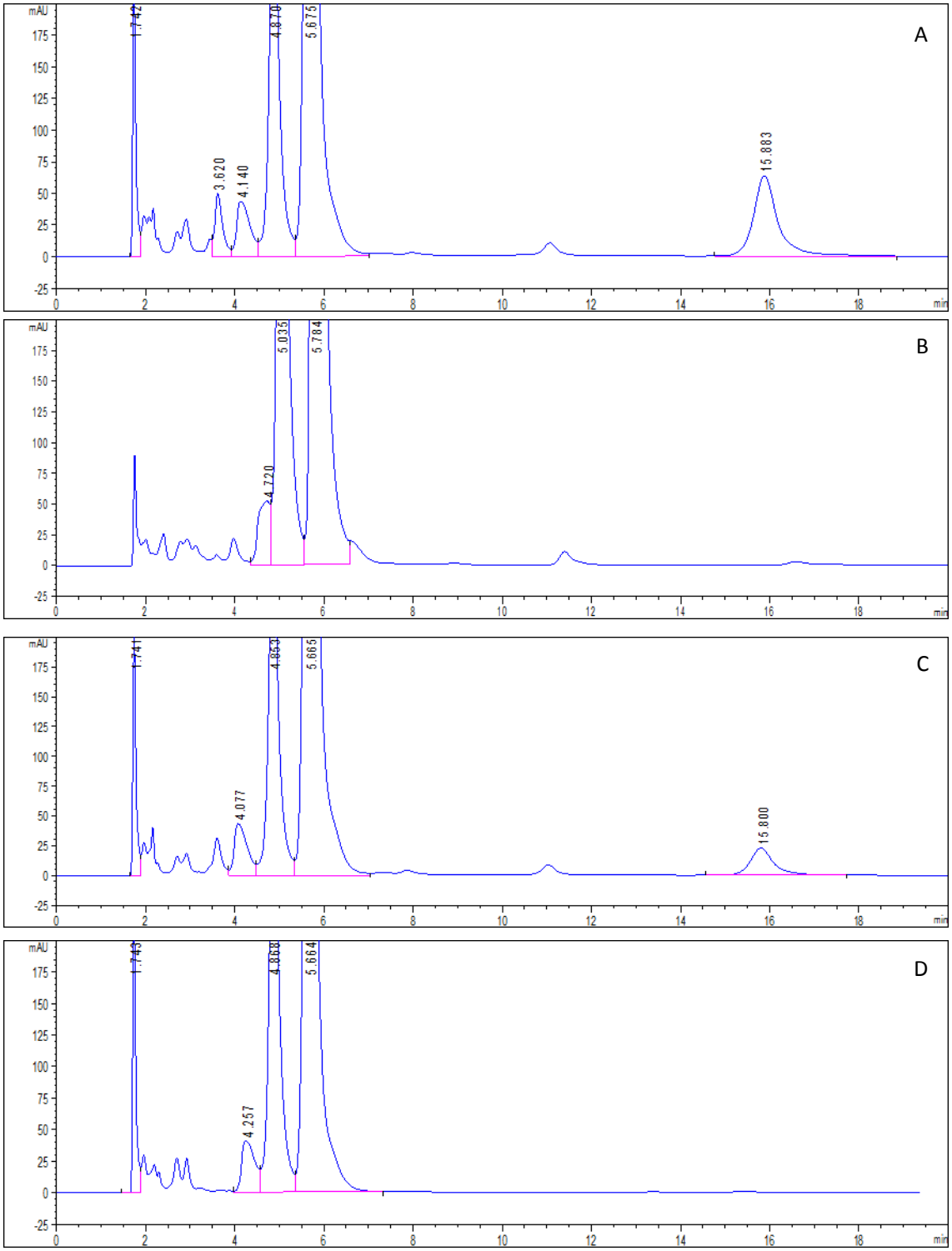
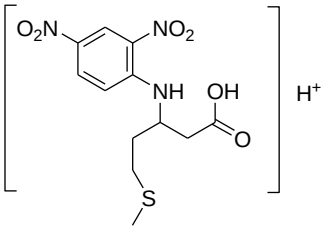


Figure S3. HPLC analysis for the product from β-AADH mutant E310G/A314Y catalyzed reductive amination of 5-(methylthio)-3-oxopentanoic acid which was generated by the hydrolysis of 5-(methylthio)-3-oxopentanenitrile with nitrilase NIT6402. The products of the reactions with no enzyme but (*R*)-β-homoMet standard (A), wild-type enzyme (B), mutant enzyme (C) and no enzyme (D), were derivatized with DNFB. The resultant mixtures were subjected to HPLC and LC-MS analysis. The retention time of (*R*)-β-homoMet was 15.8 min.



$C_{12}H_{16}N_3O_6S^+$
Exact Mass: 330.0754
Mol. Wt.: 330.3364
m/e: 330.0760 (100.0%), 331.0793 (13.0%), 332.0718 (4.5%), 332.0802 (1.2%), 331.0730 (1.1%)
C, 43.63; H, 4.88; N, 12.72; O, 29.06; S, 9.71

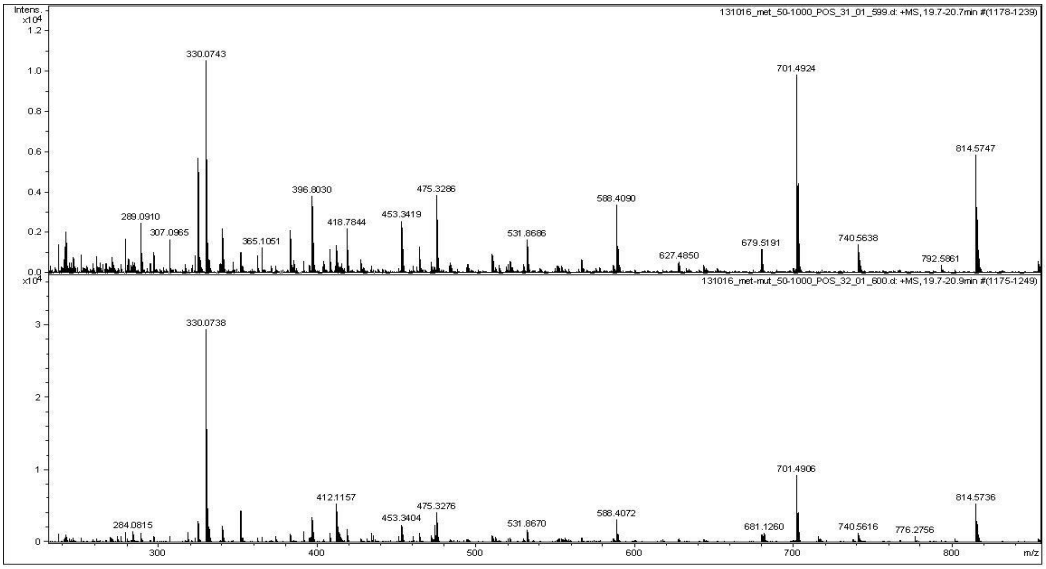


Figure S4. LC-MS analysis of the reductive amination product β-homoMet generated from 5-(methylthio)-3-oxopentanenitrile catalyzed by β-AADH mutant E310G/A314Y coupled nitrilase NIT6402.

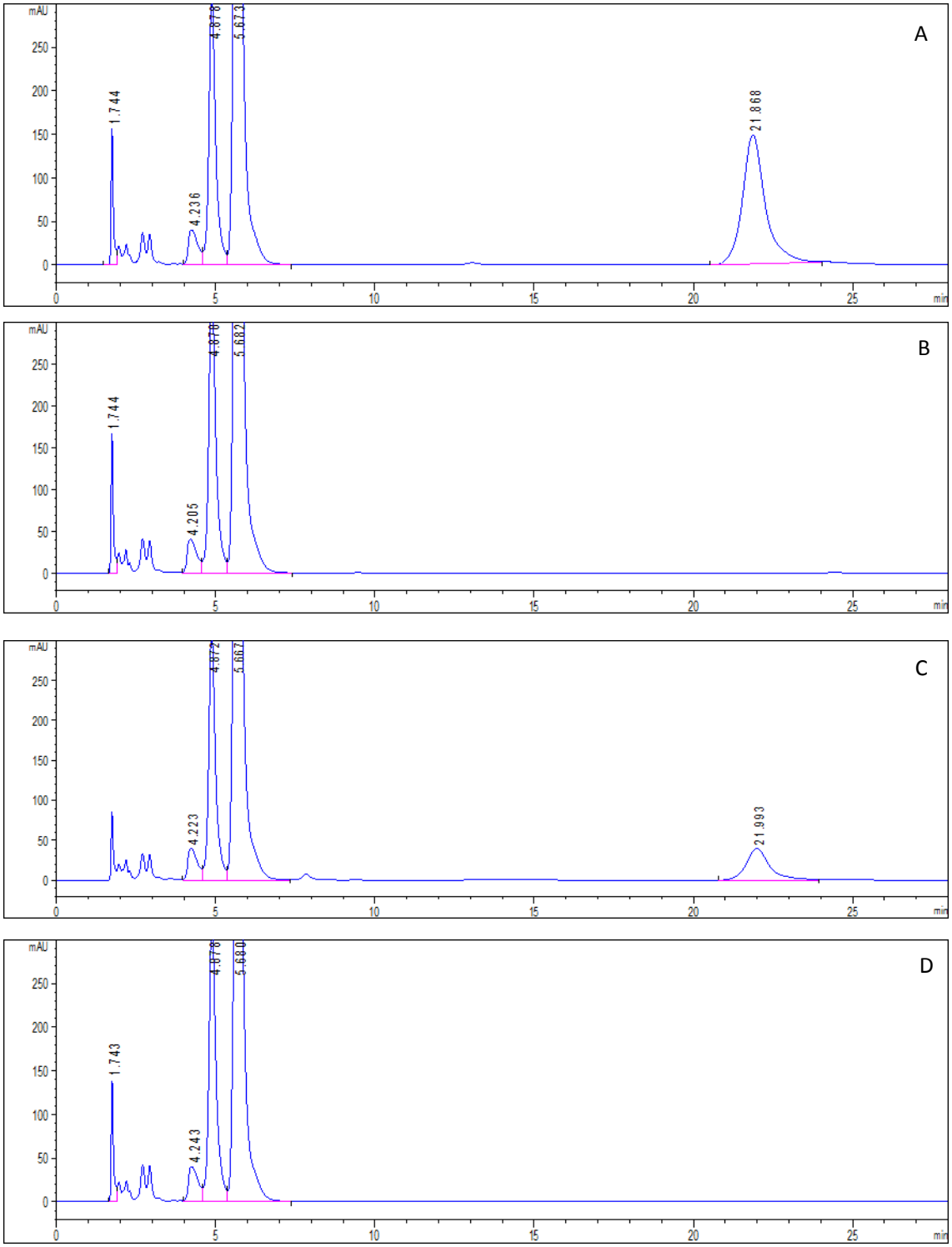
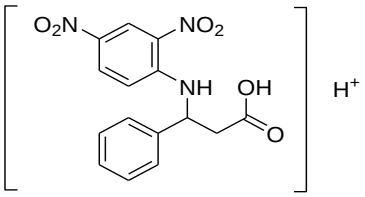


Figure S5. HPLC analysis for the product from β-AADH mutant E310G/A314Y catalyzed reductive amination of benzoylacetic acid, which was generated by the hydrolysis of ethyl benzoylacetate with lipase Novozyme 435. The products of the reactions with no enzyme but (*R*)-β-Phe standard (A), wild-type enzyme (B), mutant enzyme (C) and no enzyme (D), were derivatized with DNFB. The resultant mixtures were subjected to HPLC and LC-MS analysis. The retention time of (*R*)-β-Phe was 21.9 min.



$C_{15}H_{14}N_3O_6^+$
Exact Mass: 332.0877
Mol. Wt.: 332.2876
m/e: 332.0883 (100.0%), 333.0916 (16.2%), 334.0925 (1.2%), 334.0950 (1.2%), 333.0853 (1.1%)
C, 54.22; H, 4.25; N, 12.65; O, 28.89

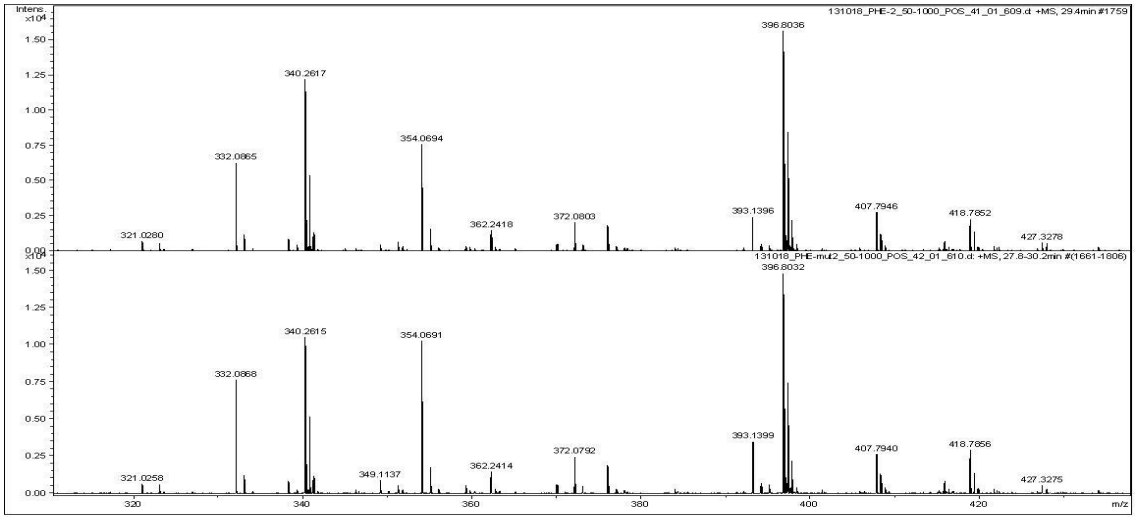


Figure S6. LC-MS analysis of the reductive amination product β-Phe generated from ethyl benzoylacetate catalyzed by β-AADH mutant E310G/A314Y coupled lipase Novozyme 435.

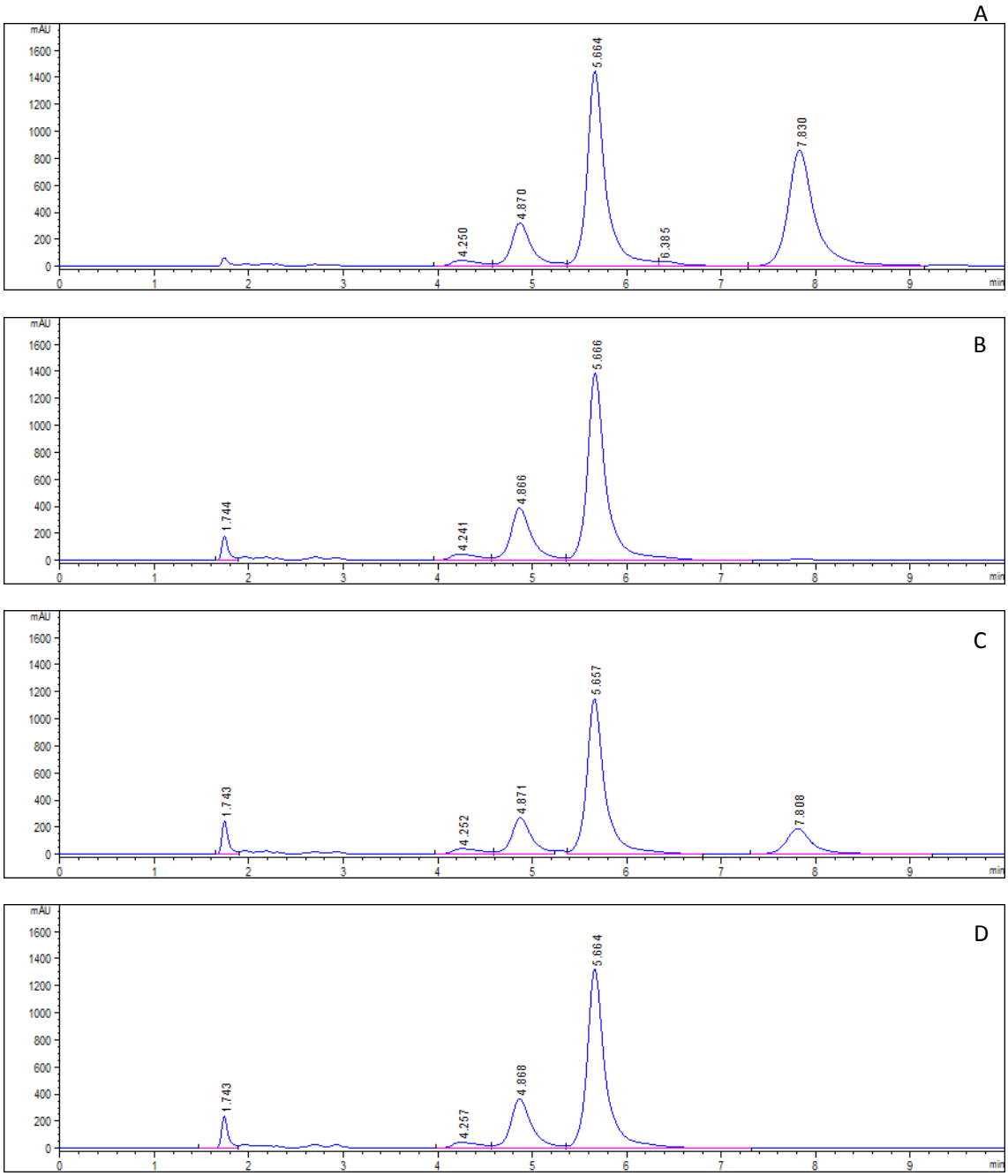
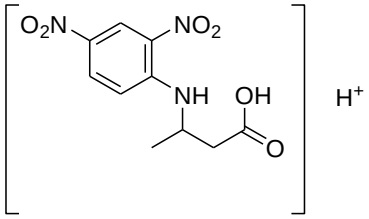


Figure S7. HPLC analysis for the product from β-AADH mutant E310G catalyzed reductive amination of 3-oxo-butyric acid, which was generated by the hydrolysis of ethyl 3-ketobutanoate with lipase Novozyme 435. The products of the reactions with no enzyme but (*S*)-β-AB standard (A), wild-type enzyme (B), mutant enzyme (C) and no enzyme (D), were derivatized with DNFB. The resultant mixtures were subjected to HPLC and LC-MS analysis. The retention time of (*S*)-β-AB was 7.8 min.



$C_{10}H_{12}N_3O_6^+$
Exact Mass: 270.0721
Mol. Wt.: 270.2182
m/e: 270.0726 (100.0%), 271.0760 (10.8%), 272.0769 (1.2%), 271.0696 (1.1%)
C, 44.45; H, 4.48; N, 15.55; O, 35.53

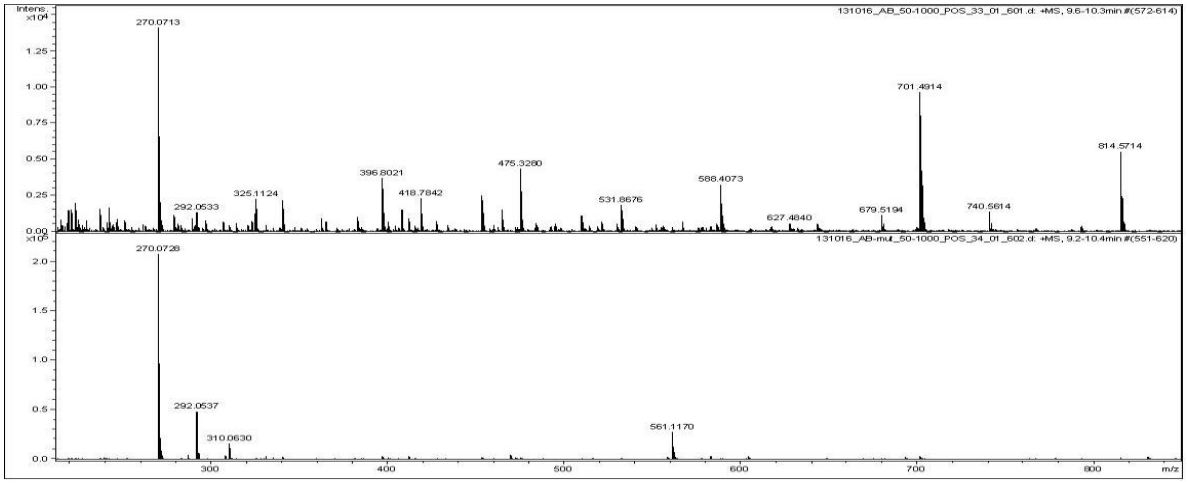


Figure S8. LC-MS analysis of the reductive amination product β-AB generated from ethyl 3-ketobutanoate catalyzed by β-AADH mutant E310G/A314Y coupled lipase Novozyme 435.

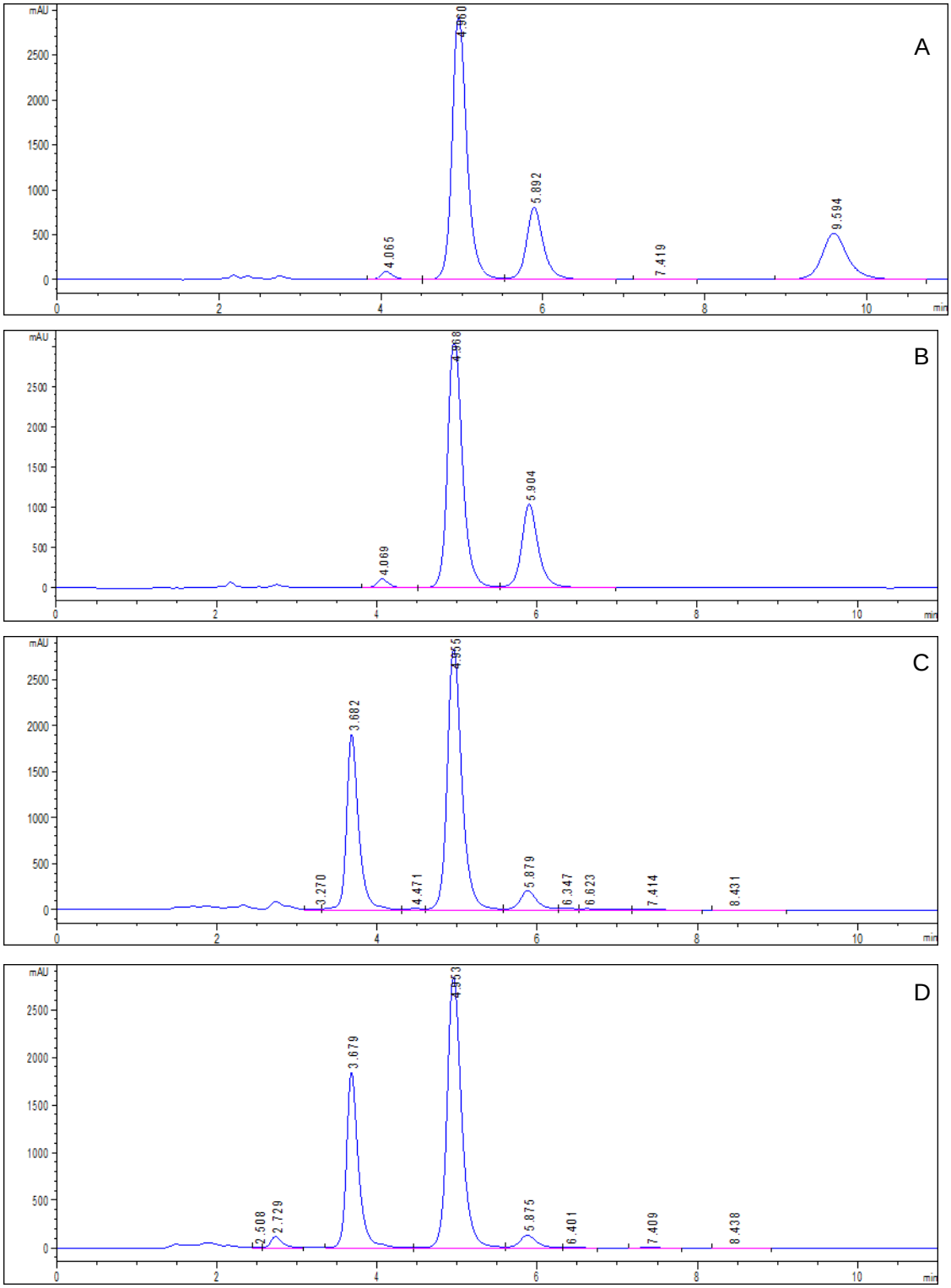


Figure S9. Determination of the configuration of β-Phe produced by β-AADH E310G. Racemic β-Phe (A), (*R*)-β-Phe (B), β-Phe generated from benzoylacetonitrile with nitrilase NIT6402 and β-AADH (C) and β-Phe generated from ethyl benzoylacetate with lipase Novozyme 435 and β-AADH (D) were derivatized with FDAA and analyzed by HPLC. The peaks at retention times 5.8 and 9.5 min were (*R*)-β-Phe and (*S*)-β-Phe, respectively.

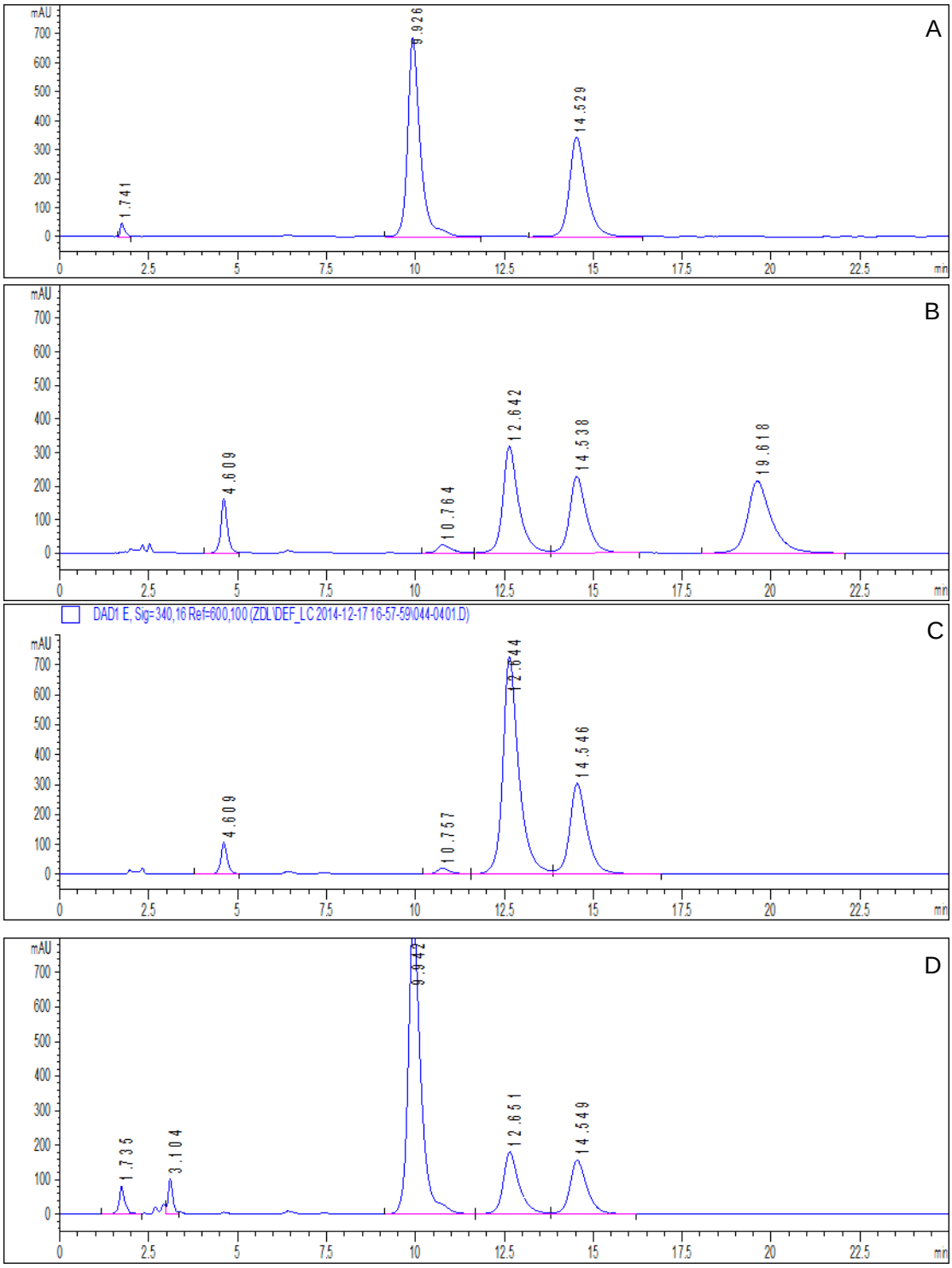


Figure S10. Determination of the configuration of β-AB produced by β-AADH E310G/A314Y. NH_4Cl (A), racemic β-AB (B), (*S*)-β-AB (C) and β-AB generated from ethyl 3-ketobutanoate with lipase Novozyme 435 and β-AADH (D) were derivatized with FDAA and analyzed by HPLC. The peaks at retention times 12.6 and 19.6 min were (*S*)-β-AB and (*R*)-β- AB, respectively.

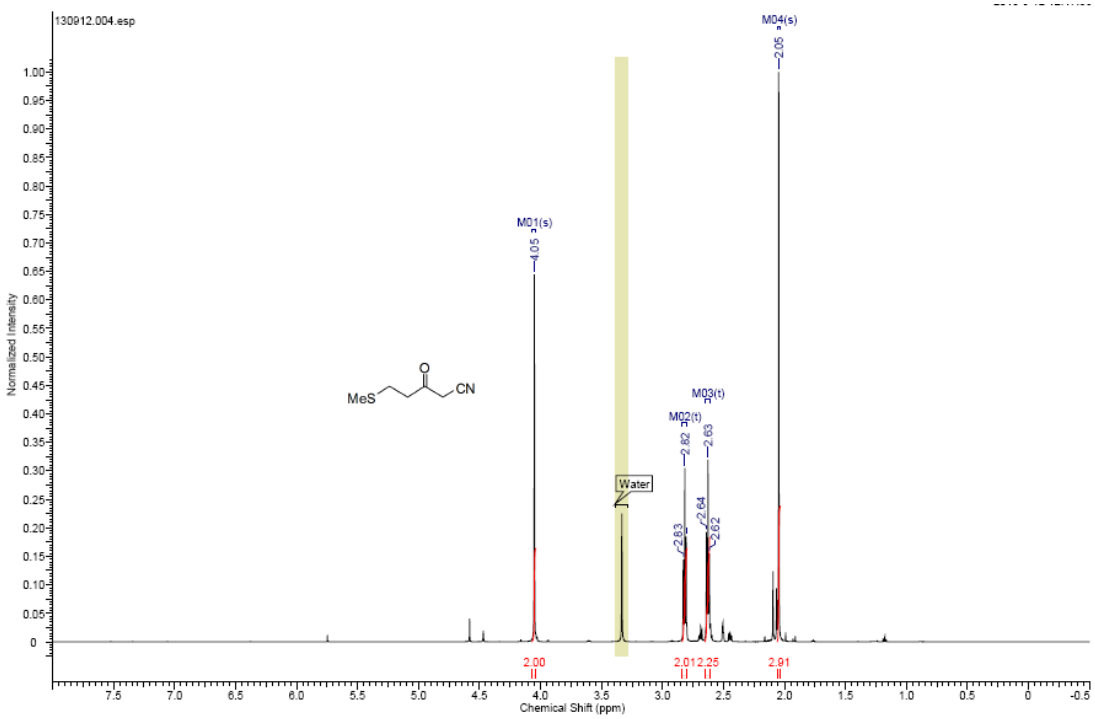


Figure S11. ^{1}H NMR spectrum of 5-(methylthio)-3-oxo pentane nitrile.

^{1}H NMR (600 MHz, *DMSO-d*$_6$): δ 4.05 (s, 2 H), 2.82 (t, *J*=7.15 Hz, 2 H), 2.63 (t, *J*=7.15 Hz, 2 H), 2.05 (s, 3 H).

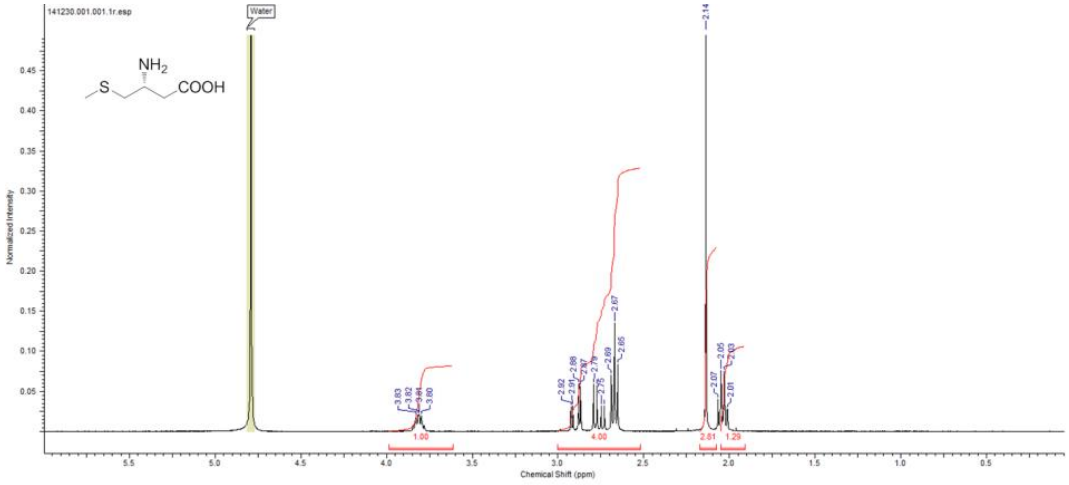


Figure S12. ^{1}H NMR spectrum of (*R*)-β-homoMet .

^{1}H NMR (D_2O, 400 Hz) δ 3.80 (m, 1H), 2.86 (dd, *J* = 4 Hz and 20 Hz,1H), 2.72 (dd, *J* = 8 Hz and 20 Hz, 1H), 2.66 (m, 2H), 2.14 (s, 3H), 1.81 (q, *J* = 4 Hz and 8 Hz, 2H). HR-MS(ES+) calcud for $C_6H_{13}NO_2S$ (M+H) 164.0745, found: 164.0732.

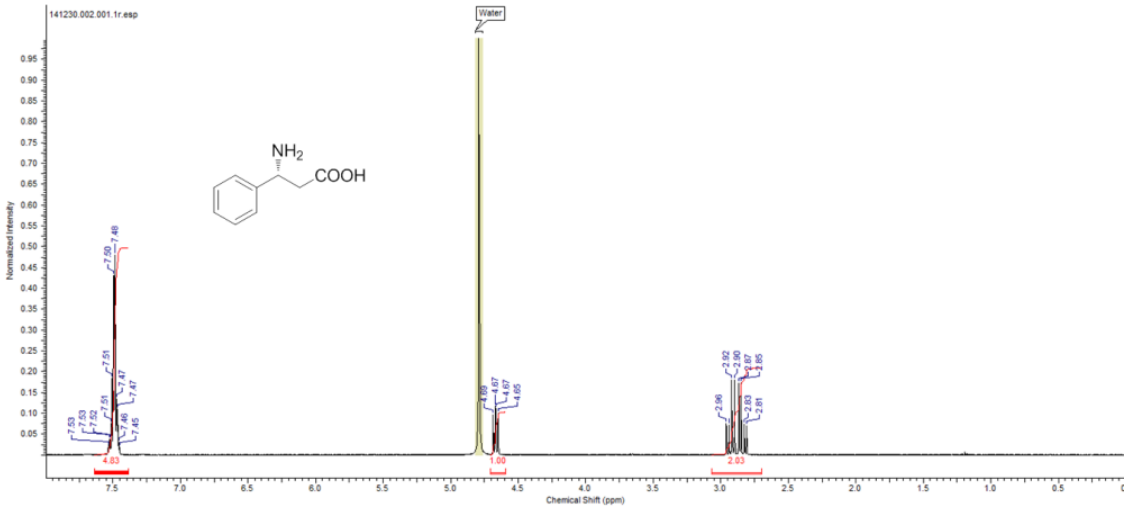


Figure S13. ^{1}H NMR spectrum of (*R*)-β-Phe.

^{1}H NMR (D_2O, 400 Hz) δ 7.48 (m, 5H), 4.64 (t, *J* = 8 Hz,1H), 2.80-2.95 (ddd, *J* = 8 Hz, 12 Hz and 20 Hz, 2H), HR-MS(ES+) calcud for $C_9H_{11}NO_2$ (M+H) 166.0868, found: 166.0866.

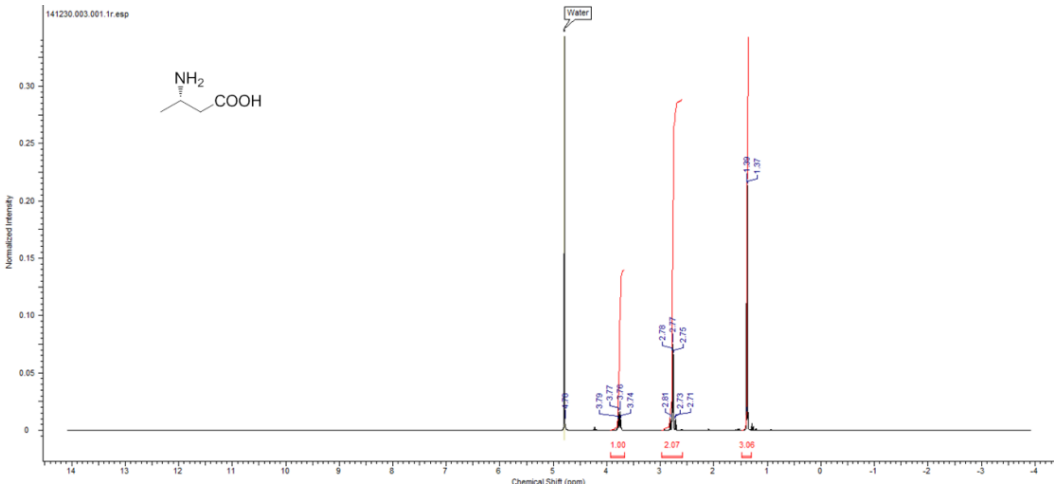


Figure S14. ^{1}H NMR spectrum of (*S*)-β-AB.

^{1}H NMR (D_2O, 400 Hz) δ 3.75 (m, 1H), 2.77 (dd, *J* = 4 Hz and 8 Hz 2H), 1.36 (d, *J* = 8 Hz, 3H), HR-MS(ES+) calcud for $C_4H_9NO_2$ (M+H) 104.0712, found: 104.0674.

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(1) Chapple, C. C. S.; Decicco, C.; Ellis, B. E. *Phytochemistry* **1988**, *27*, 3461-3463.

(2) Kreimeyer, A.; Perret, A.; Lechaplais, C.; Vallenet, D.; Medigue, C.; Salanoubat, M.; Weissenbach, J. *J. Biol. Chem.* **2007**, *282*, 7191-7197.

(3) Barker, H. A.; Kahn, J. M.; Chew, S. *J. Bacteriol*. **1980**, *143*, 1165-1170.