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Supporting Information

Engineering the activity of Amine Dehydrogenase in the Asymmetric Reductive Amination of Hydroxyl Ketones

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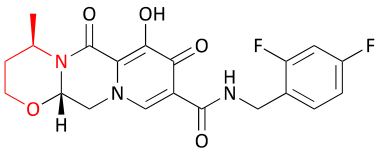
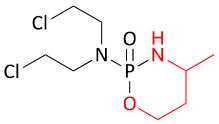
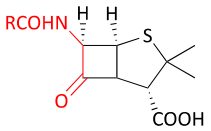


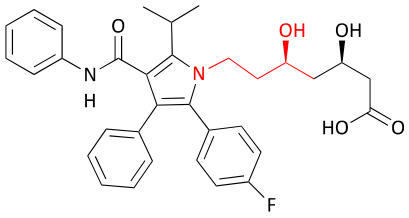
Dolutegravir
(Anti-HIV)



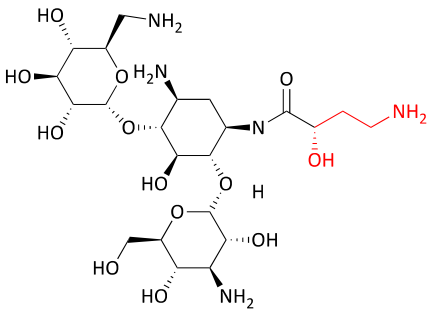
4-methylcyclophosphamide
(Anti-tumor)



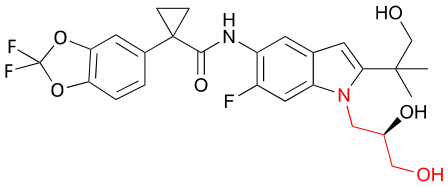
β-lactam antibiotics
(Anti-infection)



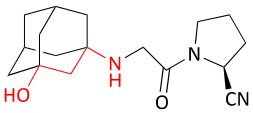
Atorvastatin
(Treatment of hypercholesterolemia)



Amikacin
(Anti-infection)



Elexacaftor
(Treatment of cystic fibrosis)



Vildagliptin
(Treatment of type 2 diabetes mellitus)

Scheme S1 Chiral amino alcohols as building blocks (colored in red) in the representative pharmaceuticals.

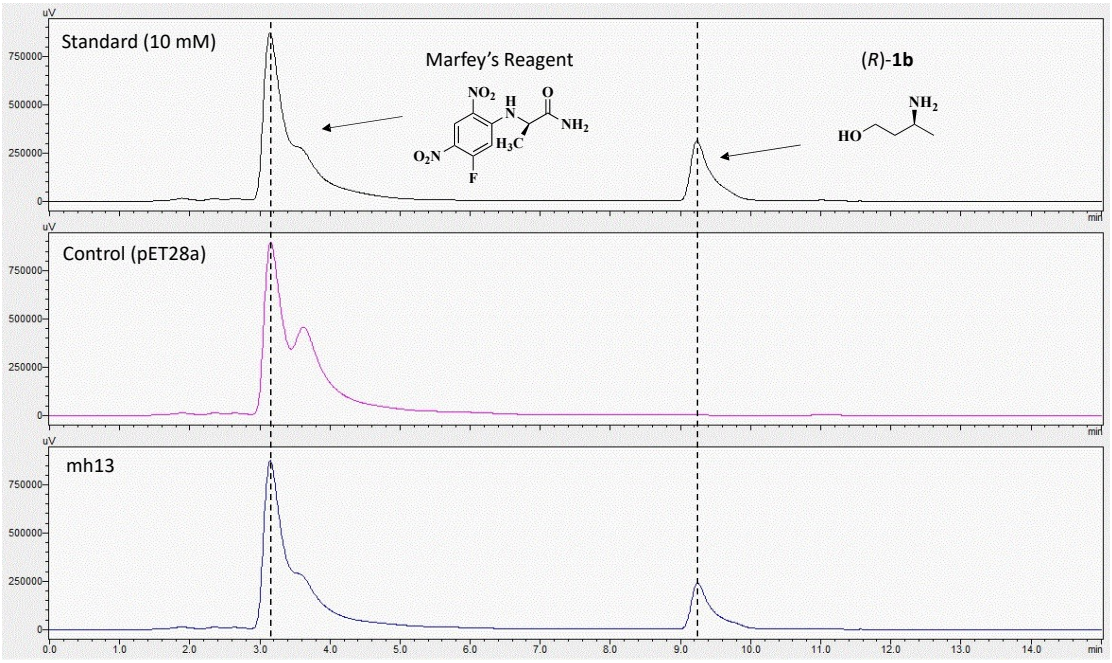


Fig. S1 HPLC profiles of *Gs*AmDH variant mh13. Standard: 10 mM (*R*)-**1b**. Control: pET28a, the excessive concentration of Marfey's reagent leads to the appearance of redundant chromatographic peak in the control group. mh13: the reaction was performed in 1 M NH_4Cl/NH_4OH buffer (pH 9.0) with 100 mM Glucose, 2 mg mL^{-1} GDH cell free extract (CFE), 1.0 mM NAD^+, 30 mM **1a**, and 0.1 g mL^{-1} whole cell loading at 40 °C for 24 h. The above reaction solution was diluted 3-fold with the same buffer and then used for HPLC detection after derivatization with Marfey's reagent.

Fig.S2 Sequence alignment between *Gs*LeuDH (PDB code 6ACF) and the variant mh13.

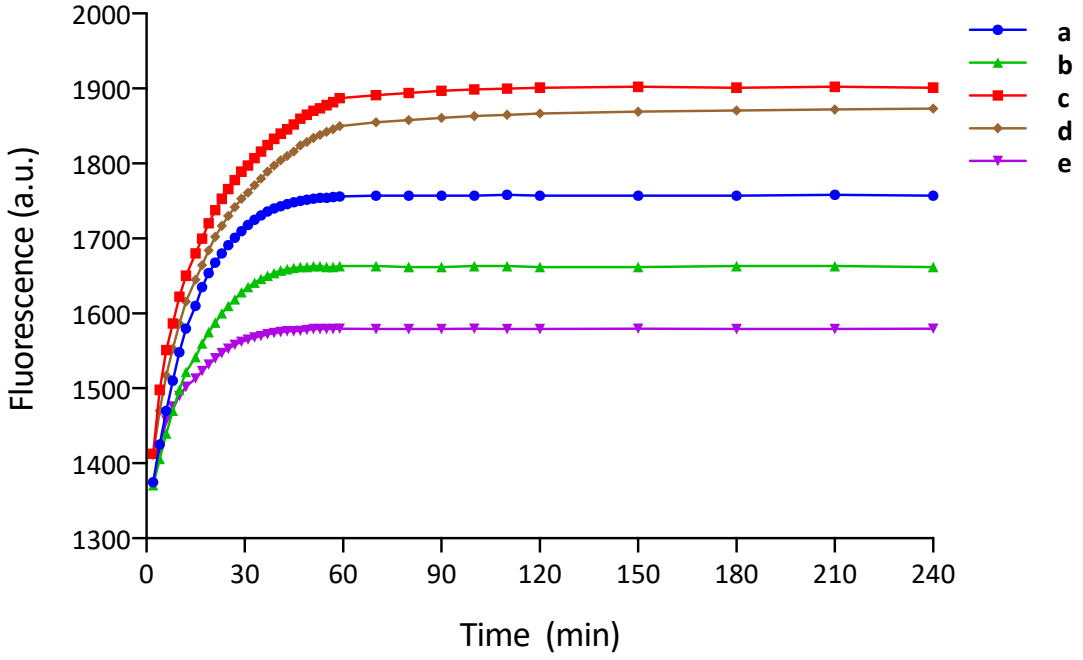


Fig S3. Fluorescence of various samples for the validation of the colorimetric assays (**a**, positive control with mh13; **b**, sample with mh174; **c**, negative control with empty vector pET28a; **d**, negative control with no cells; **e**, negative control with no substrate). Assay conditions: **a***:* mh13 on pET28a using BL21(DE3) cells, $NH_4Cl/NH_3{\cdot}H_2O$ buffer (1 M, pH 9.0), 10 mM substate **1a**, 100 mM glucose, 2 mg mL^{-1} NADH-dependent glucose dehydrogenase (GDH) cell free extract (CFE) and 1 mM nicotinamide adenine dinucleotide (NAD^+); **b**: the same conditions with **a** except the cells were replaced with mh174 on pET28a using BL21(DE3) cells; **c**: the same conditions with **a** except the cells were replaced with pET28a in BL21(DE3) cells; **d***:* the same conditions with **a** without cells; **e**: the same conditions with **a** without the substrate.

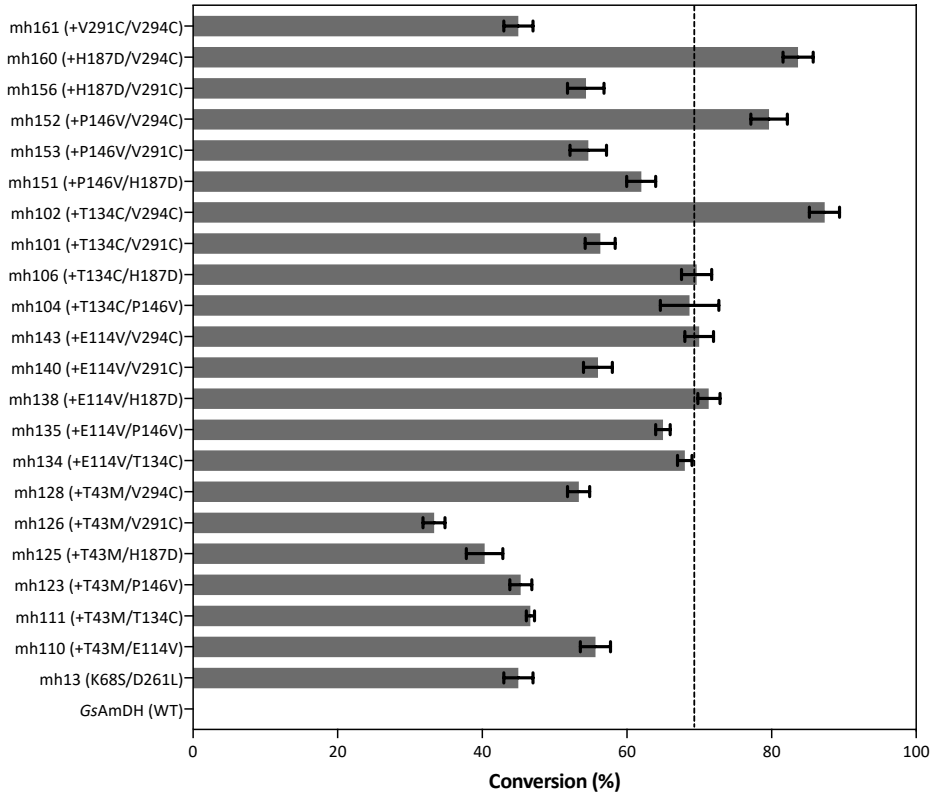


Fig. S4 The conversions of positive mutants obtained from the second round of mutagenesis. The reaction was performed in 1 M $NH_4Cl/NH_3{\cdot}H_2O$ buffer (pH 9.0) containing 1 mM NAD^+, 100 mM Glucose, 2 mg mL^{-1} GDH cell free extract, 0.1 g mL^{-1} whole cell and 30 mM substrate **1a**, at 40 °C for 24 h. +: by iterating new substitutions based on mh13 (K68S/D261L).

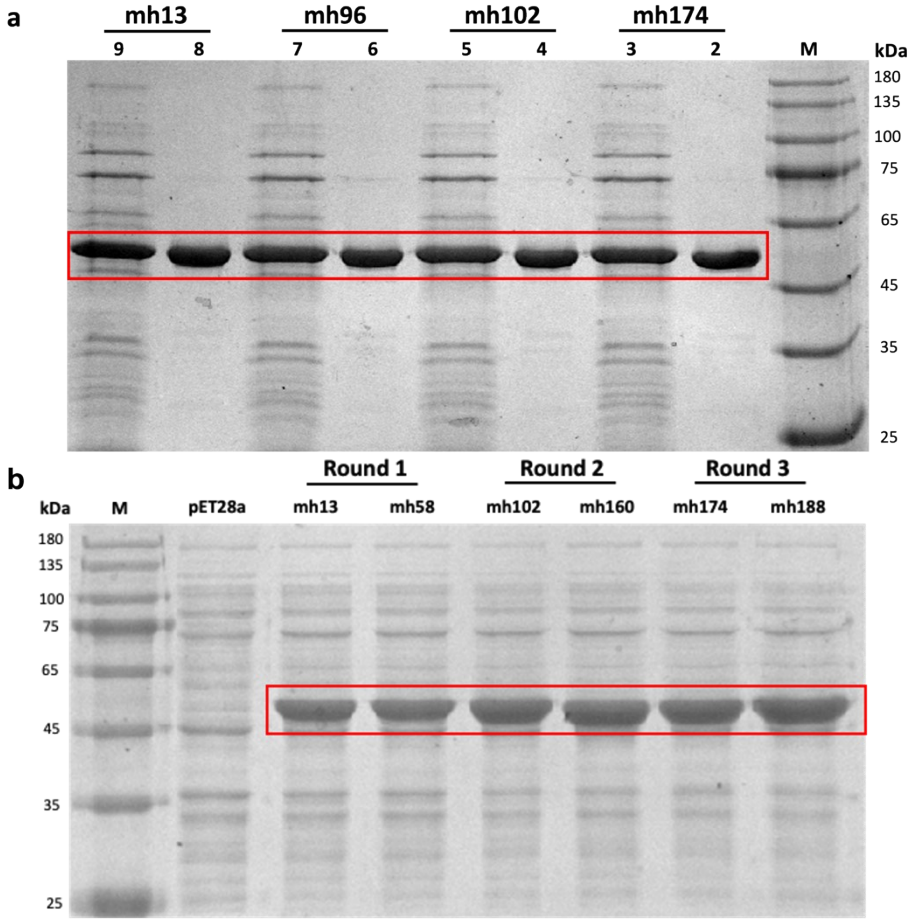


Fig. S5 SDS-PAGE analysis of the protein expression and purification of *Gs*AmDH mutants. (a) Molecular marker is depicted as M. Lanes 9~8, 7~6, 5~4 and 3~2 represent the crude and purified enzymes, respectively. The expected size of *Gs*AmDH (47.7 kDa) is indicated by red box. (b) SDS-PAGE analysis of the protein expression levels of a selection of mutants.

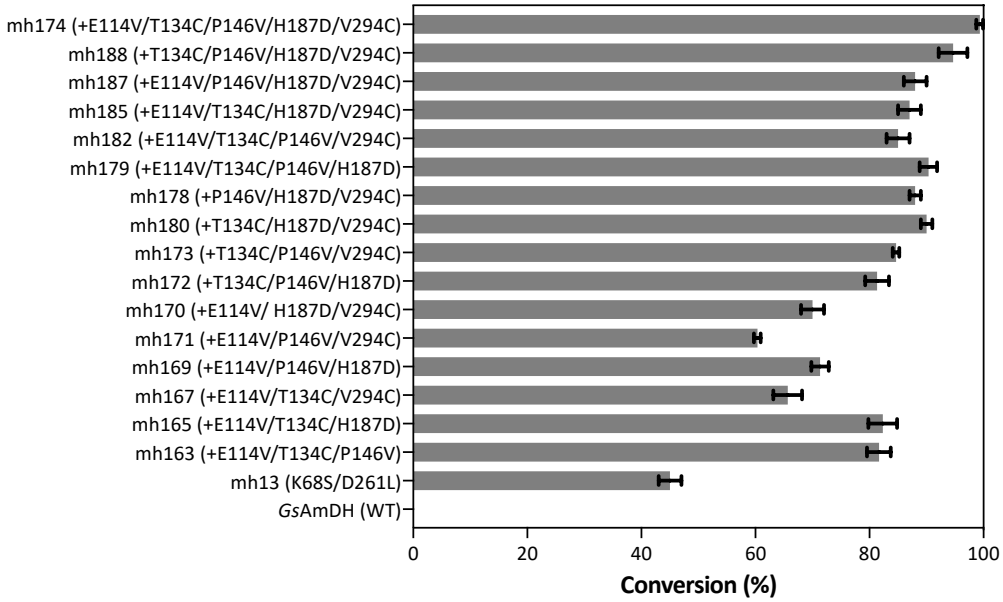


Fig. S6 The conversion of positive mutants obtained from the third round of mutagenesis. The reaction was performed in 1 M $NH_4Cl/NH_3{\cdot}H_2O$ buffer (pH 9.0) containing 1 mM NAD^+, 100 mM Glucose, 2 mg mL^{-1} GDH cell free extract (CFE), 0.1 g mL^{-1} whole cell and 30 mM substrate **1a**, at 40 °C for 24 h. +: by iterating new substitutions based on mh13 (K68S/D261L).

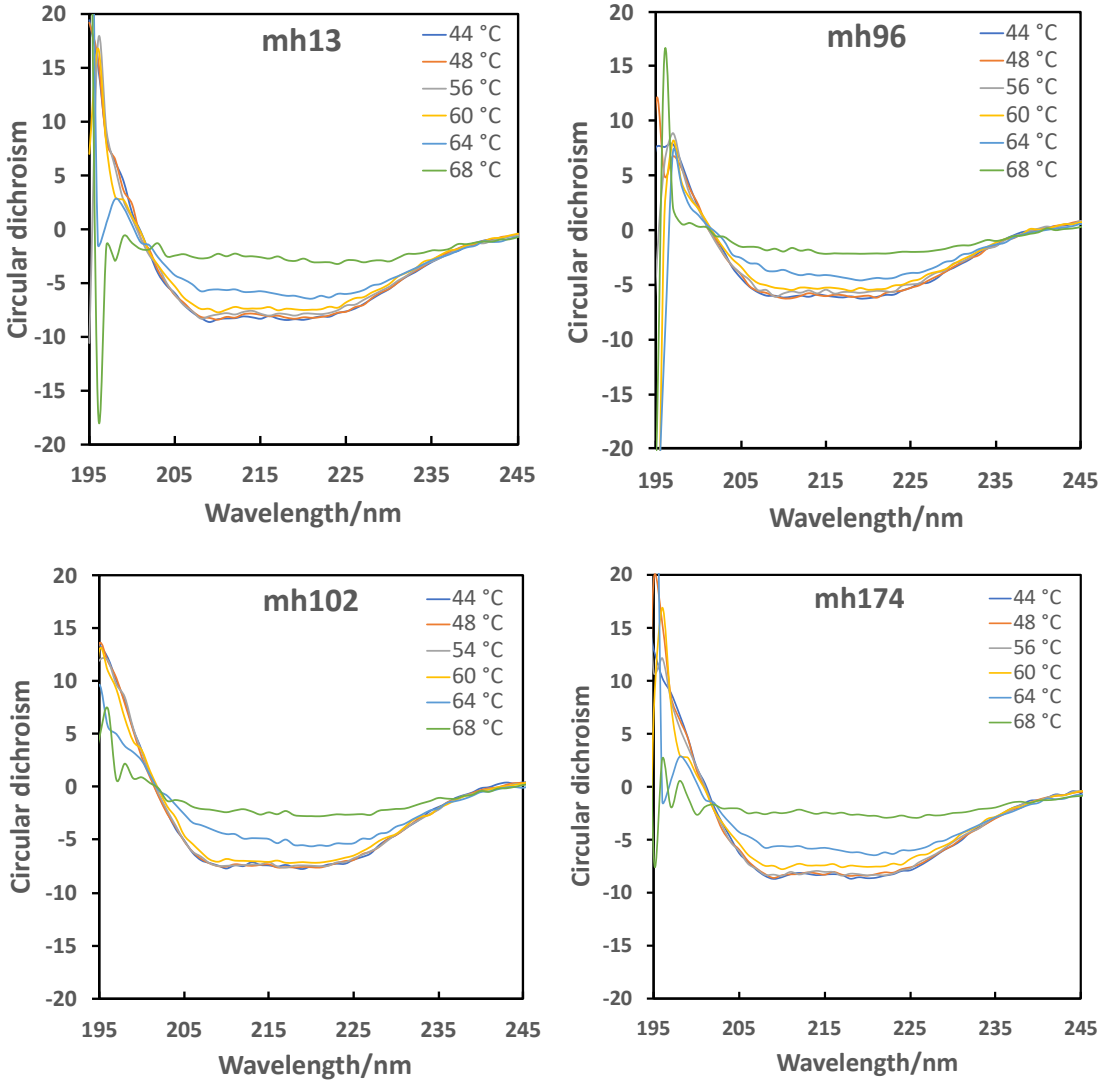
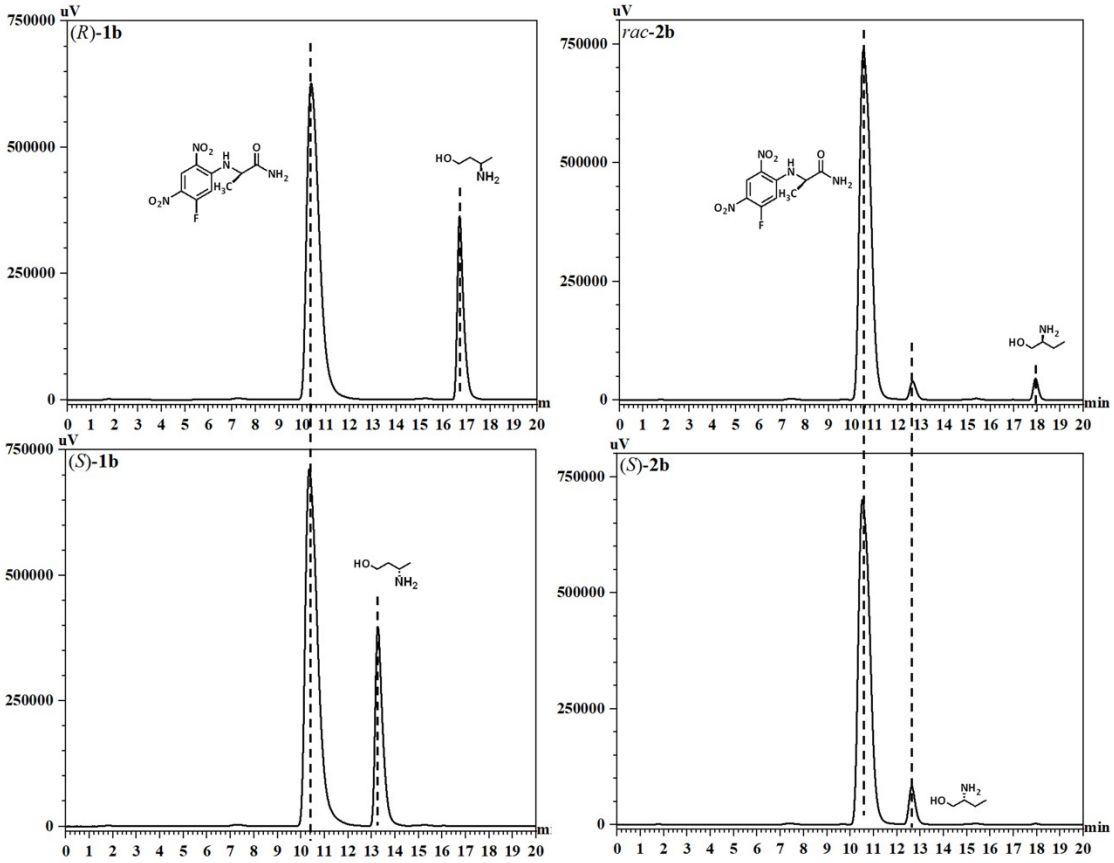
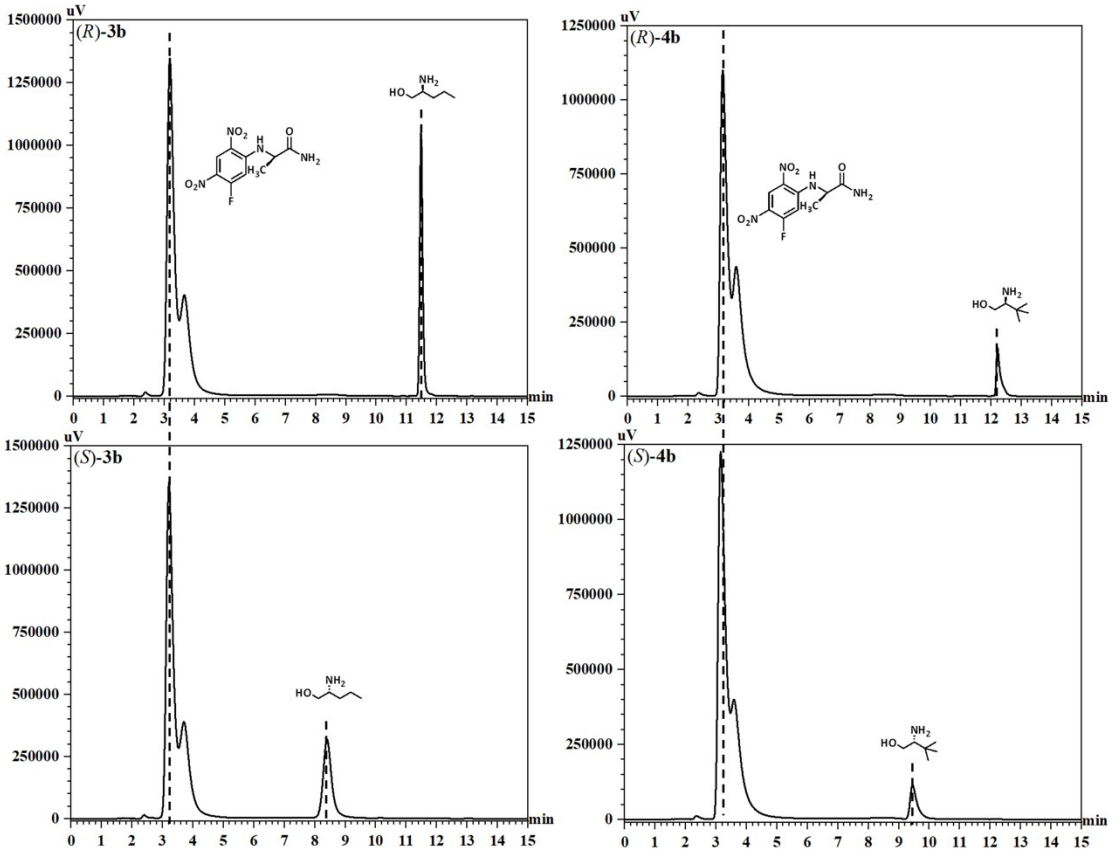
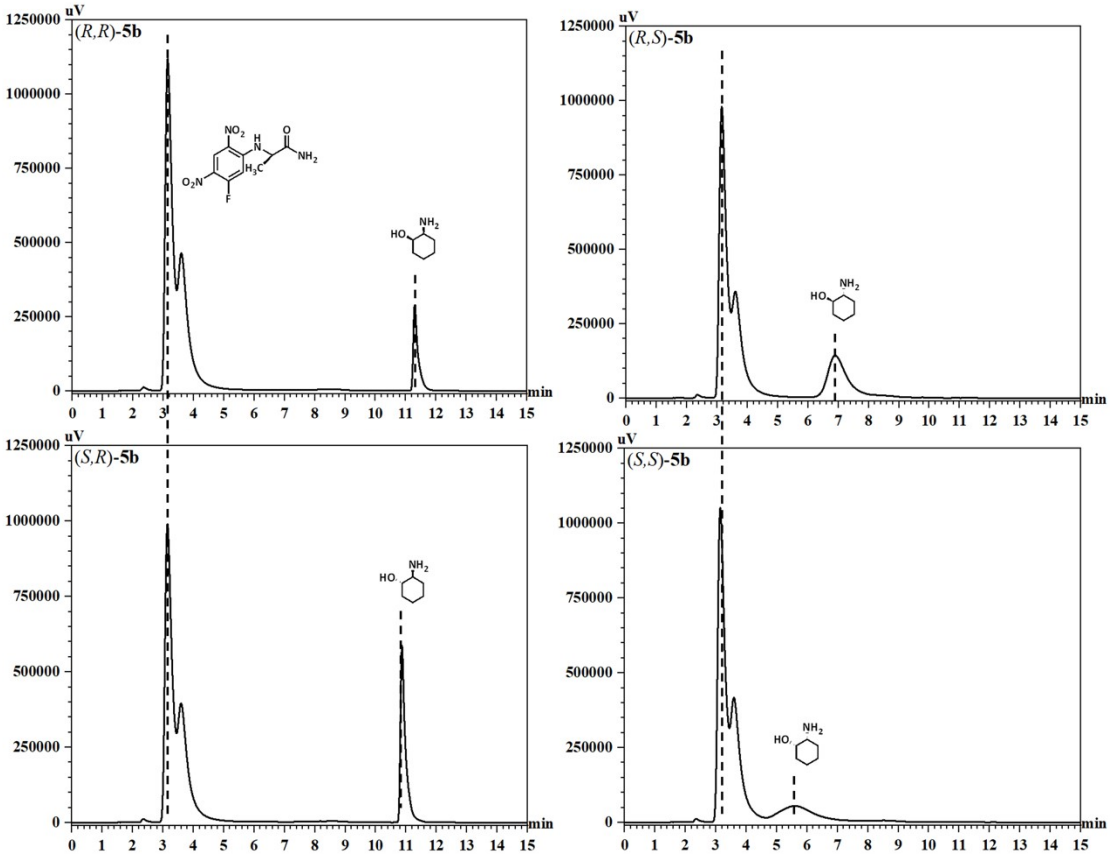
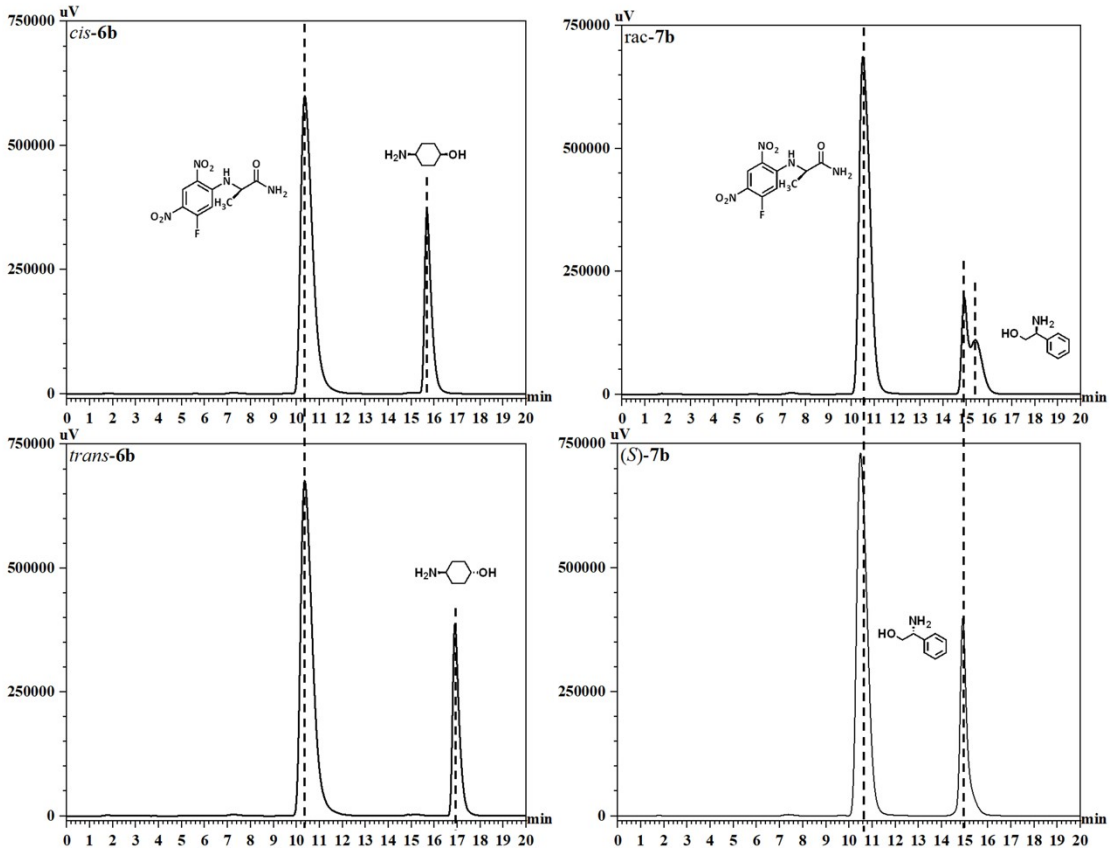


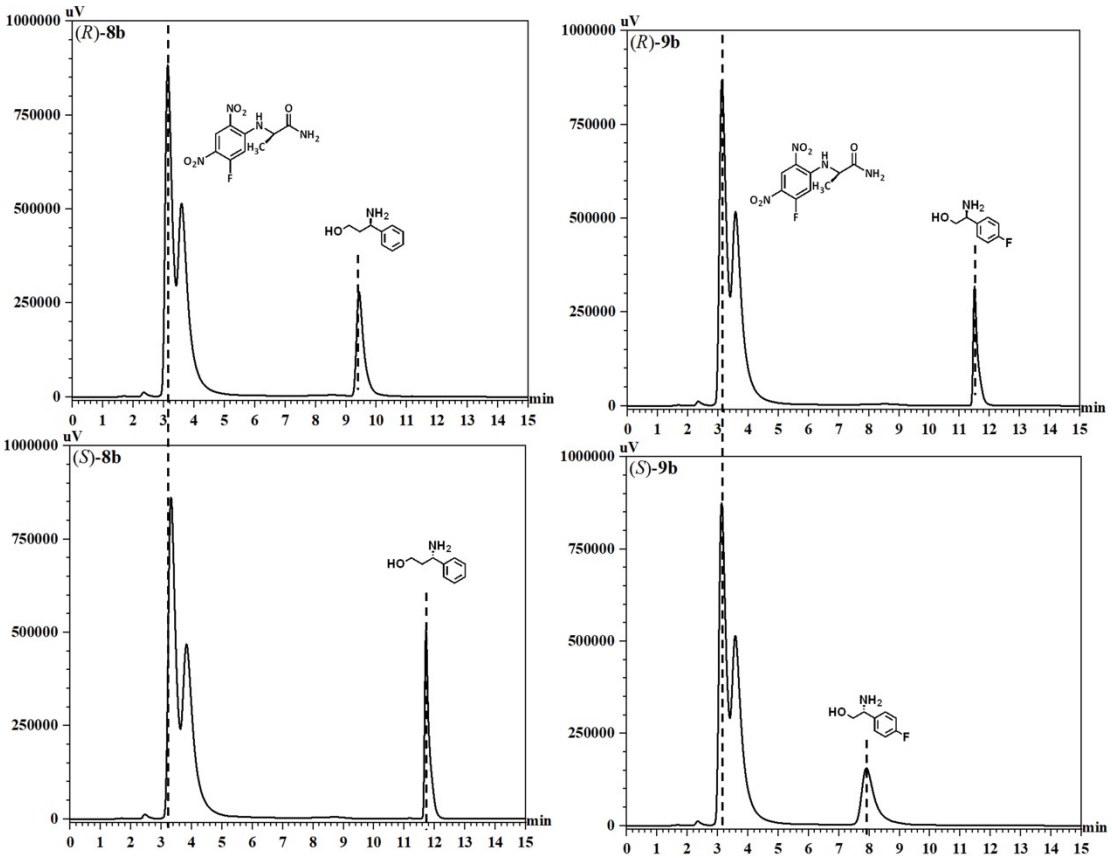
Fig. S7 Circular dichroism spectrum of mh13, mh96, mh102 and mh174.


(R)-1b
rac-2b
(S)-1b
(S)-2b
uV
min


(R)-3b
(R)-4b
(S)-3b
(S)-4b
uV
min


uV
(R,R)-5b
(R,S)-5b
(S,R)-5b
(S,S)-5b
1250000
1000000
750000
500000
250000
0
min
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15


uV
cis-6b
trans-6b
rac-7b
(S)-7b
min


uV
(R)-8b
(S)-8b
(R)-9b
(S)-9b
min

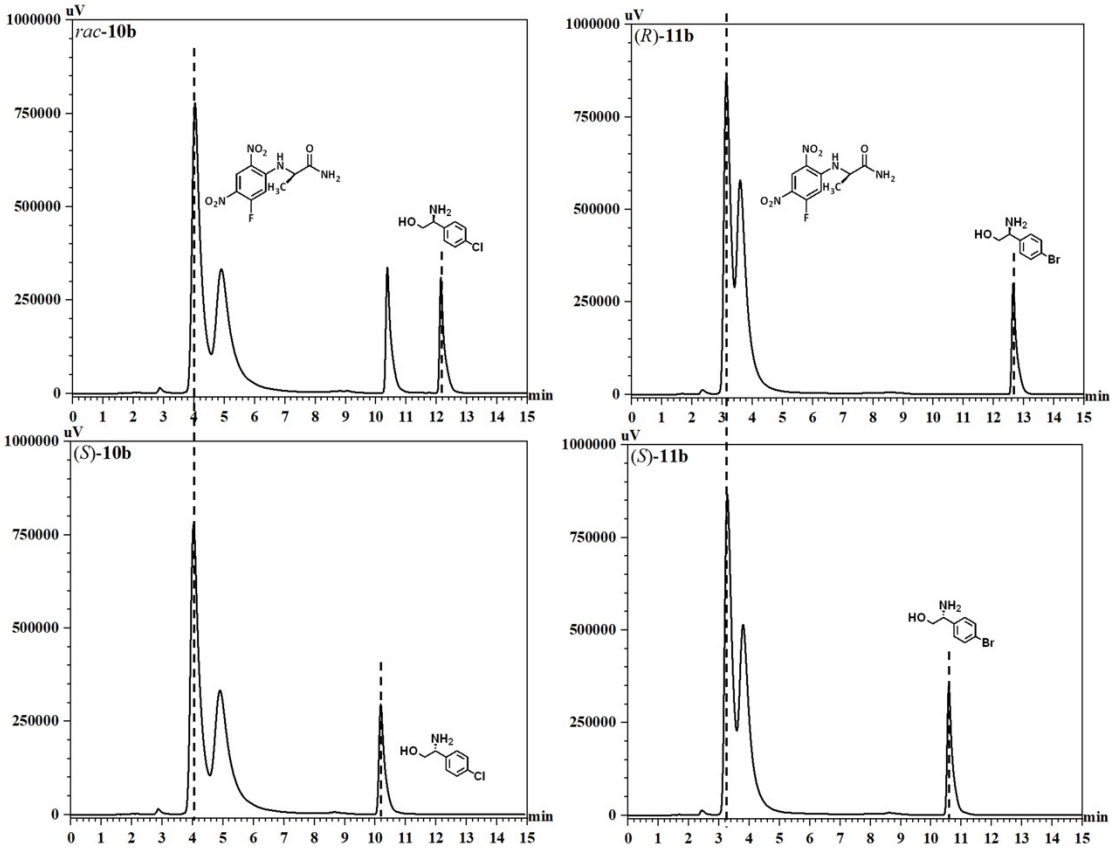


Fig. S8 HPLC profiles of the amino alcohols standards ((*R*)-**1b**, (*S*)-**1b**, *rac*-**2b**, (*S*)-**2b**, (*R*)-**3b**, (*S*)-**3b**, (*R*)-**4b**, (*S*)-**4b**, (*1R,2R*)-**5b**, (*1S,2R*)-**5b**, (*1R,2S*)-**5b**, (*1S,2S*)-**5b,** *cis*-**6b**, *trans*-**6b**, *rac*-**7b**, (*S*)-**7b**, (*R*)-**8b**, (*S*)-**8b**, (*R*)-**9b**, (*S*)-**9b**, *rac*-**10b**, (*S*)-**10b**, (*R*)-**11b**, (*S*)-**11b**).

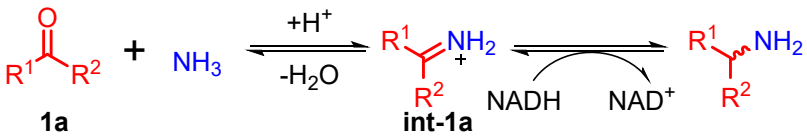


Fig. S9 Mechanism of overall asymmetric reductive amination catalyzed by AmDHs/AADHs. Adapted from previous studies[1].

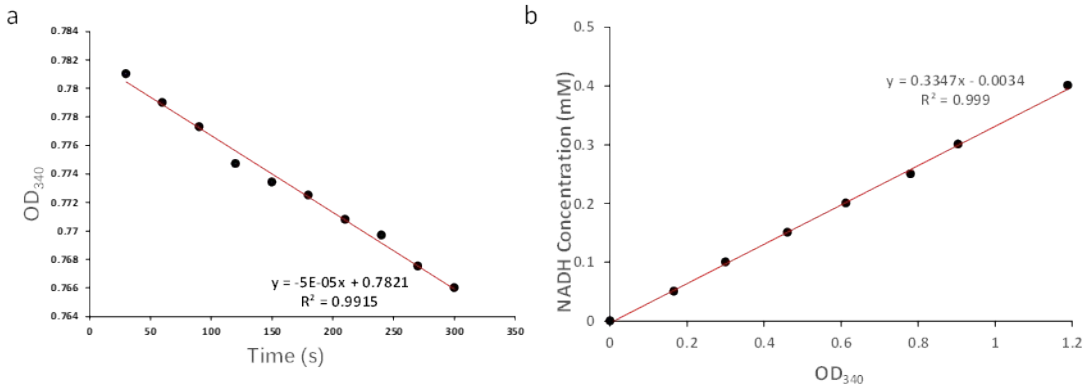


Fig. S10 Calibration curve of NADH consumption at 20 mM substrate concentration. Conditions: (a) mh174 on pET28a using BL21(DE3) cells, $NH_4Cl/NH_3{\cdot}H_2O$ buffer (1 M, pH 9.0), 20 mM **1a**, 100 mM glucose, 0.2 mM NADH at 40 °C for 5 min; (b) 1 M NH_4Cl/NH_4OH buffer (pH 9.0) containing NADH (0.1-0.4 mM).

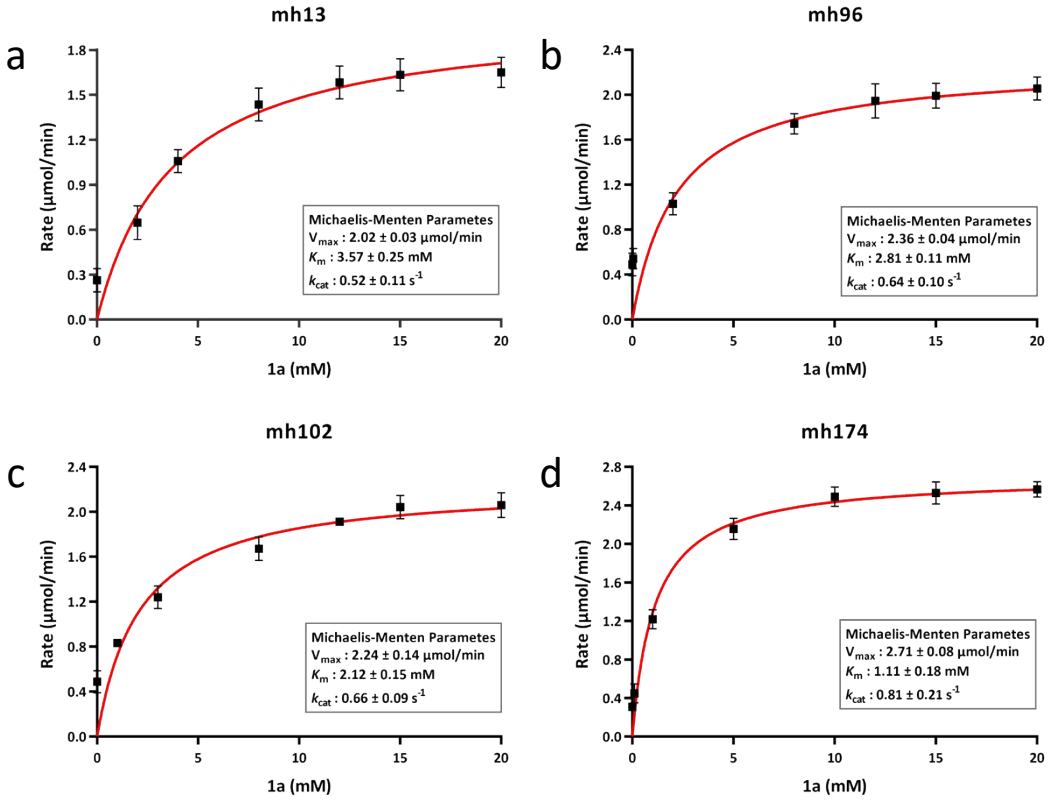


Fig. S11 Determination of kinetic parameters of variants mh13 (a), mh96 (b), mh102 (c) and mh174 (d). All experiments were conducted in triplicate.

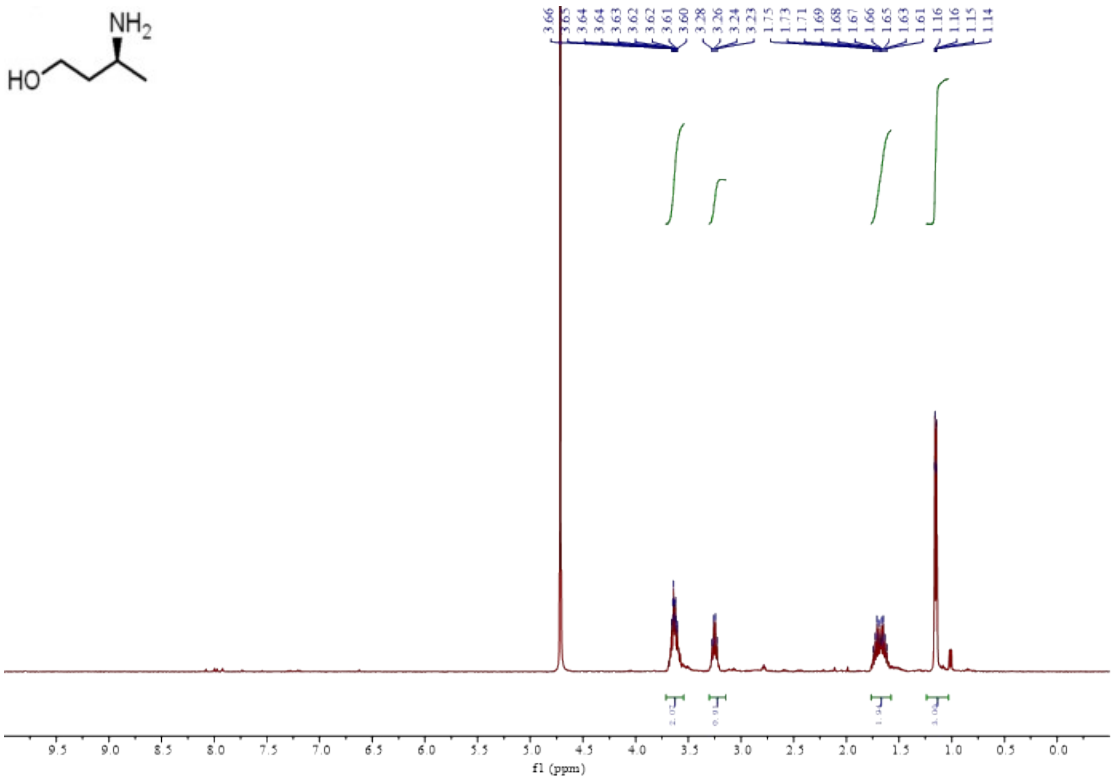


Fig. S12 NMR spectra of (*R*)-3-amino-1-butanol**.** ^{1}H NMR (400 MHz, Deuterium Oxide) δ 3.84 – 3.38 (m, 2H), 3.25 (q, *J* = 6.7 Hz, 1H), 1.68 (dp, *J* = 24.2, 7.2 Hz, 2H), 1.15 (dd, *J* = 6.6, 1.5 Hz, 3H)[2].

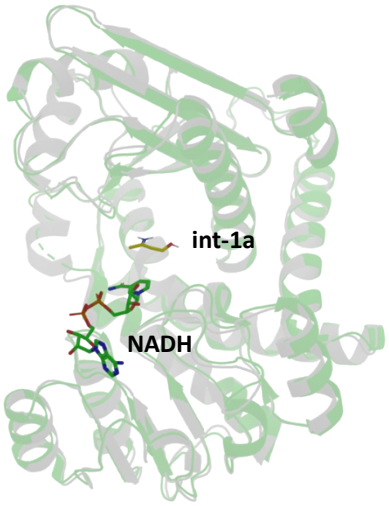


Fig. S13 Overlay of the homology model (mh174, grey) and crystal structure of wild-type leucine dehydrogenase from *Geobacillus stearothermophilus* (PDB ID 6ACH, chain a, green). The root-mean-square distance (RMSD) between them is 0.612 Å. The coordinates of cofactor NADH is superimposed from 6ACH, while the compound **int-1a** is docked into the substrate binding pocket.

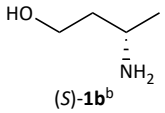
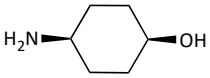
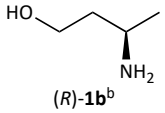
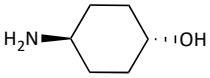
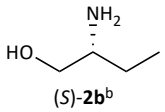
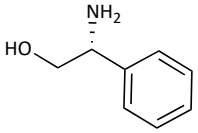
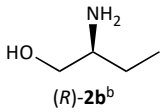
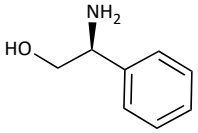
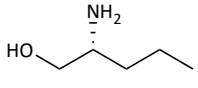
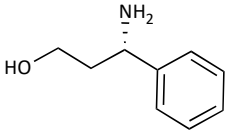
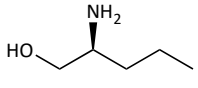
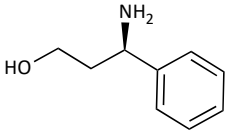
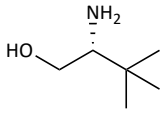
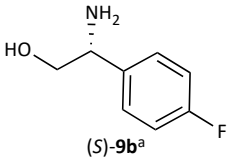
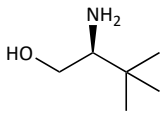
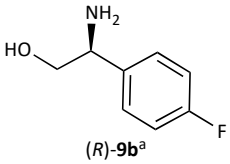
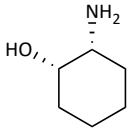
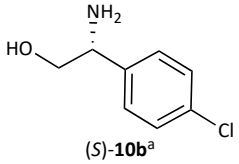
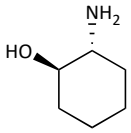
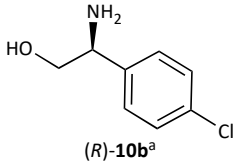
Table S1 Key residues selected for engineering *Gs*AmDH based on published data.

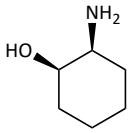
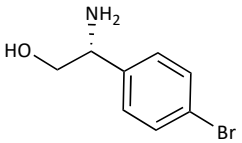
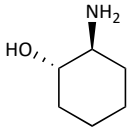
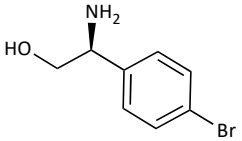
Entry	*Gs*AmDH site	Original site	Comments	Refs.
1	L40	L40	Binding pocket residues	3
2	G41	G41	Binding pocket residues	3
3	G42	G42	Binding pocket residues	3
4	T43	T41	Within 6 Å from substrate	4
5	L61	L61	Within 9-14 Å from substrate	5
6	M65	M65	Binding pocket residues	3
7	K68	K66	Interaction with the carboxylate group of natural substrate or responsible for altering the substrate specificity	4
8	N69	M67	Within 6 Å from substrate	4
9	I111	W114	Within 6 Å from substrate	4
10	T112	T115	Within 6 Å from substrate	4
11	A113	A113	Changing the substrate specificity or enlarging the active pocket	3
12	E114	E114	Altering the substrate specificity	3
13	D115	D115	Essential to the catalytic	3
14	V116	V116	Altering the substrate specificity	3
15	T134	T134	Enlarging the active pocket	6
16	P146	S149	Within 6 Å from substrate	4
17	T150	T153	Within 6 Å from substrate	4
18	H187	A187	Changing the substrate specificity	3
19	L239	L239	Within 9-14 Å from substrate	5
20	D261	N261	Responsible for altering the substrate specificity	6
21	N262	N262	Interact with the carboxyl group of the natural substrate	4
22	N287	N288	Within 6 Å from substrate	4
23	A288	A289	Within 6 Å from substrate	4
24	G290	G291	Within 6 Å from substrate	4
25	V291	V291	Binding pocket residues	3
26	I292	I292	Changing the substrate specificity	3
27	V294	V294	Binding pocket residues	3
28	A295	A295	Within 5 Å from substrate	5
29	E297	V294	Changing the substrate specificity	3

Table S2 Primes for construction of single-point saturation mutagenesis libraries.

Site	Primers
L40-F	CTGGGCCCGGCCNDT/VMA/ATG/TGGGGTGGTACACGTATGTG
G41-F	GGCCCGGCCCTGNDT/VMA/ATG/TGGGGTACACGTATGTGGATG
G42-F	GGCCCGGCCCTGGGTNDT/VMA/ATG/TGGACACGTATGTGGATG
T43-F	GCCCTGGGTGGTNDT/VMA/ATG/TGGCGTATGTGGATGTATAATAG
Y110-R	CACATCTTCGGCGGTAATATAGCGACCATTC
L61-F	GAAGATGCCCTGCGCNDT/VMA/ATG/TGGGCCCGCGGTATGACCTA
M65-F	CGCCTGGCCCGCGGTNDT/VMA/ATG/TGGACCTATAGCAATGCAGC
K68-F	CGCGGTATGACCTATNDT/VMA/ATG/TGGAATGCAGCAGCCGGT
N69-F	GGTATGACCTATAGCNDT/VMA/ATG/TGGGCAGCAGCCGGTCTG
I111-F	CTGAATGGTCGCTATNDT/VMA/ATG/TGGACCGCCGAAGATGTG
T112-F	GAATGGTCGCTATATTNDT/VMA/ATG/TGGGCCGAAGATGTGGG
A113-F	GGTCGCTATATTACCNDT/VMA/ATG/TGGGAAGATGTGGGTAC
E114-F	CGCTATATTACCGCCNDT/VMA/ATG/TGGGATGTGGGTACAACC
D115-F	GCTATATTACCGCCGAANDT/VMA/ATG/TGGGTGGGTACAACCGTG
V116-F	CTATATTACCGCCGAAGATNDT/VMA/ATG/TGGGGTACAACCGTGG
G180-R	GATATGCCACATTACCCACACCCTGCACGGCAAC
T134-F	GAAACCGATTATGTGNDT/VMA/ATG/TGGGGCATTAGTCCGGAATTTG
P146-F	GGTAGCAGCGGCAATNDT/VMA/ATG/TGGAGCCCGGCCACCG
T150-F	CAATCCGAGCCCGGCCNDT/VMA/ATG/TGGGCATACGGTGTGTATC
H187-F	GGTAATGTGGCATATNDT/VMA/ATG/TGGCTGTGTCGTCATCTG
L264-R	GACGCGGTTCTTTCAGCTGATTCAGTGCACTG
L239-F	GCGATATTTTTGCACCGTGCGCCNDT/VMA/ATG/TGGGGTGGCATTATTAATGATC
D261-F	GTTATTGCCGGCAGTGCANDT/VMA/ATG/TGGAATCAGCTGAAAG
N262-F	GCCGGCAGTGCACTGNDT/VMA/ATG/TGCAGCTGAAAGAACCG
N287-F	GATTATGTTATTNDT/VMA/ATG/TGGGCCGGCGGTGTGATTAATGTTGC
A288-F	GATTATGTTATTAATNDT/VMA/ATG/TGGGGCGGTGTGATTAATG
G290-F	GTTATTAATGCCGGCNDT/VMA/ATG/TGGGTGATTAATGTTGCAG
V291-F	GTTATTAATGCCGGCGGTNDT/VMA/ATG/TGGATTAATGTTGCAGATG
I292-F	AATGCCGGCGGTGTGNDT/VMA/ATG/TGGAATGTTGCAGATGAAC
V294-F	GGCGGTGTGATTAATNDT/VMA/ATG/TGGGCAGATGAACTGTATGG
A295-F	GCGGTGTGATTAATGTTNDT/VMA/ATG/TGGGATGAACTGTATGG
E297-F	GATTAATGTTGCAGATNDT/VMA/ATG/TGGCTGTATGGTTATAATCG
I360-R	GGACGACGACTCAGAATATGATGGCCATTC

Table S3 Parameters for HPLC analysis.

Substrates	Products	Retention time (min)	Substrates	Products	Retention time (min)
1a	 (*S*)-**1b**[b]	13.27	**6a**	 *cis*-**6b**[b]	15.68
	 (*R*)-**1b**[b]	16.70		 *trans*-**6b**[b]	16.89
2a	 (*S*)-**2b**[b]	12.63	**7a**	 (*S*)-**7b**[b]	14.91
	 (*R*)-**2b**[b]	17.94		 (*R*)-**7b**[b]	15.40
3a	 (*S*)-**3b**[a]	8.40	**8a**	 (*S*)-**8b**[a]	11.72
	 (*R*)-**3b**[a]	11.48		 (*R*)-**8b**[a]	9.43
4a	 (*S*)-**4b**[a]	9.45	**9a**	 (*S*)-**9b**[a]	7.91
	 (*R*)-**4b**[a]	12.21		 (*R*)-**9b**[a]	11.50
5a	 (*1S,2S*)-**5b**[a]	5.60	**10a**	 (*S*)-**10b**[a]	10.16
	 (*1R,2S*)-**5b**[a]	6.90		 (*R*)-**10b**[a]	12.46

	 (*1R*,*2R*)-**5b**[a]	11.30	**11a**	 (*S*)-**11b**[a]	10.59
	 (*1S*,*2R*)-**5b**[a]	10.87		 (*R*)-**11b**[a]	12.65

[a] HPLC conditions: Zorbax SB-C18 column (4.6 × 150 mm, 5 μm), detection wavelength: 340 nm, temperature: 25 °C, flow rate: 1 mL min^{-1}, loading volume: 10 μL, mobile phase buffer A: ddH_2O (0.1% trifluoroacetic acid), buffer B: methanol (0.1% trifluoroacetic acid), gradient program: 40% B; hold for 3 min; increase B to 60% in 4 min, increase B to 80% in 3 min; hold for 3 min; decrease B to 60% in 2 min.

[b] HPLC conditions: Zorbax SB-C18 column (4.6 × 150 mm, 5 μm), detection wavelength: 340 nm, temperature: 25 °C, flow rate: 1 mL min^{-1}, loading volume: 10 μL, mobile phase buffer A: ddH_2O (0.1% trifluoroacetic acid), buffer B: methanol (0.1% trifluoroacetic acid), gradient program: 40% B; hold for 6 min; increase B to 60% in 9 min; hold for 3 min; decrease B to 40% in 2 min.

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