Supplementary Material

Kinetic Resolution of Racemic Amines to Enantiopure (*S*)-amines by a Biocatalytic Cascade Employing Amine Dehydrogenase and Alanine Dehydrogenase

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The codon optimized gene sequence of *RS*-AmDH:

ATGAGCATCGATAGCGCGCTGAACTGGGATGGTGAAATGACCGTTACCCGTTTCGATGCGATGACCGGTGCG
CACTTCGTTATCCGCCTGGATAGCACCCAGCTGGGCCCGGCGGCGGGCGGTACCCGTGCAGCGCAGTACTCT
AACCTGGCTGATGCGCTGACCGACGCGGGTAAACTGGCGGGCGCGATGACCCTGCAGATGGCGGTGTCTAA
CCTGCCGATGGGTGGTGGTAAAAGCGTTATCGCGCTGCCGGCGCCGCGTCACAGCATCGACCCGTCTACCTG
GGCGCGTATCCTGCGTATCCACGCGGAAAACATCGATAAACTGTCTGGCAACTACTGGACCGGTCCGGACGT
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TGGCGGCGCAGGTAGCGGCGCGTTCACCACCGCCGTTGGCGTGTTCGAAGCGATGAAAGCTACCGTTGCGC
ACCGTGGTCTGGGTTCTCTGGATGGTCTGACCGTGCTGGTTCAGGGTCTGGGTGCGGTTGGCGGCTCTCTGGC
GTCTCTGGCGGCAGAAGCGGGTGCGCAGCTGCTGGTTGCGGATACCGATACCGAACGTGTGGCTCACGCGGT
TGCTCTGGGCCACACCGCTGTTGCGCTGGAAGATGTTCTGTCCACCCCGTGCGATGTATTCGCGCCGTGTGCT
ATGGGTGGCGTTATTACCACCGAAGTGGCTCGTACCTTGGATTGTTCTGTAGTAGCTGGTGCAGCATGTAACG
TTATTGCTGACGAAGCAGCTTCTGATATTTTACACGCACGTGGTATCTTATATGCACCAGATTTTGTTGCAAA
CGCTGGCGGTGCAATTCATTTAGTAGGTCGTGAAGTTTTAGGTTGGAGTGAAAGTGTTGTACACGAACGTGC
AGTGGCAATCGGTGATACTTTGAACCAGGTTTTTGAAATTTCCGATAACGATGGCGTGACCCCGGATGAAGC
GGCACGTACCCTGGCTGGTCGTCGTGCTCGTGAAGCAAGCACCACCACCGCGACCGCG

The amino acid sequence of *RS*-AmDH [1]:

MSIDSALNWDGEMTVTRFDAMTGAHFVIRLDSTQLGPAAGGTRAAQYSNLADALTDAGKL

AGAMTLQMAVSNLPMGGGKSVIALPAPRHSIDPSTWARILRIHAENIDKLSGNYWTGPDV

NTNSADMDTLNDTTEFVFGRSLERGGAGSGAFTTAVGVFEAMKATVAHRGLGSLDGLTVL

VQGLGAVGGSLASLAAEAGAQLLVADTDTERVAHAVALGHTAVALEDVLSTPCDVFAPCA

MGGVITTEVARTLDCSVVAGAACNVIADEAASDILHARGILYAPDFVANAGGAIHLVGRE

VLGWSESVVHERAVAIGDTLNQVFEISDNDGVTPDEAARTLAGRRAREASTTTATA

***Chi*-AmDH** [2]

The gene encoding *Chi*-AmDH was synthesized by Bionics (Seoul, Korea) and then inserted into IPTG-inducible pET-duet vector using restriction enzymes *Bam*H1 and *Sac*1. The expression and purification were performed as discussed in the previous section.

The codon optimized gene sequence of *Chi*-AmDH:

TCCCTGGTCGAAAAGACCTCTATCATCAAGGACTTCACCCTGTTCGAGAAGATGTCCGAGCACGAGCAGGTG
GTGTTCTGCAACGACCCAGCAACTGGTCTGCGTGCTATTATCGCAATCCACGACACTACTCTGGGTCCTGCAC
TGGGTGGTTGTCGTATGCAACCTTACAACTCTGTCGAGGAGGCTCTGGAAGATGCACTGCGTCTGTCTAAGG
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ATCGCGGGCTCCGCCCTGAATCAGCTGAAAGAACCGCGTCATGGCGATATGATTCACGAAATGGGCATCGTT
TACGCGCCGGATTATGTTATCAACGCCGGCGGCTGCATTAACGTTGCGGATGAACTGTACGGCTATAACCGT
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TAACATTCCGACGTATGTAGCCGCGGATCGCATGGCGGAAGAACGCATTGAAACCATGCGCAAAGCGCGTT
CCCAGTTTCTGCAGAACGGCCACCACATTCTGAGCCGCCGTCGTGCGCGCTAA

The amino acid sequence of *Chi*-AmDH:

SLVEKTSIIKDFTLFEKMSEHEQVVFCNDPATGLRAIIAIHDTTLGPALGGCRMQPYNSV

EEALEDALRLSKGMTYSCAASDVDFGGGKAVIIGDPQKDKSPELFRAFGQFVDSLGGRFY

TGTDMGTNMEDFIHAMKETNCIVGVPEAYGSSGNPSPATAYGVYRGMKAAAKEAFGSDSL

EGKVVAVQGVGNVAYHLCRHLHEEGAKLIVTDINKEAVARAVEEFGAKAVDPNDIYGVEC

DIFAPCALGGIINDQTIPQLKAKVIAGSALNQLKEPRHGDMIHEMGIVYAPDYVINAGGC

INVADELYGYNRERAMKKIEQIYDNIEKVFAIAKRDNIPTYVAADRMAEERIETMRKARS

QFLQNGHHILSRRRAR

The codon optimized gene sequence of AlaDH

ATGATCATCGGTGTTCCGAAAGAAATCAAAAACAATGAGAACCGCGTAGCACTGA
CTCCGGGTGGCGTGAGCCAGCTGATTAGTAATGGCCATAGGGTGCTAGTTGAAACCGGT
GCGGGGTTAGGTAGCGGTTTTGAAAACGAAGCCTACGAATCCGCGGGCGCTGAAATCA
TCGCTGACCCGAAACAGGTTTGGGATGCTGAAATGGTTATGAAAGTGAAGGAACCGCT
GCCGGAAGAATACGTGTACTTCCGCAAAGGCCTGGTTCTGTTCACCTACCTGCACCTGG
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TGCGCTGTGCTGATTCCGGGGGCAAAAGCCCCGACTCTTGTTACCGAAGAAATGGTTAA
ACAGATGAAACCAGGCTCTGTTATCGTTGATGTTGCTATTGACCAGGGTGGTATTGTTGA
AACCGTTGATCACATTACGACCCATGATCAGCCAACTTACGAAAAACACGGTGTTGTTC
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AACGTAACTGTTCCGTATGCATTGCAGATTGCTAACAAAGGCGCCGTAAAAGCGCTGG
CTGATAACACCGCTCTGCGTGCTGGTCTGAACACCGCTAACGGTCACGTGACTTATGAA
GCGGTAGCGCGTGATTTAGGTTATGAATATGTTCCGGCAGAAAAAGCACTGCAGGATG
AATCTAGCGTTGCAGGTGCA TAA

Enzyme Expression and Purification

The gene encoding *Chi*-AmDH [2] and *Rs*-AmDH [1] was synthesized by Bionics (Seoul, Korea) and then inserted into IPTG-inducible pET-24ma vector. After the transformation of the plasmids into *E. coli* (BL21) cells, they were grown in 0.5 L LB-containing Ampicillin (50 μg/mL) at 37 ˚C. The cells were induced with 0.1 mM IPTG when OD_{600} reached 0.6-0.8. The cells were harvested by centrifugation (4000 rpm, 10 min) after the overnight induction at 20°C. The harvested cells were washed twice with 50 mM Tris buffer (pH 7.0). The washed cells were collected by centrifugation and resuspended in 10 mL lysis buffer [Na_2HPO_4 (50 mM), NaCl (300 mM), Imidazole (5 mM, pH 8.0). The cell suspension was then disrupted by a probe sonicator (Sonics& Material Inc, USA). Sample was held in ice bath to control the temperature during sonication. The pulse of a sonicator was set at 5 sec on and 5 sec off. The His6-tagged enzyme from the cell lysate was purified at 4°C on a Ni-NTA agarose resin [Qiagen, Hilden, Germany] [4]. The enzymes were eluted with the increasing gradient of imidazole [50-250 mM]. The eluted solution containing the purified protein was dialyzed against 20 mM Tris-HCl buffer (pH 8.0) and concentrated using an Amicon PM-10 ultrafiltration unit [5]. Glycerol (25% v/v) was added to the purified enzyme solution and stored at −20 °C for further study.

Co-expression of *Chi*-AmDH and AlaDH

The gene encoding AmDH was cloned into pET24ma vector and AlaDH in pET-duet vector. Both the plasmids were transformed to *E. coli* BL21 cells by heat shock method. The cells were grown in the presence of Ampicillin (100 μg/mL) and kanamycin (50 μg/mL). Once OD_{600} of the cells reached 0.6-0.8, IPTG was added (0.1 mM final concentration) and cells were cultivated overnight. The cells were harvested by centrifugation and washed with tris buffer (20 mM, pH 7.0). Cells were centrifuged again, resuspended in Glycine buffer (100 mM, pH 10.0) and used for whole-cell transformations.

Enzyme Assay

The activities of AmDHs and AlaDH were determined using an NADH-dependent (340 nm, λ 340 = 6220 M^{-1} cm^{-1}) spectrophotometric assay as previously reported [3]. Activity was calculated from the stoichiometric reduction of NAD^+ or oxidation of NADH as measured by the change in absorbance over time. One unit of activity is defined as the amount enzyme that catalyzes the formation of 1 μmol of NADH or NAD^+ in 1 min [2].

Analysis of Amines

The analysis of kinetic resolution of racemic amines to their enantiopure (S)-amines was performed as previously reported [3,5]. Briefly, the conversion and ee analysis of amines was performed by HPLC using a Crownpak CR (Daicel Co., Japan) column at 210 nm with an elution of pH 1.5 perchloric acid solution (0.6 mL min^{-1}). Quantitative chiral analysis of enantiomers not separated by these conditions was performed using a C18 symmetry column (Waters, MA) with Waters HPLC system at 254 nm after derivatization with GITC. Separation of each enantiomer was achieved through an isocratic elution with a mixture of 50% methanol and 50% water (0.1% TFA) at a flow rate of 1.0 mL min^{-1} .

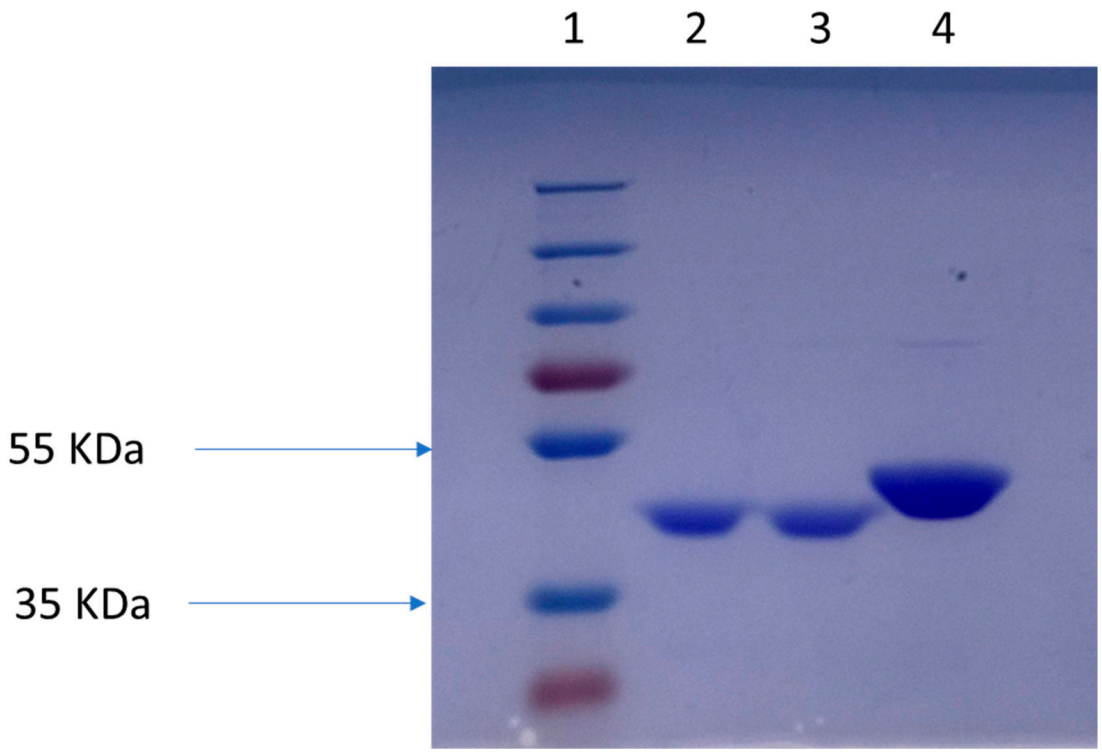

Figure S1: SDS-PAGE analysis of purified AmDHs and AlaDH [Lane 1: Molecular weight markers; Lane2: *Chi*-AmDH; Lane 3: *Rs*-AmDH; Lane 4: AlaDH]

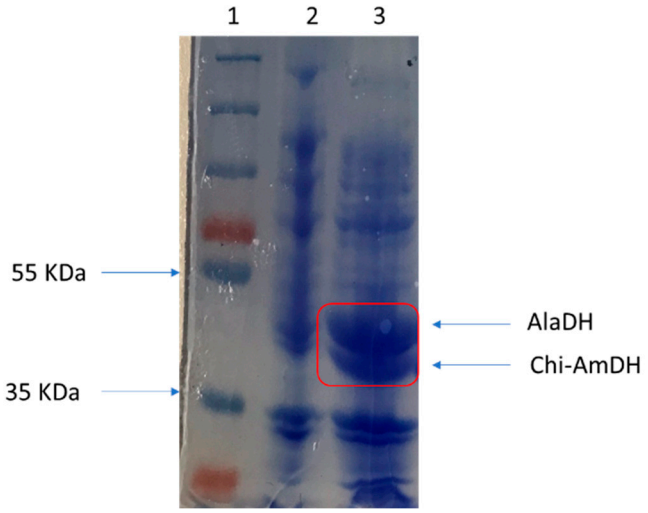

Figure S2: SDS-PAGE analysis of whole-cells expressing AmDHs and AlaDH [Lane 1: Molecular weight markers; Lane2: uninduced *E. coli* cells; Lane 3: *E. coli* cells co-expressing *Chi*-AmDH and AlaDH]

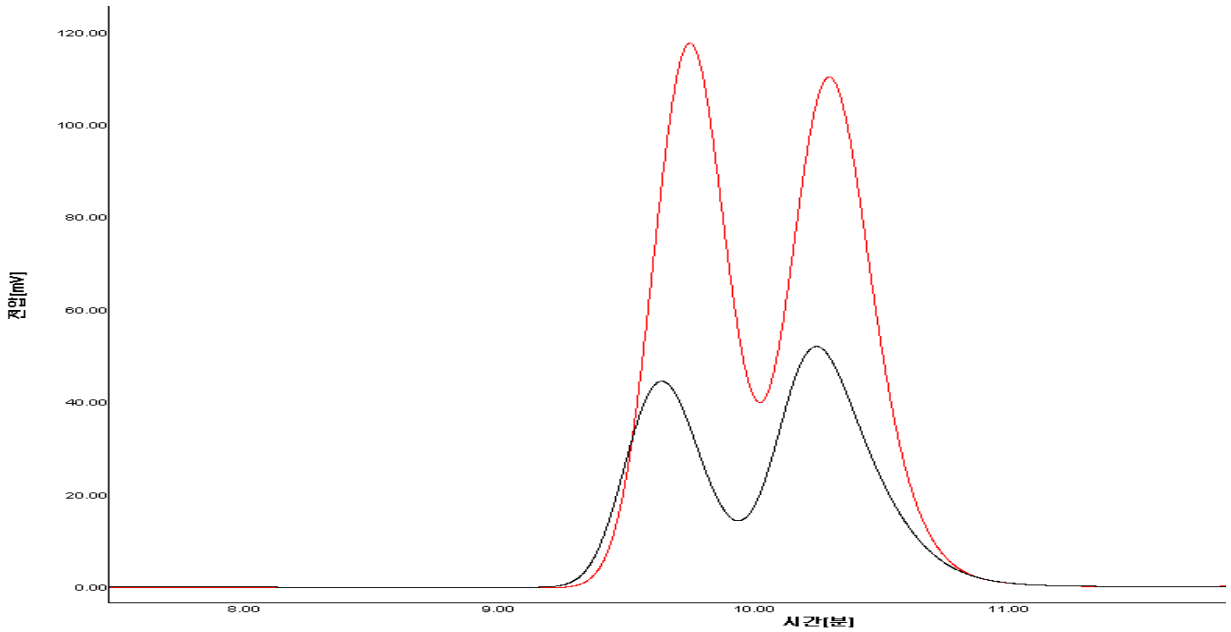

Figure S3: HPLC analysis of kinetic resolution of *rac*-**a1** (red line) to (*S*)- **a1** (black line)

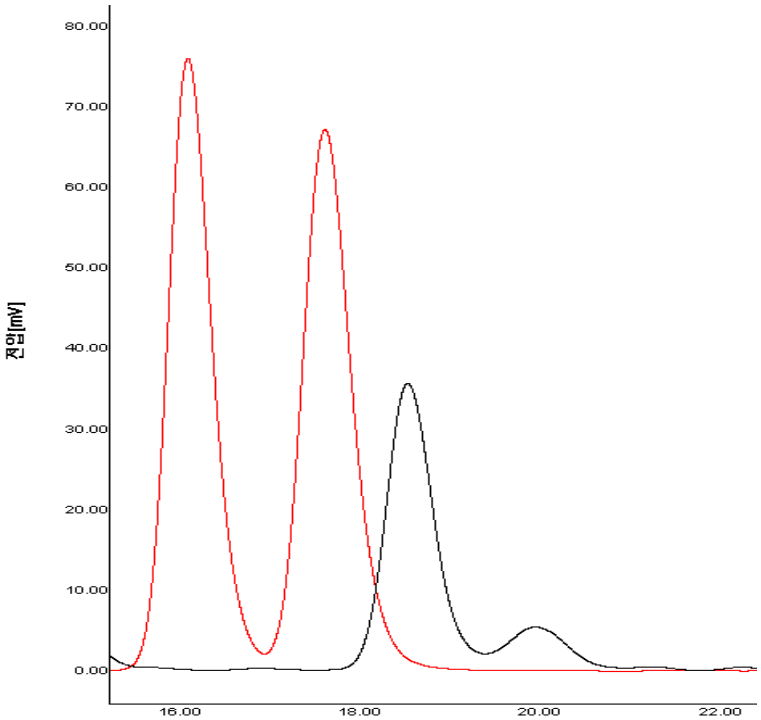

Figure S4: HPLC analysis of kinetic resolution of *rac*-**a2** (red line) to (*S*)- **a2** (black line)

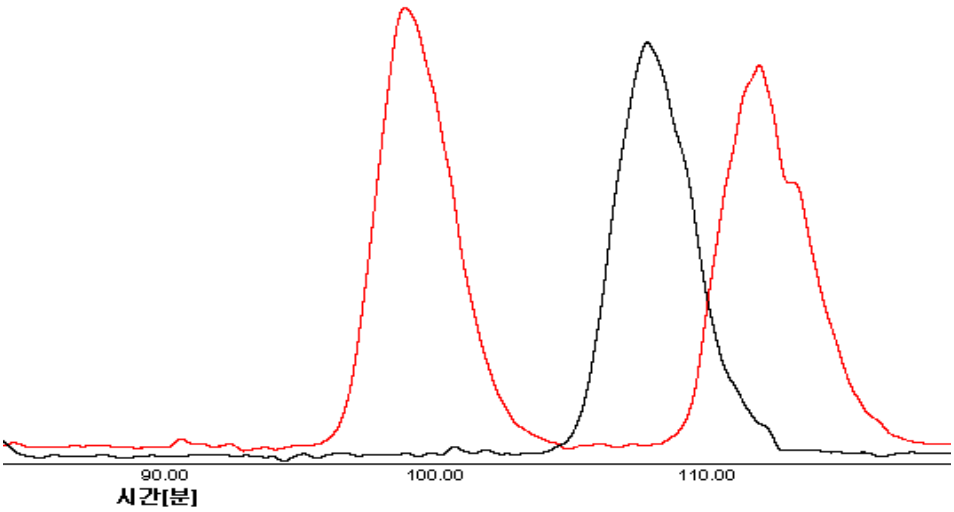

Figure S5: HPLC analysis of kinetic resolution of *rac*-**a3** (red line) to enantiopure (*S*)- **a3** (black line)

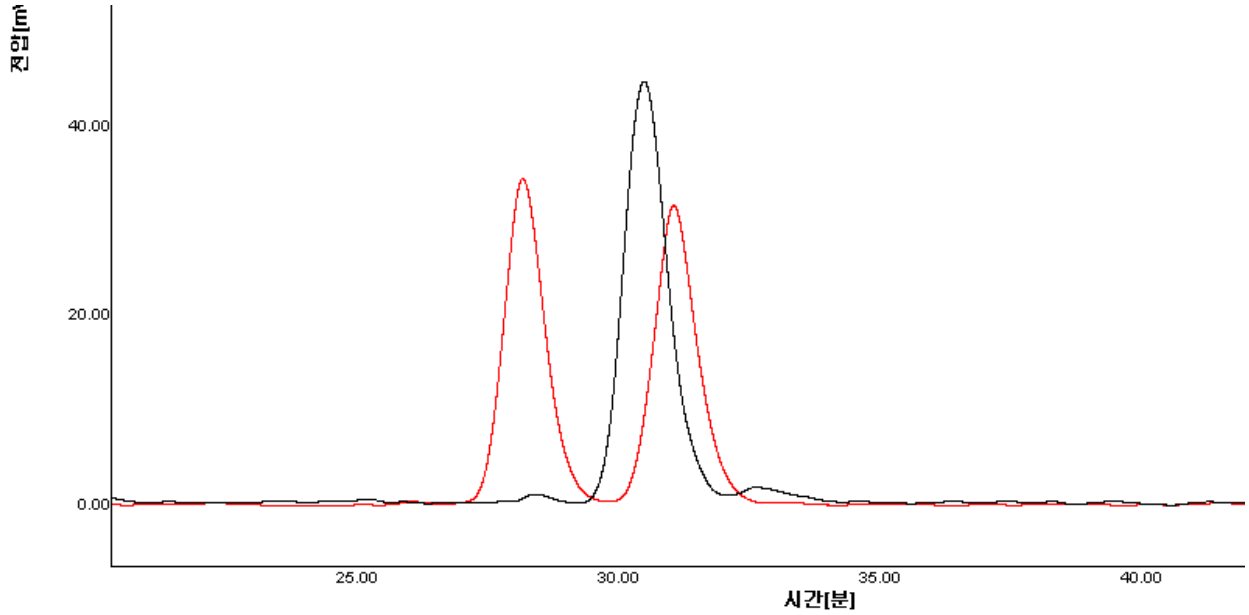

Figure S6: HPLC analysis of kinetic resolution of *rac*-**a4** (red line) to enantiopure (*S*)- **a4** (black line)

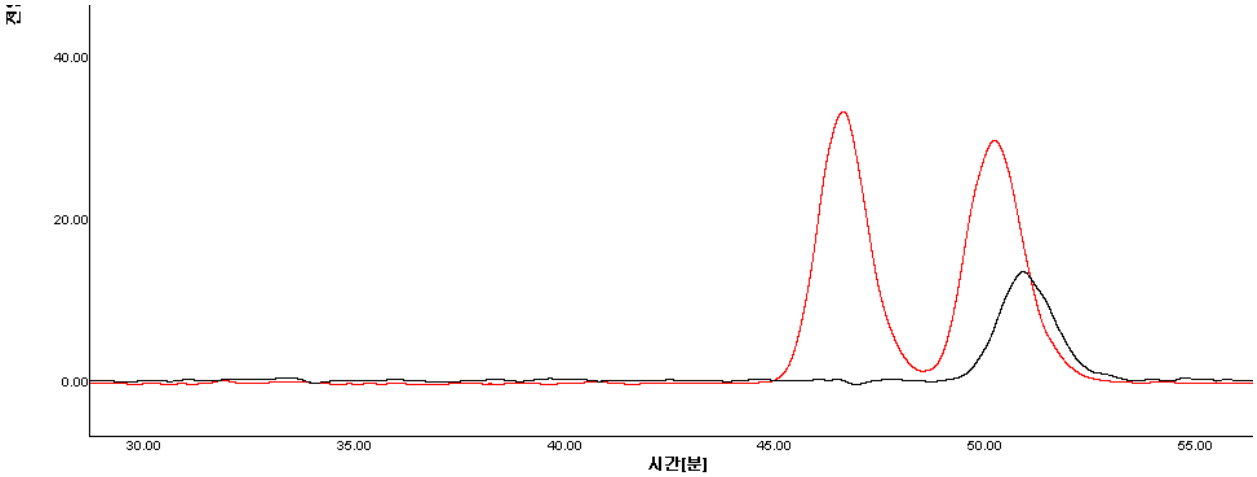

Figure S7: HPLC analysis of kinetic resolution of *rac*-**a5** (red line) to enantiopure (*S*)- **a5** (black line)

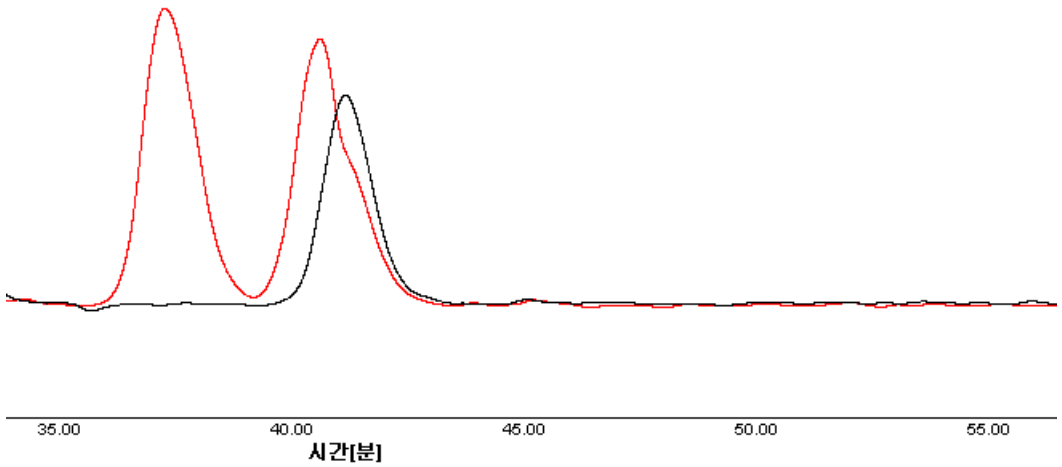

Figure S8: HPLC analysis of kinetic resolution of *rac*-**a6** (red line) to enantiopure (*S*)- **a6** (black line)

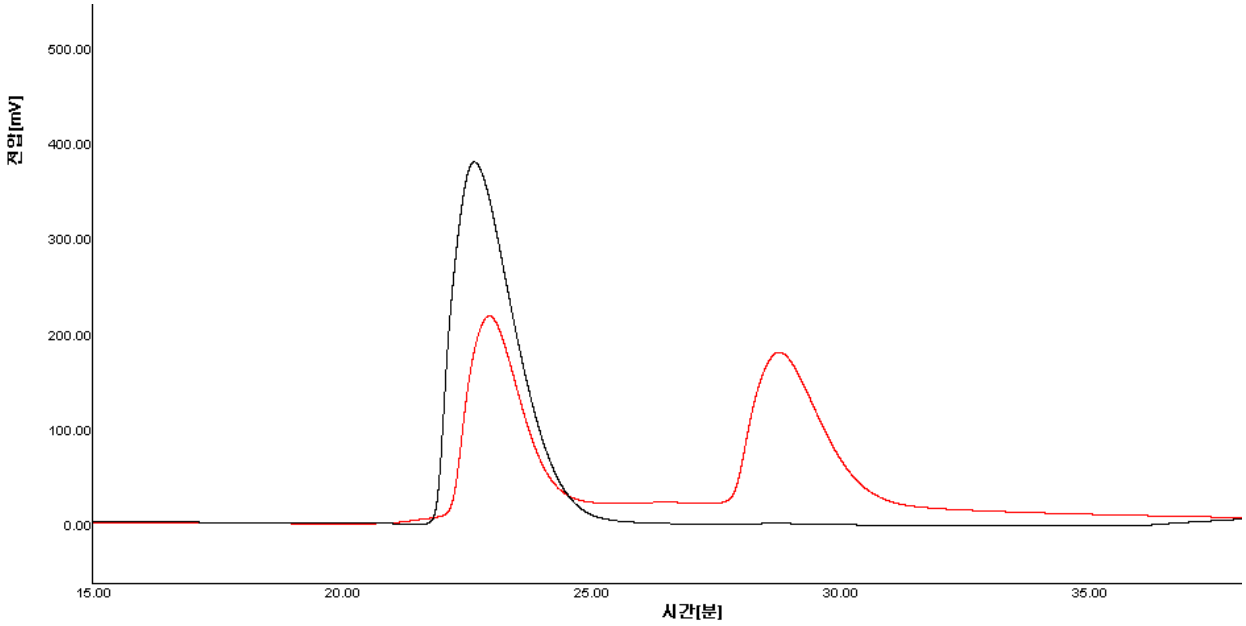

Figure S9: HPLC analysis of kinetic resolution of *rac*-**a7** (red line) to enantiopure (*S*)- **a7** (black line)

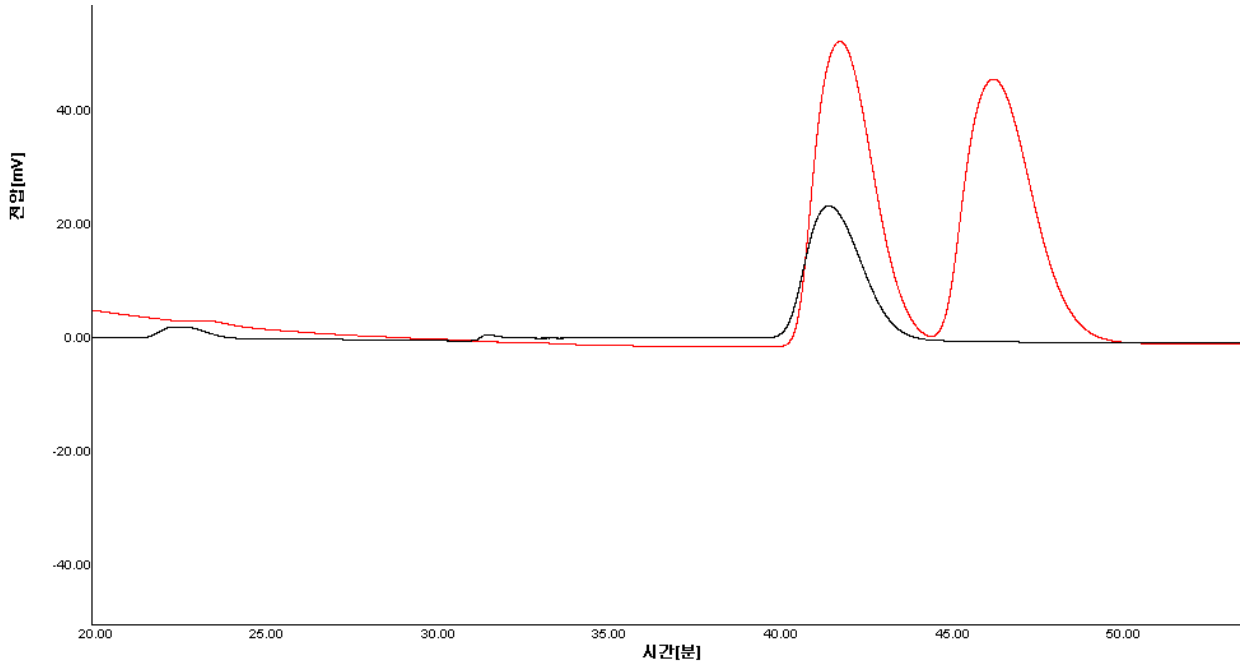

Figure S10: HPLC analysis of kinetic resolution of *rac*-**a8** (red line) to enantiopure (*S*)- **a8** (black line)

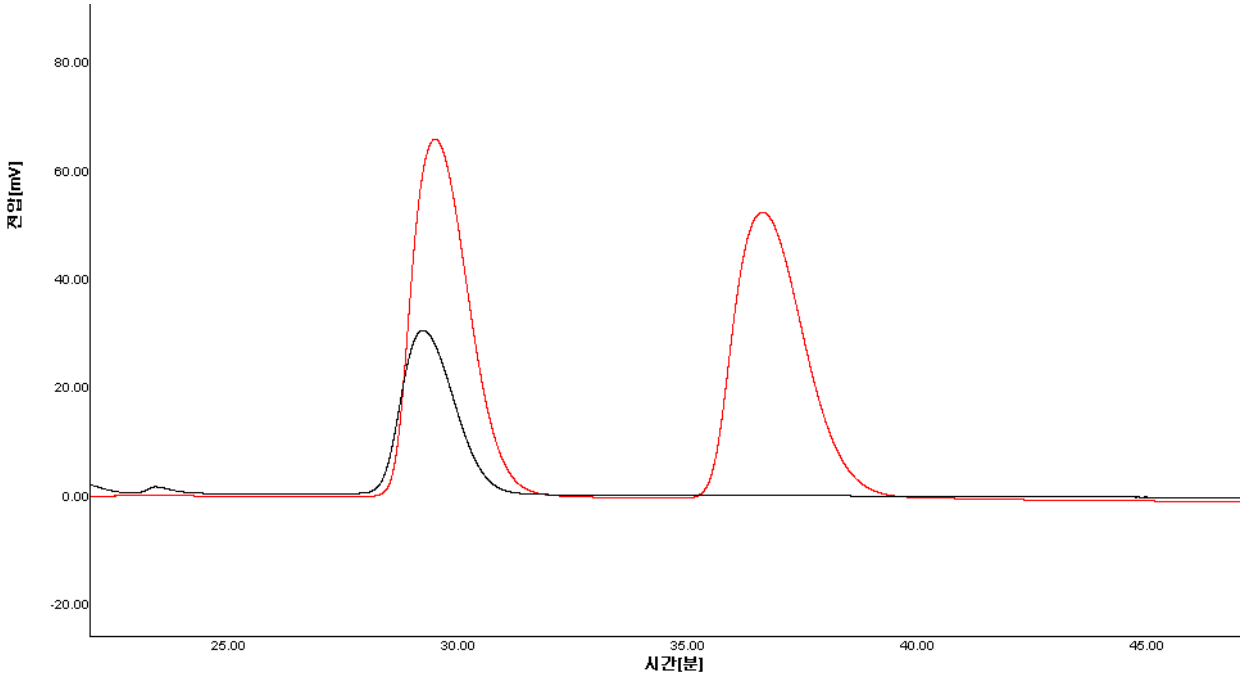

Figure S11: HPLC analysis of kinetic resolution of *rac*-**a9**(red line) to enantiopure (*S*)- **a9** (black line)

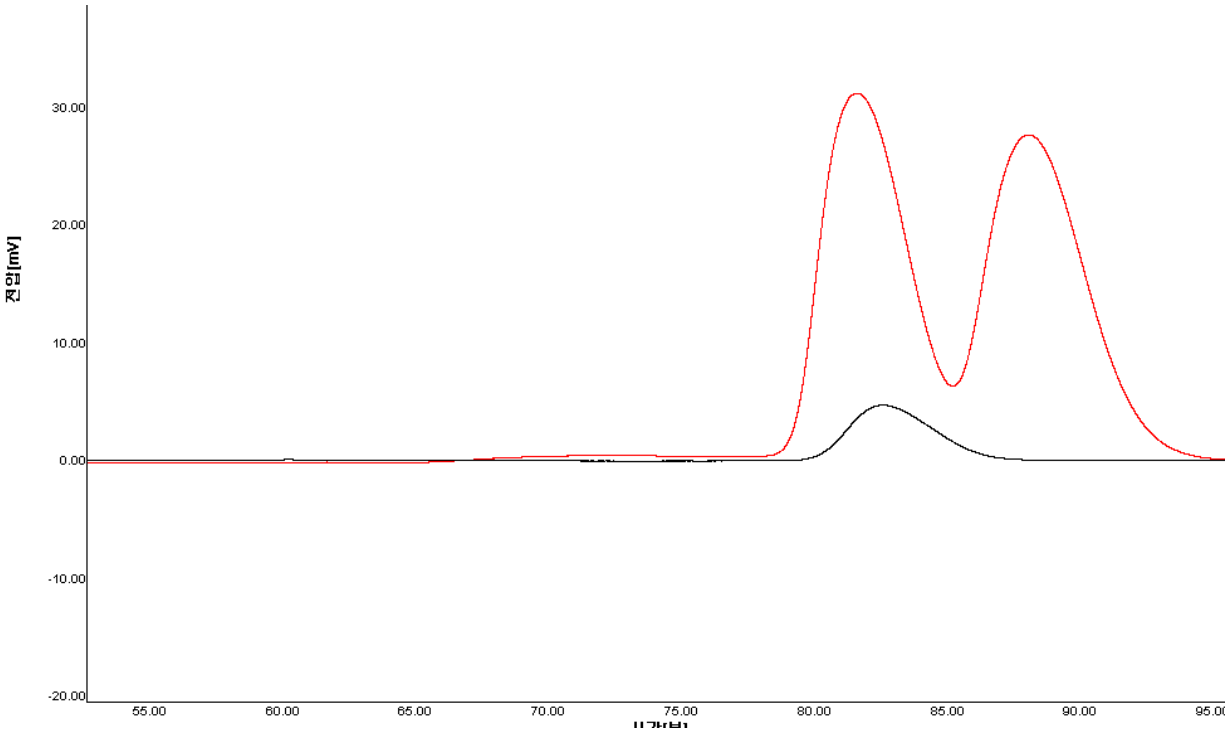

Figure S12: HPLC analysis of kinetic resolution of *rac*-**a10** (red line) to enantiopure (*S*)- **a10** (black line)

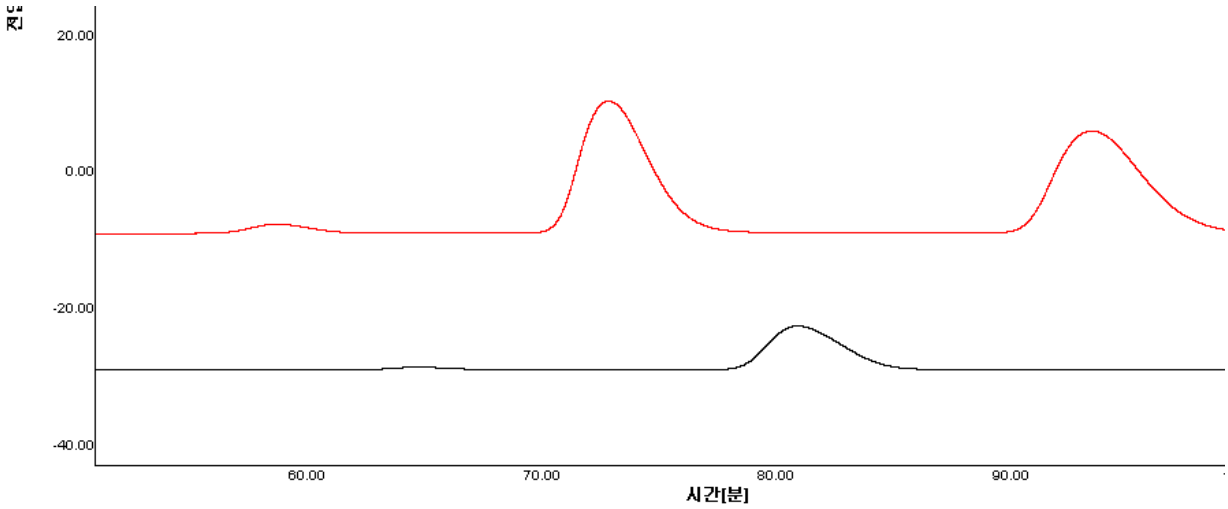

Figure S13: HPLC analysis of kinetic resolution of *rac*-**a11** (red line) to enantiopure (*S*)- **a11** (black line)

Table S1. Amine retention times

Substrate	Retention time (min)	
	(S)	*(R)*
a1[b]	9.5	110.5
a2[b]	19	10
a3[b]	100	108
a4[b]	27	31
a5[b]	46	51
a6[b]	36	42
a7[c]	22	29
a8[b]	41	46
a9[b]	29	37
a10[c]	82	89
a11[c]	71	91

[b] sample was analyzed using Crownpak CR(+) column
[c] sample was analyzed using C18 symmetry column after GITC derivatization [3]

References

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2. Bommarius, B.R., Schürmann, M. and Bommarius, A.S. A novel chimeric amine dehydrogenase shows altered substrate specificity compared to its parent enzymes. *Chemical Communications*, **2014**, *50*, 14953-14955.
3. Jeon, H.; Yoon, S.; Ahsan, M.; Sung, S.; Kim, G.H.; Sundaramoorthy, U.; Rhee, S.K.; Yun, H. The Kinetic resolution of racemic amines using a whole-cell biocatalyst co-expressing amine dehydrogenase and NADH oxidase. *Catalysts*, **2017**, *7*, 251.
4. Kim, G.H.; Jeon, H.; Khobragade, T.P.; Patil, M.D.; Sung, S.; Yoon, S.; Won, Y.; Choi, I.S.; Yun, H. Enzymatic synthesis of sitagliptin intermediate using a novel ω-transaminase. *Enzyme Microb. Technol.* **2019**, *120*, 52-60.
5. Yoon, S.; Patil, M.D.; Sarak, S.; Jeon, H.; Kim, G.H.; Khobragade, T.P.; Sung, S.; Yun, H. Deracemization of Racemic Amines to Enantiopure (*R*)-and (*S*)-amines by Biocatalytic Cascade Employing ω-Transaminase and Amine Dehydrogenase. *ChemCatChem* **2019**, *11*, 1898-1902.