Supplementary Information

Kinetic resolution of aromatic β-amino acids by ω-transaminase

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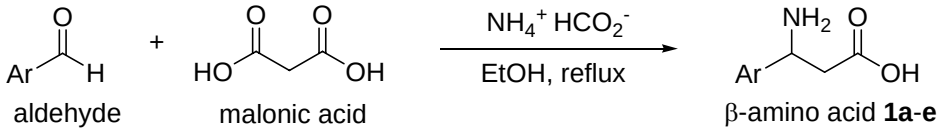
1. Materials

Lipase from *Candida rugosa* (no. L1754), ethyl 3-oxo-3-phenylpropionate(no. 12980), Isopropyl-β-D-thiogalactopyranoside (IPTG), 2,3,4,6-tetra-*o*-acetyl-α-D-glucopyranosyl isothiocyanate (GITC), *rac*-β-homoalanine, *rac*-β-homoleucine, (*S*)-α-MBA, pyruvate, α-ketoglutarate, β-alanine, benzaldehyde, 2-oxobutyrate, fluoropyruvate were from Sigma-Aldrich, Korea. The rest of the chemicals were of analytical or reagent grade.



2. General procedure for the synthesis of racemic β-amino acids (1a–e)

To a 100 mL round-bottomed flask equipped with a magnetic stirrer and reflux condenser, malonic acid (3.3 g, 25 mmol), ammonium formate (3.2 g, 50 mmol), aromatic aldehyde (25 mmol) and ethanol (50 mL) were added. The resulting mixture was then refluxed for 6 h. The reaction mixture was allowed to cool to room temperature and then evaporated to dryness. To this residue acetone was added (30 mL), and the resulting white solid was collected by filtration. The crude product was further purified by recrystallization (water–ethanol) to provide the product as a white crystalline solid.

3-Amino-3-phenylpropanoic acid (1a) yield: (3.8 g, 23 mmol, 92%); ^{1}H NMR (400 MHz, D_2O): δ 7.33-7.28 (m, 5H) 4.50-4.43 (m, 1H) 2.77-2.59 (m, 2H). The NMR data are in accordance with those reported in the literature. [1]

3-Amino-3-(4-fluorophenyl)propanoic acid (1b) yield: (1.98 g, 20.8 mmol, 83%);^{1}H NMR (400 MHz, D_2O): δ 7.36-7.24 (m, 2H), 7.10-6.98 (m, 2H), 4.54-4.46 (m, 1H), 2.76 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.65 (dd, *J* = 16.0, 6.8 Hz, 1H). The NMR data are in accordance with those reported in the literature. [1b,2]

3-Amino-3-(4-methoxyphenyl)propanoic acid (1c) yield: (3.96 g 20.3 mmol, 81%);^{1}H NMR (400 MHz, D_2O): δ 7.32-7.22 (m, 2H), 6.94-6.88 (m, 2H), 4.46 (dd, *J* = 8.0, 6.8 Hz, 1H), 3.69 (s, 3H), 2.76 (dd, *J* = 16.2, 8.2 Hz, 1H), 2.64 (dd, *J* = 16.0, 6.4 Hz, 1H; ^{13}C NMR (100 MHz, D_2O): δ 177.4, 159.5, 128.7, 114.7, 55.6, 52.4, 40.5. The NMR data are in accordance with those reported in the literature. [3]

3-Amino-3-(3,4-dimethoxyphenyl)propanoic acid (1d) yield: (4.39 g, 19.5 mmol, 78%); ^{1}H NMR (400 MHz, D_2O): δ 6.94-6.83 (m, 3H), 4.43 (dd, *J* = 8.4, 6.4 Hz, 1H), 3.70 (s, 3H), 3.68 (s, 3H), 2.74 (dd, *J* = 16.4, 8.4 Hz, 1H), 2.62 (dd, *J* = 16.0, 6.4 Hz, 1H). The NMR data are in accordance with those

reported in the literature.[4]

3-Amino-3-benzo[1,3]dioxol-5-ylpropanoic acid (1e) yield: (1.55 g 7.41 mmol, 57%);^{1}H NMR (400 MHz, D_2O): δ 6.87-6.75 (m, 3H), 5.86 (s, 2H), 4.43 (t, *J* = 7.5 Hz, 1H), 2.74 (dd, *J* = 16.4, 8.4 Hz, 1H), 2.64 (dd, *J* = 16.0, 6.4 Hz, 1H); ^{13}C NMR (100 MHz, D_2O): δ 177.3, 147.92, 147.88, 129.9, 121.3, 107.4, 101.7, 52.8, 40.6. The NMR data are in accordance with those reported in the literature.[4]

3. Enzyme expression and purification

To express Po ω-TA with a His-tagged polypeptide, the coding region of the enzyme was amplified by PCR using the primers P1 (5′-CGCCATATGAACAAGCCGTCCACGTCTTCCATG-3′) and P2 (5′-CCCAAGCTTGACCTGCAACGGGCAACAGCG -3′) from the genomic DNA of *Polaromonas* sp. strain JS666. The PCR product was digested with *Nde*I and *Hind*III and inserted into the IPTG-inducible expression vector pET24ma.[5] The plasmid was then introduced into the *E. coli* (BL21) and transformants were grown at 37°C in 1L LB broth containing 50 μg mL^{-1} of kanamycin. When the OD_{600} reached 0.25, IPTG was added to a final concentration of 0.5 mM. After 6 h of induction, the cells were harvested and washed twice with 50 mM phosphate buffer (pH 8.0). After centrifugation, cell pellet was resuspended in 2 volumes of 20 mM phosphate buffer (pH 7.0) containing 20 μM pyridoxal 5′-phosphate (PLP), 2 mM EDTA, 1 mM PMSF and 10% glycerol. It was then subjected to ultrasonic disruption for 15 min.

The C-terminal His_6-tagged fusion protein was purified at 4°C on a Ni-NTA agarose resin obtained from Qiagen (Hilden, Germany). Briefly, the crude extract was passed directly over a column containing 3 mL of Ni-NTA agarose resin. The column was then washed with 50 mL of phosphate buffer (pH 8.0) containing 20 mM imidazole, and the C-terminal His_6-tagged protein was eluted with phosphate buffer (pH 8.0) containing 200 mM imidazole. The eluted solution containing purified protein was dialyzed against 50 mM potassium phosphate buffer (pH 7.0) containing 20 μM PLP and concentrated using an Amicon PM-10 ultrafiltration unit. The glycerol was added to the purified enzyme solution (25% glycerol) and stored at –20°C for further study (Figure S1).

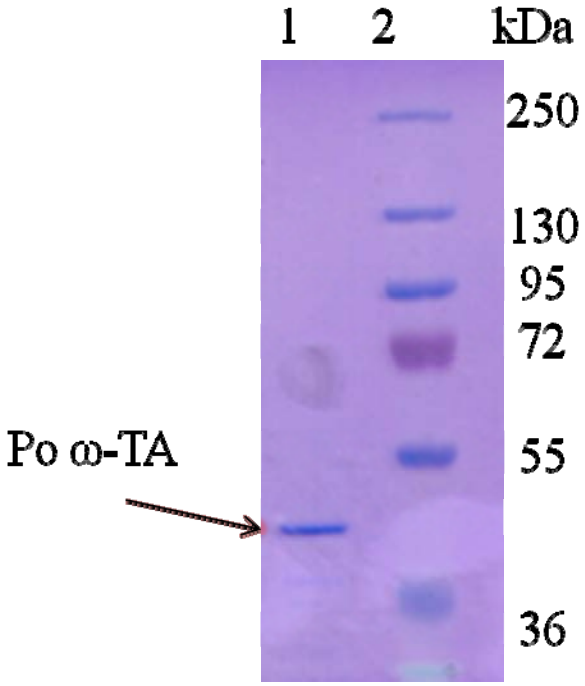


Figure S1. SDS-PAGE of purified enzyme. Proteins were separated on a 10% polyacrylamide gel in the presence of 1% SDS. Lane 1, purified enzyme (47 kDa); lane 2, molecular marker.

4. Enzyme assay

One unit of enzyme is defined as the amount that catalyzes the product formation of 1 μmol within 1 min at the defined condition (see Table 1 on the main text) at 37°C. To determine the optimal pH, initial reaction rate was measured by analyzing the formation of L-Ala in the presence of 10 mM (*S*)-**1a** and 10 mM pyruvate within a pH range of 6.0 to 10 [100 mM phosphate buffer (pH 6.0–8.0) and 100 mM Tri/HCl buffer (pH 7.5–10.0)]. Reaction condition; 1 mL of reaction volume, 10 mM pyruvate, 10 mM (*S*)-**1a**, the enzyme (3.15 μg mL^{-1}) and various buffer and incubation at 37°C for 30 min. Po ω-TA showed the highest activity at pH 8.5.

5. Analytical methods

5.1 The analysis of β-amino acids (1a-e)

The conversion analysis of β-amino acids was carried 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}). Each enantiomer was not separated in this analytical condition but the exact conversion could be calculated (Table S1). Quantitative chiral analysis of β-amino acids was performed using a C_{18} Symmetry column (Waters, MA) with a Waters HPLC system at 254 nm after the derivatization of sample with GITC. [6] Separation of each enantiomer was achieved through an isocratic elution with a mixture of 55% methanol and 45% water (0.1% TFA) at a flow rate of 1.0 mL min^{-1}.

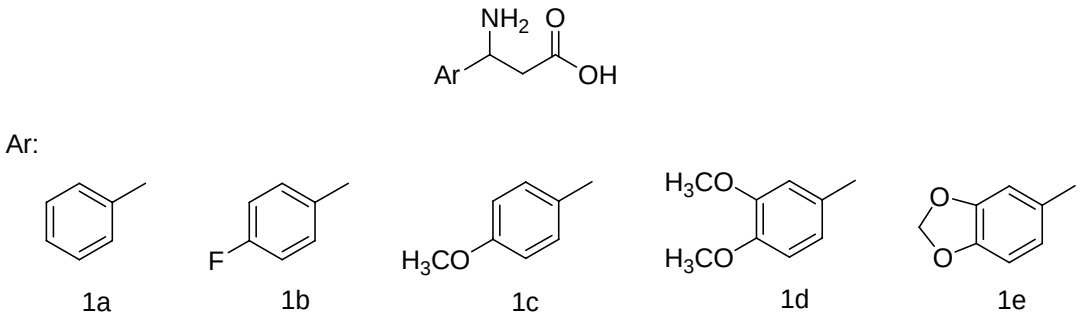


Figure S2. The structure of β-amino acids.

Table S1 The retention time of β-amino acid **1a**–**e**.

Substrate	Retention time (min)[a]		Retention time (min)[b]
	(*R*)	(*S*)	
1a	16.6	21.0	37
1b	20.5	26.6	43
1c	18.2	22.3	58
1d	12.2	14.2	63
1e	17.7	22.2	53

[a] sample was analyzed by C_{18} Symmetry column after GITC derivatization.

[b] sample was analyzed by Crownpak CR column.

5.2 The analysis of α-amino acids, pyruvate, and acetophenone

Quantitative chiral analysis of Ala, 2-amino butyric acid, 3-fluoroalanine was performed using a C_{18} Symmetry column (Waters, MA) with a Waters HPLC system at 254 nm after the derivatization of sample with GITC.[6] Separation of each enantiomer was achieved through an isocratic elution with a mixture of 55% methanol and 45% water (0.1% TFA) at a flow rate of 1.0 mL min^{-1}. To quantify each enantiomer, an appropriate amount of valine (an internal standard) was added to the stop solution, and the mixture was derivatized with GITC. After derivatization, the retention times for L-Ala, D-Ala, (*R*)-3-fluoroalanine, (*S*)-3-fluoroalanine and L-Val were observed to be 4.25, 5.49, 5.20, 6.81 and 9.01 min, respectively. The chirality of (*R*)-3-fluoroalanine, (*S*)-3-fluoroalanine was determined based on the retention times. Generally, L-amino acids have shorter retention time than that of D-amino acid after derivatization. In the case of 2-amino butyric acid, GITC derivatized-samples are analyzed with an eluent (50% methanol/50% water (0.1% TFA)). After derivatization, the retention times of L-2-amino butyric acid and D-2-amino butyric acid were observed to be 8.5 and 11.8 min, respectively. Pyruvate was analyzed using an Aminex HPX-87H HPLC column (Bio-Rad, CA) with an elution of 5 mM sulfuric acid solution at UV 210 nm. Acetophenone was analyzed using a C_{18} Symmetry column (Waters, MA) with an elution mixture of 50% methanol and 50% water (0.1% TFA) at a flow rate of 1.0 mL min^{-1}.[6]

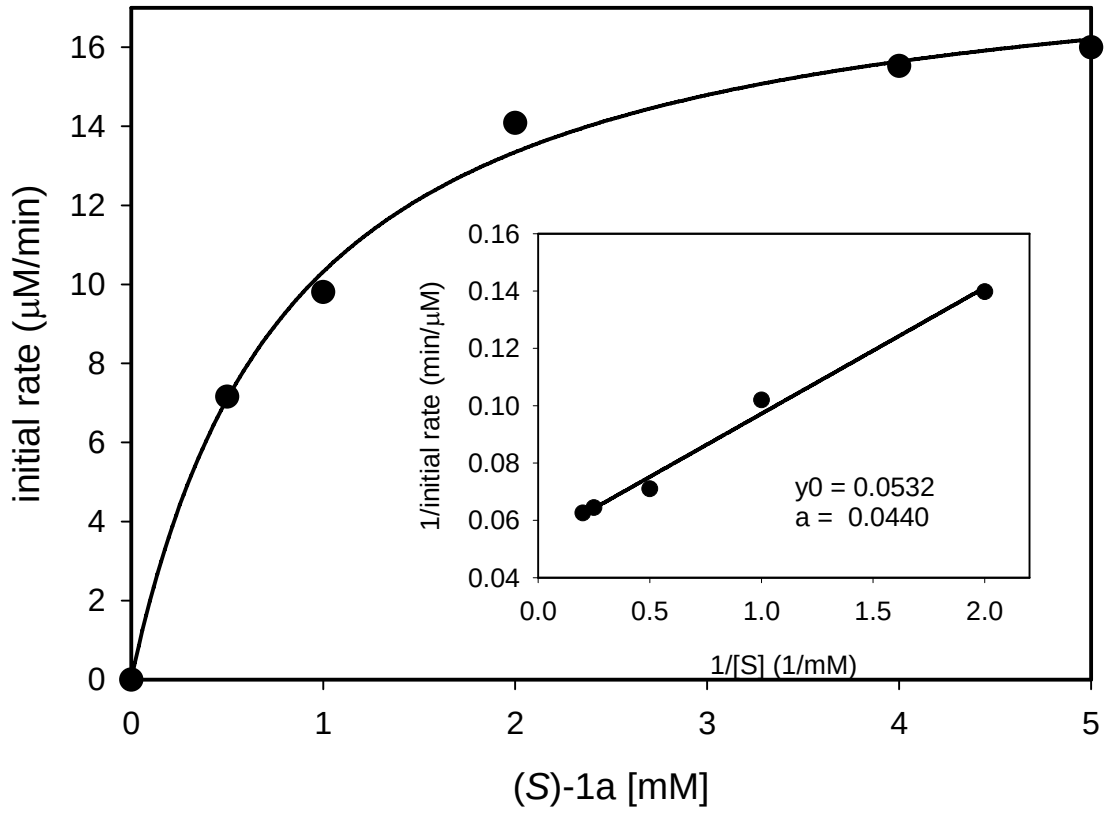


Figure S3. Michaelis–Menten plot of the reaction of Po ω-TA on (*S*)-**1a** and the Lineweaver–Burk plot (k_{cat} = 292 min^{-1} and K_m = 0.83 mM). Reaction conditions; 1 mL of reaction volume, 10 mM pyruvate, (*S*)-**1a** (0–5 mM), the enzyme (3.15 μg mL^{-1}), 100 mM Tris/HCl buffer (pH 8.5) and incubation at 37°C for 30 min.

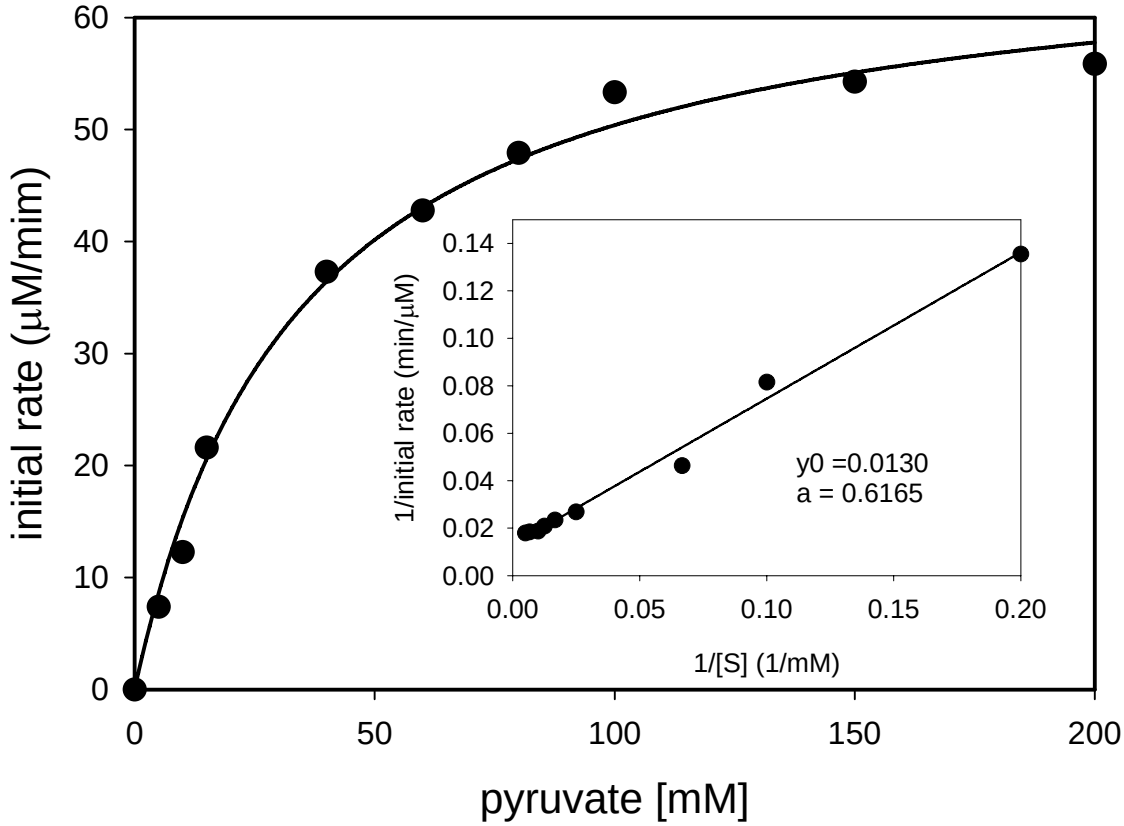


Figure S4. Michaelis–Menten plot of the reaction of Po ω-TA on pyruvate and the Lineweaver–Burk plot (k_{cat} = 1190 mim^{-1} and K_m = 47.4 mM). Reaction conditions; 1 mL of reaction volume, 10 mM (*S*)-**1a**, pyruvate (0–200 mM), the enzyme (3.15 μg mL^{-1}), 100 mM Tris/HCl buffer (pH 8.5) and incubation at 37°C for 30 min..

Table S2. Preparation of (*R*)-**1a–e** using Po ω-TA-catalyzed kinetic resolution of racemic substrates.

Substrate	Reaction time (h)	Conversion (%)	*ee* (%)	*E*
1a	3	50.5	>99	>100
1b	3	51.0	>99	>100
1c	3	50.7	>99	>100
1d	3	50.5	>99	>100
1e	3	51.0	>99	>100

The reaction conditions; 1 mL of reaction volume, 10 mM *rac*-**1a–e**, 10 mM pyruvate, Po ω-TA (15.7 μg mL^{-1}), 100 mM Tris/HCl buffer (pH 8.5) and incubation at 37°C.

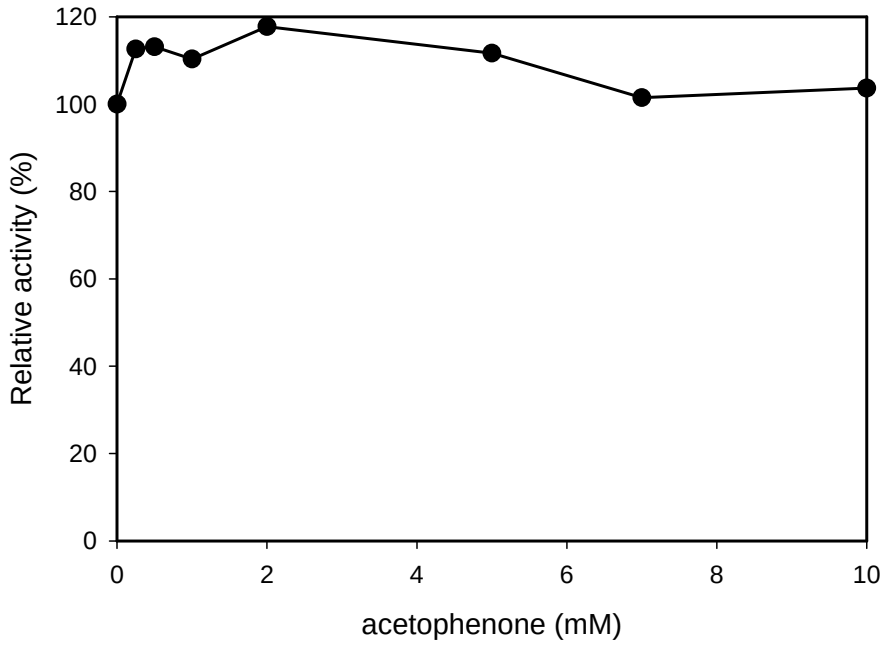


Figure S6. The product inhibition of Po ω-TA by acetophenone. The initial rate was determined by measuring the alanine produced. Reaction condition: 1 mL of reaction volume, 10 mM (*S*)-**1a**, 10 mM pyruvate, Po ω-TA (3.15 μg mL^{-1}), various concentrations of acetophenone (0–10mM) and 100 mM Tris/HCl buffer (pH 8.5).

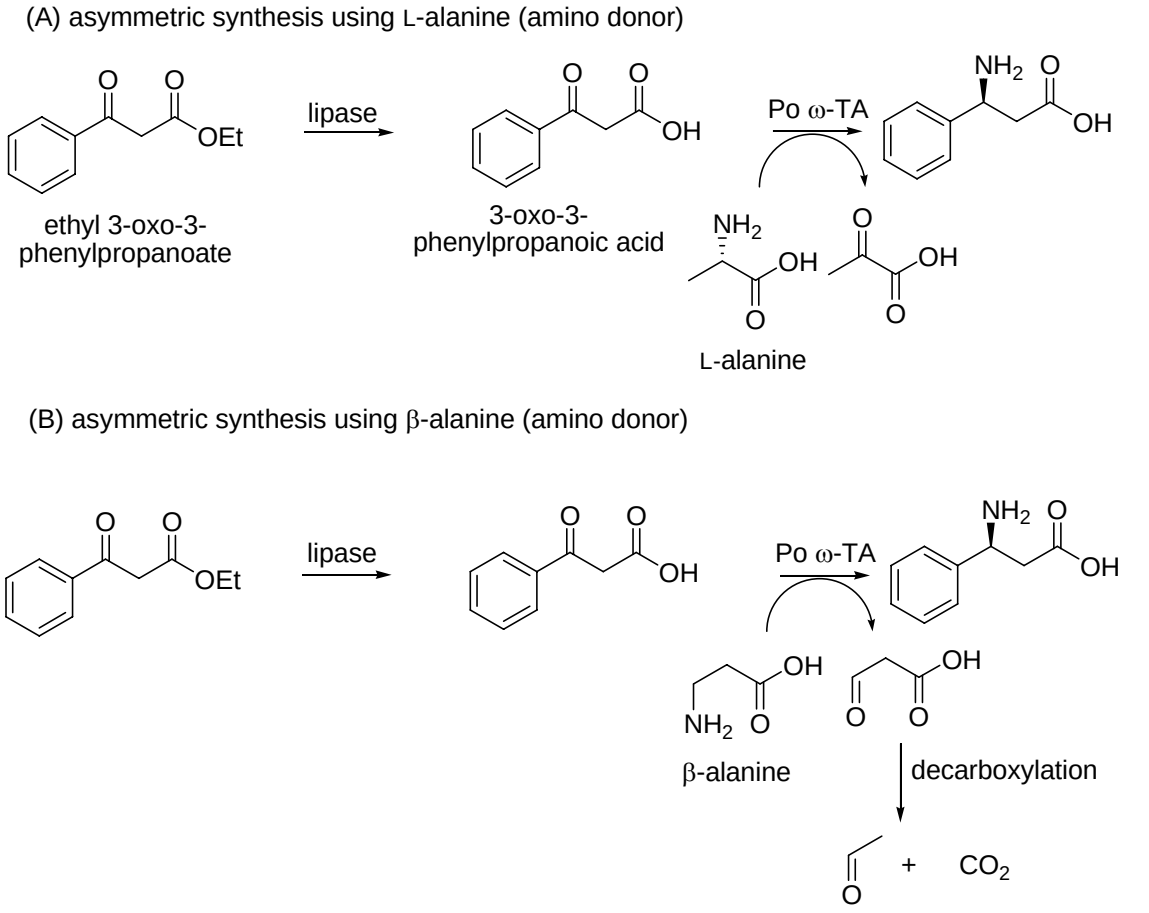


Figure S7. Reaction scheme for the asymmetric synthesis of enantiomerically pure (*S*)-**1a.**[7] (A) L-alanine is the amino donor used for the Po ω-TA reaction. (B) β-alanine is the amino donor used for the Po ω-TA reaction. Reaction conditions: 10 mL of reaction volume, 10 mM ethyl 3-oxo-3-phenylpropanoate, 200 mM amino donor (L-alanine or β-alanine), *Candida rugosa* lipase (0.14 mg mL^{-1}, Sigma, L1754), Po ω-TA (0.077 mg mL^{-1}), 100 mM phosphate buffer (pH 7.0) and shaking for 24 h at 37°C.

References

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