Experimental

Preparation of Gly74Cys single mutant by site-directed mutagenesis of AMDase

A pUC19-based plasmid pAMD101 was used as the templates for PCR-based site-directed mutagenesis. The template plasmid was previously prepared in our laboratory (ref X in the article). To introduce mutation, partially overlapping PCR products were generated spanning the 5' and 3' end of the gene. The synthetic oligonucleotides used for the preparation of the 5' fragment were the forward primer which corresponds to the anti-codon of the multi-cloning site sequence of the vector (P5: 5'-CAGGAAACAGCTATGACC-3', 18 mer), and the reverse primer containing the mutation site to change the amino acid residue of 74th glycine to cysteine (G74Creverse: 5'-GCTGAGCGAGGTGCACATCAGCGAAACCAC-3', 30 mer). The forward primer, which contained mutation site (G74Cfowrawd: 5'-GTTTCGCTGATGTGCACCTCGCTCAGCTTC-3', 30 mer), and the reverse primer corresponding to the vector sequence (P1: 5'-GTAAAACGACGGCCAGTG-3', 18 mer) were used to prepare the 3' fragment.

After preparation of the 5' and 3' PCR fragments for mutation, they were combined by mixing and second PCR amplification with the same primers, P5 and P1. The final PCR product was digested with the restriction enzymes, HindIII and Pst I. This mutated gene was connected with pUC19 which was digested with the same restriction enzymes. Then *E. coli* JM109 was transformed by the mutant plasmid of pAMD101. The transformant harboring the mutant plasmid was cultivated in 1.5 L of LB-broth (pH 7.0, containing 150 mg/L of ampicillin) at 30 °C with shaking (200 rpm). After cultivation for 2 h, starting from 1/100 volume of over-night preculture inoculum, IPTG (isopropyl-β-D-galactopyranoside) was added at 0.1 mM. The cultivation was continued for additional 16 h.

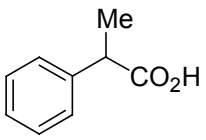
Purification of AMDase

The potassium phosphate buffer of various concentrations containing 0.5 mM EDTA and 5 mM β-mercaptoethanol was used throughout the purification. All the procedures for the purification of the enzyme were performed below 4 °C. The *E. coli* cells containing AMDase were collected by centrifugation at 6000 g for 20 min. The cells were suspended in 200 mL of 100 mM buffer (pH 7.0) and homogenized by French press (1500 kg/cm^2). This suspension was centrifuged (12,000 g, 20 min) to remove the insoluble precipitates. To the resulting solution, was added 1% volume of aqueous solution of protamine sulfate (conc. 2%), and the mixture was stirred for 30 min. The resulting precipitate was removed by centrifugation (12,000 g, 20 min). To the obtained

enzyme solution, was added ammonium sulfate to the concentration of 60% saturation and stirred for 1 h. The precipitated protein was isolated by centrifugation (12,000 g, 20 min) and dissolved in 10 mM Tris–HCl buffer (pH 8.0) and dialyzed against the same buffer. The enzyme solution was charged to a DEAE-Toyopearl column, which had been equilibrated with 10 mM Tris–HCl buffer (pH 8.0) and eluted with aqueous solution of NaCl with a linear gradient of 10–50 mM. The active fractions were collected and concentrated to 30 mL by ultrafiltration. Ammonium sulfate was added to this solution to 25% saturation and this mixture was applied to a butyl-toyopearl column which had been equilibrated with 25% ammonium sulfate solution of 10 mM Tris–HCl buffer (pH 8.0). The enzyme was eluted with an aqueous solution of ammonium sulfate with a linear gradient of 25–15%. The active fractions were combined and dialyzed against 10 mM Tris–HCl buffer.

The concentration of protein was determined by Bio-Rad protein Assay kit or from the absorbance at 280 nm. As to the mutant enzymes, the protein which was identical with AMDase on SDS-PAGE was recovered after the purification by column chromatography.

Preparation of the Substrates



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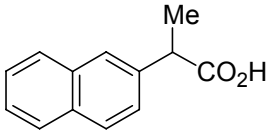

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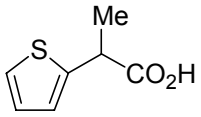

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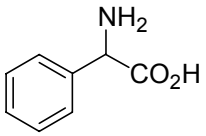

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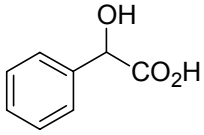

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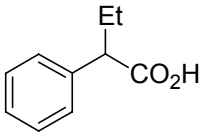

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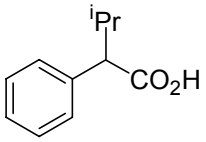

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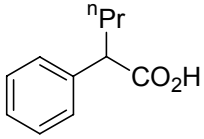

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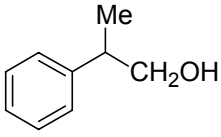

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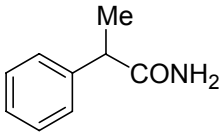

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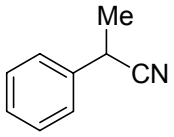

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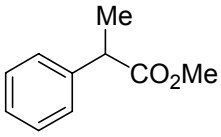

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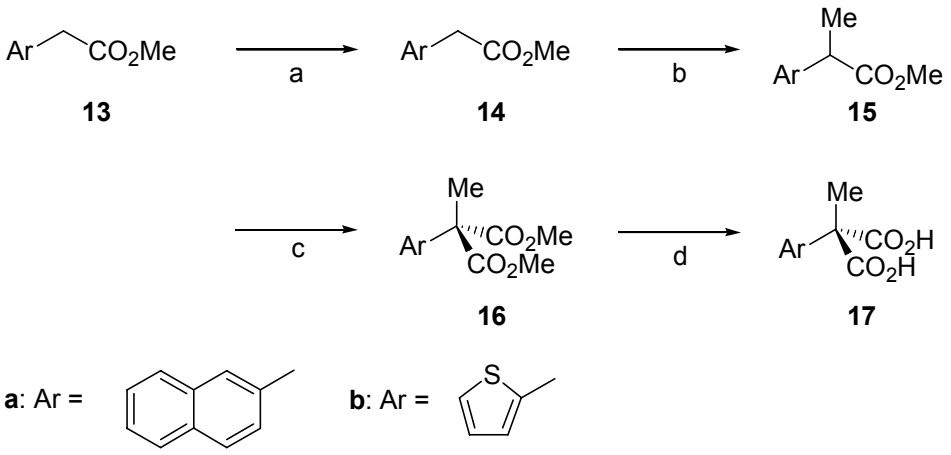
The enantiomerically pure substrates such as (*R*) and (*S*)-2-phenylpropionic acid (**1**), phenylglycine (**4**), mandelic acid (**5**), 2-phenylbutylic acid (**6**), and 2-phenylpropanol (**9**) were commercially available.

(*R*) and (*S*)-2-(2-naphthyl)propionic acid (**2**) and 2-(2-thienyl)propionic acid (**3**) were prepared from the corresponding malonates by enantioselective decarboxylation by the aid of our unique enzyme, wlid type and mutant AMDase.[1]

Preparation of corresponding malonates



Reagents: a;H^+, MeOH b; LDA, MeI, THF c; LDA, $ClCO_2Me$, THF d; KOH, EtOH

Methyl 2-naphthylacetate (**14a**)

Commercially available 2-naphthylacetic acid (**13a**) (5.00 g, 26.9 mmol) in MeOH (40 ml) and catalytic amount of *conc.* H_2SO_4 were heated under reflux for 3 h using Dean-Stark trap. After evaporation of the solvent, the residue was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography (hexane/ethyl acetate = 3/1) to afford 5.35 g of **14a** as a colorless oil (99 % yield). ^{1}H-NMR (270MHz, $CDCl_3$) δ : 3.69 (3H, s), 3.78 (2H, s), 7.38~7.81 (7H, m) ; IR ν_{max} cm^{-1}: 3456, 3054, 2951, 2842, 1736, 1601, 1509, 1435, 1263, 1161, 1016, 819, 759, 705, 637.

Methyl α-(2-naphtyhl)propionate (**15a**)

To a solution of diisopropylamine (2.10 mL, 15 mmol) in dry THF (20 mL) was added 1.57 M solution of *n*-BuLi in hexane (8.22 mL, 15 mmol) with stirring at –78 °C over a period of 30 min. Methyl 2-naphthylacetate (**14a**) (2.00 g, 10 mmol) in THF (20 mL) was added at –78 °C and the mixture was stirred for 30 min. Then methyl iodide

(0.93 mL, 15 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was quenched with 2 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 4 / 1) to give 2.08 g of **15a** as a colorless oil (97 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 1.60 (3H, d, J=7.3 Hz), 3.67 (3H, s), 3.90 (1H, q, J=7.1 Hz), 7.42~7.83 (7H, m); IR ν_{max}: 3449, 3056, 2979, 2951, 1919, 1732, 1601, 1508, 1455, 1434, 1378, 1331, 1198, 1093, 1066, 949, 859, 822, 795, 748, 709 cm^{-1}

Dimethyl α-methyl-α-(2-naphthyl)malonate (**16a**)

To a solution of diisopropylamine (1.47 mL, 11.5 mmol) in dry THF (10 mL) was added 1.57 M solution of *n*-BuLi in hexane (5.76 mL, 11.5 mmol) with stirring at –78 °C for 30 min. Methyl α-(2-naphthyl)propionate (**15a**) (1.50 g, 7 mmol) in THF (10 mL) was added at –78 °C and the mixture was stirred for 30 min. Then methyl chloroformate (0.90 mL, 11.5 mmol) was added and the mixture was allowed to warm to room temperature with stirring for 1 h. The mixture was quenched with 2 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 4 / 1) to give 1.73 g of **16a** as colorless oil (95 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 1.99 (3H, s), 3.79 (6H, s), 7.46~7.83 (7H, m); IR ν_{max}: 3458, 3000, 2952, 1733, 1434, 1378, 1255, 1113, 820, 750 cm^{-1}

α-Methyl-α-(2-naphthyl)malonic acid (**17a**)

A solution of dimethyl α-methyl-α-(2-naphthyl)malonate (**16a**) (0.67 g, 2.5 mmol) in ethanol (5 mL) was added to a solution of potassium hydroxide (2.24 g, 25 mmol) in ethanol (20 mL). The mixture was stirred at 0 °C for 1 h. After acidification of the mixture with 6 M HCl, ethanol was evaporated and the residue was extracted with diethyl ether. The ether layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by recrystallization from ethyl acetate / hexane to give 0.48 g of **17a** as colorless crystals (80 % yield). Mp : 170~ 172 °C ;

^{1}H-NMR (400MHz, $CDCl_3$)δ: 1.97 (3H, s), 7.48~7.97 (7H, m) ; IR ν_{max} : 3529, 3060, 2629, 1715, 1455, 1287, 1129, 815, 751, 707 cm^{-1}

Methyl 2-thienylacetate (**14b**)

Commercially available 2-thienyllacetic acid (**13b**) (5.00 g, 40.0 mmol) in MeOH (40 ml) and catalytic amount of *conc.* H_2SO_4 were heated under reflux for 3 h using Dean-Stark trap. After evaporation of the solvent, the residue was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography (hexane/ethyl acetate = 5/1) to afford 5.49 g of **14b** as a colorless oil (99 % yield). ^{1}H-NMR (270MHz, $CDCl_3$) δ : 3.73 (3H, s), 3.85 (2H, s), 6.95~7.23 (3H, m) ; IR ν_{max} cm^{-1}: 3108, 3002, 2950, 1730, 1440, 1329, 1172, 1011, 850, 706

Methyl α-(2-thienyl)propionate (**15b**)

To a solution of diisopropylamine (2.53 mL, 18 mmol) in dry THF (20 mL) was added 1.57 M solution of *n*-BuLi in hexane (9.87 mL, 18 mmol) with stirring at –78 °C over a period of 30 min. Methyl 2-thienylacetate (**14b**) (2.34 g, 12 mmol) in THF (20 mL) was added at –78 °C and the mixture was stirred for 30 min. Then methyl iodide (1.40 mL, 18 mmol) was added, the mixture was allowed to warm to room temperature, and stirred for 1 h. The mixture was quenched with 2 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 5 / 1) to give 2.19 g of **16b** as a colorless oil (86 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 1.59 (3H, d, J=7.2 Hz), 3.71 (3H, s), 4.02 (1H, q, J=7.1 Hz), 6.95~7.23 (3H, m); IR ν_{max}: 3108, 2982, 2952, 1739, 1532, 1435, 1377, 1327, 1200, 1059, 853, 702 cm^{-1}

Dimethyl α-methyl-α-(2-thienyl)malonate (**16b**)

To a solution of diisopropylamine (1.05 mL, 7.5 mmol) in dry THF (10 mL) was added 1.57 M solution of *n*-BuLi in hexane (4.11 mL, 7.5 mmol) with stirring at –78 °C over a period of 30 min. Methyl α-(2-thenyl)propionate (**15b**) (0.85 g, 5 mmol) in THF (10 mL) was added at –78 °C and the mixture was stirred for 30 min. Then methyl chloroformate (0.58 mL, 7.5 mmol) was added, the mixture was allowed to warm to room temperature, and stirred for 1 h. The mixture was quenched with 2 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 9 / 1) to give 1.10 g of **16b** as a colorless oil (97 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 1.94 (3H, s), 3.78

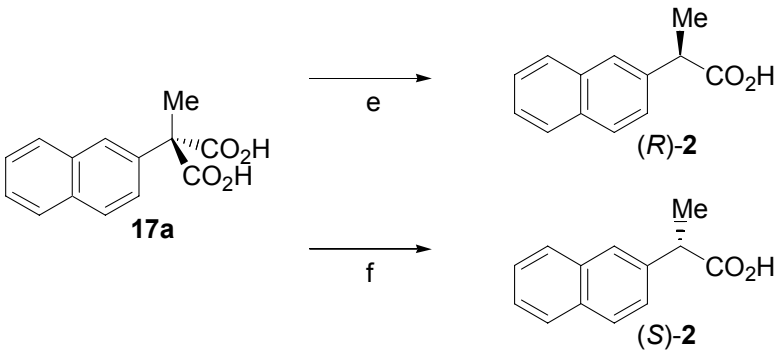
(6H, s), 6.96~7.31 (3H, m); IR ν_{max}: 3478, 3108, 3002, 2953, 1731, 1434, 1377, 1276, 1119, 983, 890, 709 cm^{-1}

α-Methyl-α-(2-thienyl)malonic acid (**17b**)

A solution of dimethyl α-methyl-α-(2-thienyl)malonate (**16b**) (0.99 g, 4.3 mmol) in ethanol (5 mL) was added to a solution of potassium hydroxide (3.18 g, 43 mmol) in ethanol (20 mL). The mixture was stirred at 0 °C for 1 h. After acidification of the mixture with 6 M HCl, ethanol was evaporated and the residue was extracted with diethyl ether. The ether layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by recrystallization from ethyl acetate and hexane to give 0.63 g of **17b** as colorless crystals (72 % yield). Mp : 157~ 159 °C ;

^{1}H-NMR (400MHz, CD_3CD)δ: 1.90 (3H, s), 6.96~7.41 (3H, m) ; IR ν_{max} : 3000, 2650, 1700, 1455, 1410, 1350, 1280, 1200, 1130, 1100, 1050, 1020, 910, 850 , 785, 745, 700 cm^{-1}

Preparation of (*R*) or (*S*)-arylpropionic acid

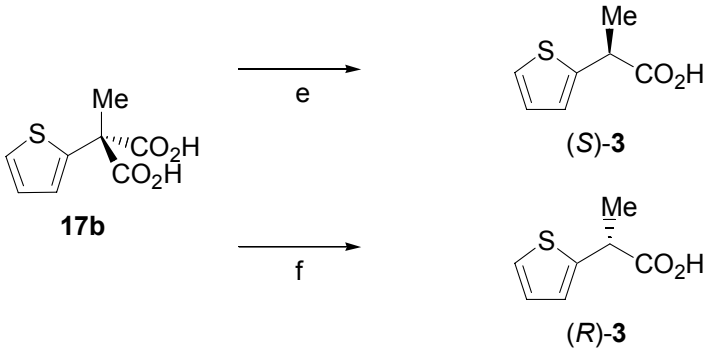


e: 100 mM Tris-HCl buffer, pH 8.5, Wild type AMDase
f: 100 mM Tris-HCl buffer, pH 8.5, S36N/G74C/C188S Mutant AMDase

(*R*) or (*S*)-α-(2-Naphthyl)propionic acid (**2**)

α-Methyl-α-(2-naphthyl)malonic acid (**17a**, 488 mg, 2 mmol) was dissolved in water (ca. 8 mL) and the pH of the mixture was adjusted to 8.0 with 2 M HCl and 2 M NaOH. Final volume was adjusted to 10 mL by adding water. To a 20-mL round-bottomed flask was added 1 mL of 10 mM Tris-HCl buffer (pH = 8.5), 2 mL of the substrate solution (97.7 mg, 0.4 mmol) and 1 mL of AMDase solution (Wild Type, 0.87 mg; S36N/G74C/C188S mutant, 0.90 mg). The mixture was stirred at 30 °C for 3 h. The mixture was quenched with 1 mL of 2 M HCl and filtrated with cerite. The filtrate was extracted with diethyl ether. The ether layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified

by preparative TLC (hexane/ethyl acetate/acetic acid =50/50/1) to give **2** as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.61 (3H, d, J=7.1 Hz), 3.93 (1H, q, J=6.9 Hz), 7.44~7.83 (7H, m) ; IR ν_{max}: 2980, 1698, 1419, 1273, 1225, 963, 747 cm^{-1}

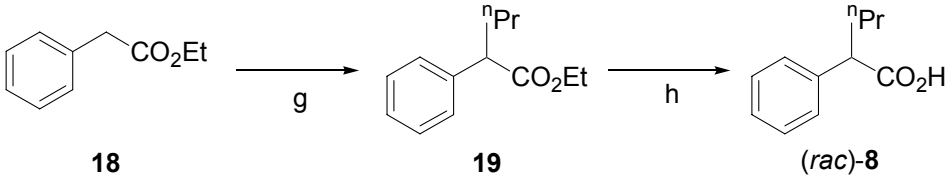


e: 100 mM Tris-HCl buffer, pH 8.5, Wild type AMDase
f: 100 mM Tris-HCl buffer, pH 8.5, S36N/G74C/C188S Mutant AMDase

(*R*) and (*S*)- α-(2-naphthyl)propionic acid (**3**)

α-Methyl-α-(2-thienyl)malonic acid (**17b**, 400 mg, 2 mmol) was dissolved in water (ca. 8 mL) and the pH of the mixture was adjusted to 8.0 with 2 M HCl and 2 M NaOH. Final volume was adjusted to 10 mL by adding water. To a 20-mL round-bottomed flask was added 1 mL of 10 mM Tris-HCl buffer (pH = 8.5), 2 mL of the substrate solution (80.0 mg, 0.4 mmol) and 1 mL of AMDase solution (Wild Type, 0.87 mg; S36N/G74C/C188S mutant, 0.90 mg). The mixture was stirred at 30 °C for 3 h. The mixture was quenched with 1 mL of 2 M HCl and filtrated with cerite. The filtrate was extracted with diethyl ether. The ether layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by preparative TLC (hexane/ethyl acetate/acetic acid =33/66/1) to give **3** as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.61 (3H, d, J=7.3 Hz), 4.04 (1H, q, J=7.3 Hz), 6.95~7.23 (3H, m) ; IR ν_{max}: 2982, 1715, 1457, 1417, 1236, 699 cm^{-1}

Preparation of 2-Phenylpentanoic acid (10)



g: LDA, *n*-PrBr, THF h: KOH, H_2O, EtOH

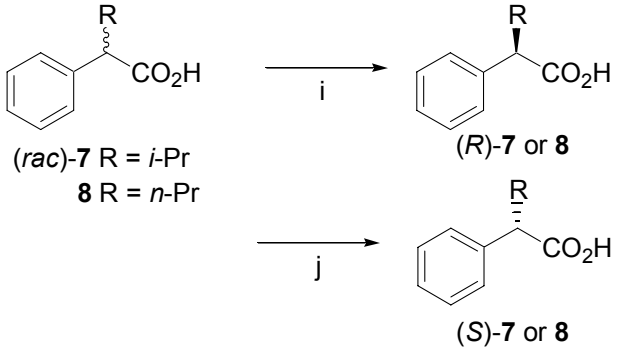
Ethyl 2-phenylpentanoate (**19**)

To a solution of diisopropylamine (0.94 mL, 6.7 mmol) in dry THF (10 mL) was added 1.59 M solution of *n*-BuLi in hexane (4.21 mL, 6.7 mmol) with stirring at –78 °C over a period of 30 min. Commercially available ethyl 2-naphthylacetate (**18**) (1.00 g, 6.1 mmol) in THF (10 mL) was added at –78 °C and the mixture was stirred for 30 min. Then 1-bromopropane (0.61 mL, 6.7 mmol) was added and the mixture was allowed to warm to room temperature, and stirred for 16 h. The mixture was quenched with 2 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 9 / 1) to give 1.09 g of **19** as a colorless oil (87 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.91 (3H, t, J= 7.4Hz), 1.20 (3H, t, J= 7.2Hz), 1.26~1.34 (2H, m), 1.69~1.81 (1H, m), 1.98~2.11 (1H, m), 3.53 (1H, t, J= 7.7Hz), 4.13 (2H, m), 7.22~7.31 (5H, m); IR ν_{max}: 2960, 2873, 1733, 1454, 1369, 1265, 1162, 1031, 732, 698 cm^{-1}

2-Phenylpentanoic acid (**8**)

A solution of ethyl 2-phenylpentanoate (**19**) (0.90 g, 4.3 mmol) in ethanol (10 mL) was added to a solution of potassium hydroxide (2.41 g, 43 mmol) in water (10 mL). The mixture was stirred at rt for 16 h. After acidification of the mixture with 6 M HCl, ethanol was evaporated and the residue was extracted with diethyl ether. The ether layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane / ethyl acetate / acetic acid = 30 / 10/ 1%) to give 0.70 g of **8** as colorless crystals (90 % yield). Mp : 51~52 °C ;

^{1}H-NMR (400MHz, $CDCl_3$)δ: 0.91 (3H, t, J= 7.3Hz), 1.24~1.36 (2H, m), 1.73~1.80 (1H, m), 1.98~2.08 (1H, m), 3.56 (1H, t, J= 7.1Hz), 7.22~7.31 (5H, m); IR ν_{max} :2956, 2659, 1697, 1455, 1413, 1249, 1214, 1186, 1070, 952, 727, 698 cm^{-1}



i:1) (*R*)-PEA, 63% ethanol solution, 2) H^+
j:1) (*S*)-PEA, 63% ethanol solution, 2) H^+

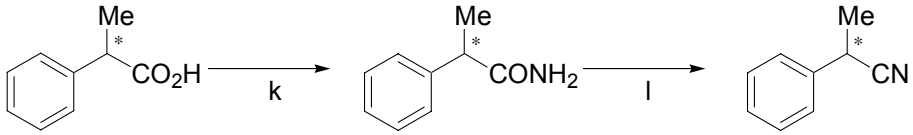
Resolution of 2-isopropyl-2-phenylacetic acid (7)[2]

Commercially available (*rac*)-2-isopropyl-2-phenylacetic acid (**7**) (115.3 mg, 0.65 mmol), (*R*)-(+)-phenylethylamine (78.3 mg, 0.65 mmo), and 63% aqueous ethanol (2.5 mL) were warmed and allowed to cool slowly. The precipitated salt (87.3 mg) was separated by filtration and recrystallized three times from 63% aqueous ethanol (2.0, 1.5, 1.0 mL respectively) to give 58.3 mg of the ammonium salt. Regeneration of free acid with 10% sulfuric acid at 0 °C gave, after extraction, drying and purification by preparative TLC, 31.2 mg of (*R*)-(-)-2-isopropyl-2-phneylacetic acid ((*R*)-**7**). The mother liquor was concentrated and partially active (+)-acid was obtained in the same way (40.0 mg). This acid was treated with (*S*)-(-)-phenylethylamine and the process was repeated to obtain the pure acid ((*S*)-**7**, 20.5 mg). Mp: 86~88 °C ;
^{1}H-NMR (400MHz, $CDCl_3$)δ: 1.14 (3H, d, J= 7.2Hz), 2.8 (1H, q, J=7.2Hz), 7.24~7.36 (5H, m); IR ν_{max} :3361, 3185, 1658, 1496, 1452, 1405, 1288, 1133, 696 cm^{-1}

Resolution of 2-phenylpentanoic acid (8)[2]

(*rac*)-2-Phenylpentanoic acid (**8**) (115.3 mg, 0.65 mmol), (*R*)-(+)-phenylethylamine (78.3 mg, 0.65 mmo), and 63% aqueous ethanol (2.5 mL) were warmed and allowed to cool slowly. The separated salt (77.7 mg) was recrystallized there times from 63% aqueous ethanol (2.0, 1.5, 1.0 mL respectively) to give 25.6 mg of pure salt. Regeneration of free acid with 10% sulfuric acid at 0 °C gave, after extraction, drying and purification by preparative TLC, 5.0 mg of (*R*)-(-)-2-phneylpentanoic acid ((*R*)-**8**). The mother liquor was concentrated, and partially active (+) acid was regenerated in the same way (40.0 mg). This acid was treated with (*S*)-(-)-phenylethylamine and the purification process was repeated to obtain the pure acid ((*S*)-**8**, 10.5 mg).

Preparation of carboxylic acid derivatives



(*R*) or (*S*)-**1** (*R*) or (*S*)-**10** (*R*) or (*S*)-**11**

k: 1) $ClCO_2Et$, Et_3N, THF 2) NH_4OH l: $SOCl_2$, DMF, benzene

(*R*) or (*S*)-2-phenylpropionamide (**10**)[3]

To a solution of (R) or (S)-2-phenylpropionic acid (**1**) (500 mg, 3.3 mmol) in THF (5 mL) was added dropwise ethyl chloroformate (2.9 mL, 3.6 mmol) in the presence of triethylamine (364 mg, 3.6 mmol) at *ca.* –15 °C over period of 20 min. To this solution was added 28% *aq.* solution of ammonium hydroxide (2.1 mL 16.6 mmol) at *ca.* –15 °C and the mixture was stirred at 0 °C for 2 h. Saturated aqueous ammonium chloride was added to the reaction mixture and the aqueous layer was extracted with diethylether. The combined organic layer was washed with brine and dried over Na_2SO_4 and evaporated. The residue was recrystallized from ethyl acetate and hexane to give 391.0 mg of (*R*)-**10**, and 390.8 mg of (*S*)-**10**. Mp : 86~88 °C ;
^{1}H-NMR (400MHz, $CDCl_3$)δ: 1.14 (3H, d, J= 7.2Hz), 2.8 (1H, q, J=7.2Hz), 7.24~7.36 (5H, m); IR ν_{max} :3361, 3185, 1658, 1496, 1452, 1405, 1288, 1133, 696 cm^{-1}

(*R*) or (*S*)-2-phenylpropionitrile (**11**)[4]

To a solution of (*R*) or (*S*)-2-phenylpropionamide (**10**) (22.4 mg, 0.15 mmol) in dry DMF (1 mL) was added a mixture of dry benzene (2 mL) and thionyl chloride (35.7 mg, 0.30 mmol). The mixture was stirred at room temperature until the amide was consumed. The reaction mixture was poured to crushed ice and extracted with benzene. The organic layer was combined and washed sequentially with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue purified by preparative TLC (hexane / ethyl acetate = 1/1) gave 6.3 mg of (*R*)-**11** or 7.4 mg of (*S*)-**11**
^{1}H-NMR (400MHz, $CDCl_3$)δ: 1.55 (3H, d, J= 7.0Hz), 3.66 (1H, q, J=7.0Hz), 7.24~7.42 (5H, m); IR ν_{max} :3032, 2938, 2244, 1711, 1600, 1494, 1462, 1379, 1284, 1199, 1081, 760, 696 cm^{-1}

Methyl (*R*) or (*S*)-2-phenylpropionate (**12**)

To a solution of (*R*) or (*S*)-2-phenylpropionic acid (**1**) (5.0 mg, 0.03 mmol) in methanol (1 mL) was added dropwise an excess amount of a solution of 1.0 M of TMS-diazomethane in hexane. The mixture was evaporated and the residue was purified by preparative TLC to give quantitative amount of (*R*) or (*S*)-**12**.

Enzymatic racemization reaction by G74C mutant AMDase

To a solution of the substrate (1.0 mg) in 100 mM Tris-Hcl buffer (pH =8.5, 300 μL) was added the purified G74C mutant AMDase solution (0.20 mg, 200 μL). The mixture was incubated at 37 °C for 14 h. After quenching with 2 M HCl, the reaction mixture was extracted with diethylether. The ether layer was washed with brine and dried over Na_2SO_4.After evaporation of the solvent, the residue was purified by preparative TLC to give the product. The enantiomeric excess of the product was examined by HPLC analysis under the following conditions.

2-Phenylpropionic acid (**1**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 98/2/0.1%, 0.5 mL/min, R_t (min); 24.4(*R*), 28.5(*S*)

2-(2-Naphthyl)propionic acid (**2**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 98/2/0.1%, 0.5 mL/min, R_t (min); 41.4(*R*), 47.8(*S*)

2-(2-Thienyl)propionic acid (**3**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 98/2/0.1%, 0.5 mL/min, R_t (min); 30.0(*S*), 38.0(*R*)

Phenylglycine (**4**)

Column; Crownpak CR(+), $HClO_4$aq, pH = 2.0, 1.0 mL/min, R_t (min); 2.3(D), 4.5(L)

Mandelic acid (**5**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 90/10/0.1%, 0.5 mL/min, R_t (min); 21.0(*S*), 26.1(*R*)

2-Phenylbutyric acid (**6**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 95/5/0.05%, 0.5 mL/min, R_t (min); 13.8(*R*), 17.0(*S*)

2-isopropyl-2-phenylacetic acid (**7**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 95/5/0.05%, 0.5 mL/min, R_t (min); 11.8(*R*), 14.4(*S*)

2-Phenylpentanoic acid (**8**)

Column; Chiralcel OD-H, hexane/2-propanol/TFA = 95/5/0.05%, 0.5 mL/min, R_t

(min); 12.6(*R*), 14.3(*S*)

2-Phenylpropanol (**9**)

Column; Chiralcel OB, hexane/2-propanol = 95/5, 0.5 mL/min, R_t (min); 17.9(*S*), 19.4(*R*)

2-Phenylpropionamide (**10**)

The enantiomeric excess of the amide was measured as methyl ester by hydrolysis and esterification as follows: amide **10** in methanol was treated with catalytic amount of *conc.* sulfuric acid under gentle reflux for 3 h to yield the corresponding ester.

Column; Chiralcel OJ, hexane/2-propanol = 90/10, 0.5 mL/min, R_t (min); 13.2(*S*), 15.0(*R*)

2-Phenylpropionenitrile (**11**)

Column; Chiralcel OB, hexane/2-propanol = 95/5, 0.5 mL/min, R_t (min); 24.7(*S*), 28.9(*R*)

Methyl 2-phenylpropionate (**12**)

Column; Chiralcel OJ, hexane/2-propanol = 90/10, 0.5 mL/min, R_t (min); 13.2(*S*), 15.0(*R*)

entry	Substrate	*e.e.* of substrate	*e.e.* of product
1	(*R*)-**1**	99	*rac*
2	(*S*)-**1**	95	*rac*
3	(*R*)-**2**	97	*rac*
4	(*S*)-**2**	96	*rac*
5	(*R*)-**3**	99	*rac*
6	(*S*)-**3**	95	*rac*
7	(*R*)-**4**	98	*83*
8	(*S*)-**4**	99	*80*
9	(*R*)-**5**	98	*rac*
10	(*S*)-**5**	99	*rac*
11	(*R*)-**6**	95	*rac*
12	(*S*)-**6**	98	*rac*
13	(*R*)-**7**	94	93
14	(*S*)-**7**	92	92
15	(*R*)-**8**	91	91
16	(*S*)-**8**	92	90
17	(*R*)-**9**	99	99
18	(*S*)-**9**	97	97
19	(*R*)-**10**	98	98
20	(*S*)-**10**	96	95
21	(*R*)-**11**	90	89
22	(*S*)-**11**	93	94
23	(*R*)-**12**	99	99
24	(*S*)-**12**	95	95

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