

Supporting information

Highly enantioselective synthesis of α,α -disubstituted malonamic acids through asymmetric hydrolysis of dinitriles with *Rhodococcus* sp. CGMCC 0497

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Materials and methods

The commercially available reagents were used without further purification. Melting points were determined on a Mettler FP62 and are uncorrected. ^1H NMR spectra were recorded on a Bruker AMX-300(300MHz) spectrometer at room temperature with TMS as internal standard. Chemical shifts in ppm were positive for upfield shifts. IR spectra were recorded neat or in KBr and measured in cm^{-1} , using a Shimadzu IR-440 IR spectrophotometer. EI-MS spectra were recorded on an HP 5989A. High-resolution mass spectra were obtained on a Finnigan MAT8430. Microanalyses were carried out on an Italian Carlo-Erba 1106. Polarimetry was carried out using an optical activity Perkin Elmer 241ML polarimeter and the measurements were made at the sodium D-line with a 10cm pathlength cell. Concentrations(c) are given in g/100ml. Enantiomeric excesses: Chiral HPLC was conducted with a PE NELSON NCI900 using Chiralpak AS or Chiralcel OJ column at a flow rate of 0.7ml/min with 2-propanol/hexane as the mobile phase.

Microorganism and cultivation

The strain *Rhodococcus* sp. CGMCC 0497 is available in CGMCC (China General Microbiological Culture Collection Center). *Rhodococcus* sp. CGMCC 0497 was subcultured at 30°C for 24 hours in a 100ml shaking flask containing 20ml of a medium consisting of 0.5g of polypepton, 0.5g of beef extract and 1g of glucose per 100ml of tap water, pH 7.0. Then the subculture was inoculated into a 5l shaking flask containing 1l of the rich medium consisting of 1g of glucose, 0.5g of beef extract, 0.25g of methacrylamide, 100mg of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 75mg of KH_2PO_4 , 10 mg of NaCl, 0.1ml of mineral medium per 100ml of tap water with methacrylamide added 24 hours later. The pH of each medium was adjusted to around 7.0-7.2 by addition of 2N NaOH or 3N HCl. After incubation at 30°C with reciprocal shaking for 48 hr. The organism was harvested by centrifugation using an HIMAC centrifuge CR20B2 (Hitachi, Japan) with a RPR9-2 rotor (6800g, 30min 10°C). Cells were washed with 100mM potassium phosphate buffer (pH=7.0) and centrifugated.

Dinitrile (Substrate) Synthesis

Method A (for 1a-1h): To a suspension of NaH (60%) 400mg (10mmol) in 40ml THF and 4ml DMF, was added drop by drop a solution of malononitrile (1.32g, 20mmol) in 5ml THF at room temperature. After 2h, benzyl bromide or chloride (10mmol) in THF (10ml) was added within 2 h. Stirring was continued at room temperature for 3-10 h. The reaction was quenched by aqueous NH₄Cl solution, extracted with ethyl acetate, dried on MgSO₄ and purified by flash chromatograph. The product was dissolved in acetone (50ml). Methyl iodide (20mmol) and solid K₂CO₃ (25mmol) were added. The mixture was stirred overnight at room temperature. After filtration and concentration, the residue was purified by flash chromatograph to yield α,α -disubstituted malononitriles.

Method B (for 1i): To a solution of malononitrile (1.32g, 20mmol) and phenylethyl bromide (10mmol) in THF (40ml) cooled by cryohydrate, was added solid *tert*-BuOK (10mmol). The mixture was stirred overnight at room temperature. The following procedure was as described in Method A..

2-benzyl-2-methyl-malononitrile 1a. m.p. 94.3-95.3°C, Lit.¹ 94.5-95.5°C; ¹H NMR (300 MHz, CDCl₃): δ 7.35-7.45(m, 5H, Ar-H), 3.22(s, 2H, CH₂), 1.81(s, 3H, CH₃); IR(KBr): 2251(CN), 765, 703; MS *m/z* (%): 170(M⁺, 1), 91(100).

2-(4'-methylbenzyl)-2-methyl-malononitrile 1b. m.p. 88.6-89.6°C; ¹H NMR (300 MHz, CDCl₃): δ 7.25, 7.20(AB, 4H, J=8.1Hz, ArH), 3.17(s, 2H, CH₂), 2.37(s, 3H, CH₃), 1.79(s, 3H, CH₃); IR(KBr): 2253(CN); MS *m/z* (%): 184(M⁺, 5), 105(100); Anal. Calcd. for C₁₂H₁₂N₂: C, 78.23; H, 6.57; N, 15.20. Found: C, 78.36; H, 6.50; N, 15.33.

2-(4'-florobenzyl)-2-methyl-malononitrile 1c. m.p. 126.1-127.1°C; ¹H NMR (300 MHz, CDCl₃): δ 7.38-7.33(m, 2H, ArH), 7.14-7.08(m, 2H, ArH), 3.19(s, 2H, CH₂), 1.82(s, 3H, CH₃); IR(KBr): 2253(CN); MS *m/z* (%): 188(M⁺, 2), 173(1), 109(100); Anal. Calcd. for C₁₁H₁₉N₂F: C, 70.20; H, 4.82; N, 14.88; F, 10.09. Found: C, 70.22; H, 4.98; N, 14.99; F, 10.05.

2-(4'-chlorobenzyl)-2-methyl-malononitrile 1d. m.p. 88.9-89.9°C; ¹H NMR (300 MHz, CHCl₃): δ 7.40, 7.31(AB, 4H, J=8.4Hz, ArH), 3.18(s, 2H, CH₂), 1.82(s, 3H, CH₃); IR(KBr): 2254(CN); MS *m/z* (%): 206(M⁺+2, 1), 204(M⁺, 4), 127(33), 125(100); Anal. Calcd. for C₁₁H₉N₂Cl: C, 64.56; H, 4.43; N, 13.69; Cl, 17.32. Found: C, 64.43; H, 4.60; N, 13.68; Cl, 17.39.

2-(4'-bromobenzyl)-2-methyl-malononitrile 1e. m.p. 86.3-87.3°C; ¹H NMR (300 MHz, CHCl₃): δ 7.55, 7.25(AB, 4H, J=8.4Hz, ArH), 3.17(s, 2H, CH₂), 1.82(s, 3H, CH₃); IR(KBr): 3065, 2995, 2247(CN), 1519, 1487, 1409, 1070, 1012, 838, 806, 726, 493; MS *m/z* (%): 250(M⁺+2, 5), 248(M⁺, 5), 171(94), 169(100); Anal. Calcd. for C₁₁H₉N₂Br: C, 53.04; H, 3.64; N, 11.25, Br, 32.08. Found: C, 53.05; H, 3.48; N, 11.06, 32.20.

2-(4'-methoxybenzyl)-2-methyl-malononitrile 1f. m.p. 68-69°C; ¹H NMR (300 MHz, CDCl₃): δ 7.28, 6.93(AB, 4H, J=9.0Hz, ArH), 3.82(s, 3H, OMe), 3.16(s, 2H, CH₂), 1.79(s, 3H, CH₃); IR(KBr): 2253(CN); MS *m/z* (%): 200(M⁺, 4), 121(100); Anal. Calcd. for C₁₂H₁₂N₂O: C, 71.98; H, 6.04; N, 13.99. Found: C, 71.87; H, 6.05; N, 13.80.

2-(3'-chlorobenzyl)-2-methyl-malononitrile 1g. m.p. 81.3-82.3°C; ¹H NMR (300 MHz, CDCl₃): δ 7.39-7.46(m, 4H, ArH), 3.19(s, 2H, CH₂), 1.84(s, 3H, CH₃); IR(KBr): 2250(CN); MS *m/z* (%): 206(M⁺+2, 4), 204(M⁺, 12), 127(34), 125(100); Anal. Calcd. for C₁₁H₉N₂Cl: C, 64.56; H, 4.43; N, 13.69; Cl, 17.32. Found: C, 64.39; H, 4.24; N, 13.72; Cl, 17.15.

2-(2'-chlorobenzyl)-2-methyl-malononitrile 1h. m.p. 61.9-62.9°C; ¹H NMR (300 MHz, CHCl₃): δ 7.55-7.51(m, 1H, ArH), 7.51-7.46(m, 1H, ArH), 7.36-7.26(m, 2H, ArH), 3.48(s, 2H, CH₂), 1.85(s, 3H, CH₃); IR(KBr): 2949, 2253(CN), 1571, 1457, 1446, 1253, 1143, 1113, 1053, 765, 739, 686, 452; MS *m/z* (%): 206(M⁺+2, 2), 204(M⁺, 6), 127(34), 125(100); Anal. Calcd. for C₁₁H₉N₂Cl: C, 64.56; H, 4.43; N, 13.69; Cl, 17.32. Found: C, 64.79; H, 4.65; N, 13.69; Cl, 17.29.

2-phenylethyl-2-methyl-malononitrile 1i. Oil; ¹H NMR (300 MHz, CDCl₃): δ 7.36-7.21(m, 5H, ArH), 3.01-2.95(m, 2H, CH₂), 2.25-2.19(m, 2H, CH₂), 1.84(d, 3H, J=1.2Hz, CH₃); IR(film): 2251(CN); MS *m/z* (%): 184(M⁺, 12), 183(9), 105(29), 104(28), 91(100); HRMS: Calcd. for (C₁₂H₁₂N₂)⁺ 184.10005, Found: 184.10047.

Hydrolysis of dinitriles by *Rhodococcus* sp. CGMCC 0497

General procedure with whole cells and determination of enantiomeric excess

A suspension of 10g washed wet cells and 80ml 0.1mM potassium phosphate buffer (pH=7.0) was incubated at 30°C or 20°C for 30mins with continuously magnetic stirring before the addition of the substrate, a solution of 100mg α,α-disubstituted malononitrile dissolved in 100μl acetone. The reaction was quenched by centrifugation. The resulting supernatant was extracted with ethyl acetate and dried over Na₂SO₄. After concentration, the residue was purified by flash chromatography on silica gel (elute: petroleum ether/EtOAc/AcOH 150:100:1).

To a solution of α,α-Disubstituted malonic acid **5** (0.1mmol) in DMF(0.1ml) was added bromoethane or iodomethane (2mmol) and anhydrous K₂CO₃ (2mmol). The reaction was carried out at room temperature for one day to achieve the esters and the esters were subjected to chiral HPLC.

(S)-2-cyano-2-methyl-3-phenylpropionamide 2. m.p. 96.5-97.5°C; [α]_D¹⁷ +46.9 (c 0.98, CHCl₃), 99%ee; ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.20(m, 5H, Ar-H), 6.04(s, br, 1H, NH), 5.52(s, br, 1H, NH), 3.26(d, 1H, J=13.5Hz, CH), 2.96(d, 1H, J=13.5Hz, CH), 1.66(s, 3H, CH₃); IR(KBr): ν 3378, 3320(NH), 2244(CN), 1666(C=O); MS *m/z* (%): 189(M⁺+1, 3), 188(M⁺, 21), 173(M⁺-CH₃, 4), 144(M⁺-CONH₂, 19), 91(100); Anal. Calcd. for C₁₁H₁₂N₂O: C, 70.19; H, 6.43; N, 14.88. Found: C, 69.98; H, 6.51; N, 14.81.

(R)-2-cyano-2-methyl-3-phenylpropionic acid 3. m.p. 94.6-95.6°C, Lit.² 84°C; [α]_D²⁵ -20.0 (c 0.40, CHCl₃), 72%ee, {Lit.² [α]_D 27.2 (c 2, CHCl₃), S}; ¹H NMR (300 MHz, CDCl₃): δ 9.83(br s, 1H, OH), 7.37-7.28(m, 5H, Ar-H), 3.27(d, 1H, J=13.5Hz, CH), 3.07(d, 1H, J=13.8Hz), 1.65(s, 3H, CH₃); IR(KBr): ν 3074(br OH), 2263(CN), 1747(C=O); MS *m/z* (%): 189(M⁺, 2), 174(M⁺-CH₃, 3), 144(M⁺-COOH, 3), 91(100).

2-benzyl-2-methyl-malonamide 4. m.p. 195.4-196.4°C, Lit.³ 202-203°C; ¹H NMR (300 MHz, [D₆]DMSO): δ 7.22-7.12(m, 9H, ArH, 2NH₂), 3.06(s, 2H, CH₂), 1.10(s, 3H, CH₃); IR(KBr): ν 3389, 3205(NH), 1692(C=O); MS *m/z* (%): 206(M⁺, 7), 162(M⁺-CONH₂, 100).

(R)-2-benzyl-2-methyl-malonamic acid 5a. m.p. 117.9-118.9°C, Lit.⁴ 120-121°C; $[\alpha]_D^{15}$ -15.01(c 1.07, MeOH), 94%ee; {Lit. $[\alpha]_D$ -4.4(c 0.5, MeOH), R}; ¹H NMR (300 MHz, [D₆]acetone): δ 7.29-7.19(m, 5H, ArH), 3.61(br s, 3H, NH₂, OH), 3.25(d, 1H, J=13.8Hz), 3.20(d, 1H, J=13.5Hz), 1.37(s, 3H, CH₃); IR(KBr): ν 3423, 3204(NH), 3034(br OH), 1745, 1659(C=O); MS *m/z* (%): 207(M⁺, 2), 91(100).

(R)-2-(4'-methylbenzyl)-2-methyl-malonamic acid 5b. m.p. 182.6-183.6°C; $[\alpha]_D^{21}$ -11.18 (c 0.96, MeOH), >99%ee; ¹H NMR (300 MHz, [D₆]acetone): δ 12.4(br s, 1H, OH), 7.27(brs, 1H, NH), 7.11, 7.05(AB, 4H, J=8.1Hz, ArH), 6.91(brs, 1H, NH), 3.19(s, 2H, CH₂), 2.27(s, 3H, CH₃), 1.41(s, 3H, CH₃); IR(KBr): ν 3451, 3329(NH), 2850-3350(br, OH), 1745, 1670(C=O); MS *m/z* (%): 221(M⁺, 3), 105(100); Anal. Calcd. for C₁₂H₁₅NO₃: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.11; H, 6.92; N, 6.20.

(R)-2-(4'-florobenzyl)-2-methyl-malonamic acid 5c. m.p. 116.9-117.9°C; $[\alpha]_D^{21}$ -9.46(c 1.46, MeOH); >99%ee; ¹H NMR (300 MHz, [D₆]acetone): δ 7.29-7.24(m, 3H, ArH, NH), 7.05-6.99(m, 2H, ArH), 6.90(br s, 1H, NH), 3.23(s, 2H, CH₂), 1.42(s, 3H, CH₃); IR(KBr): ν 3445, 3421(NH), 3202(br, OH), 1656(C=O); MS *m/z* (%): 226(M⁺+1, 5), 225(M⁺, 4), 109(100); Anal. Calcd. for C₁₁H₁₂NO₃F: C, 58.66; H, 5.37; N, 6.22; F, 8.44. Found: C, 58.78; H, 5.52; N, 5.88; F, 8.44.

(R)-2-(4'-chlorobenzyl)-2-methyl-malonamic acid 5d. m.p. 114.8-115.8°C; $[\alpha]_D^{21}$ -12.99 (c 0.83, MeOH), >99%ee; ¹H NMR (300 MHz, [D₆]acetone): δ 12.1(br s, 1H, OH), 7.31-7.23(m, 5H, ArH, NH), 6.91(br s, 1H, NH), 3.25(d, 1H, J=13.8Hz, CH), 3.20(d, 1H, J=13.2Hz, CH), 1.41(s, 3H, CH₃); IR(KBr): ν 3403, 3227(NH), 2800-3300(br OH), 1727, 1688(C=O); MS *m/z* (%): 243(M⁺+2, 1), 241(M⁺, 1), 197(M⁺-CONH₂, 25), 127(33), 125(100); Anal. Calcd. for C₁₁H₁₂NO₃Cl: C, 54.67; H, 5.00; N, 5.80; Cl, 14.67. Found: C, 54.72; H, 5.22; N, 5.61; Cl, 14.86.

(R)-2-(4'-bromoenzyl)-2-methyl-malonamic acid 5e. m.p. 119.5-120.5°C; $[\alpha]_D^{20}$ -11.41 (c 1.17, MeOH), >99%ee; ¹H NMR (300 MHz, [D₆]acetone): δ 12.0(br s, 1H, OH), 7.43, 7.19(AB, 4H, J=8.1Hz, ArH), 7.26(br s, 1H, NH), 6.93(br s, 1H, NH), 3.21(s, 2H, CH₂), 1.41(s, 3H, CH₃); IR(KBr): ν 3407, 3227(NH), 2800-3200(br OH), 1725, 1685(C=O); MS *m/z* (%): 287(M⁺+2, 9), 285(M⁺, 10), 43(100); Anal. Calcd. for C₁₁H₁₂NO₃Br: C, 46.18; H, 4.23; N, 4.90; Br, 27.93. Found: C, 46.09; H, 4.20; N, 4.72; Br, 27.64.

(R)-2-(4'-methoxybenzyl)-2-methyl-malonamic acid 5f. m.p. 105.5-106.5°C; $[\alpha]_D^{22}$ -10.75 (c 1.06, MeOH), >99%ee; ¹H NMR (300 MHz, [D₆]acetone): δ 12.5(br s, 1H, OH), 7.29(brs, 1H, NH), 7.14, 6.81(AB, 4H, J=8.7Hz, ArH), 6.93(brs, 1H, NH), 3.75(s, 3H, OMe), 3.17(s, 2H, CH₂), 1.41(s, 3H, CH₃); IR(KBr): ν 3428, 3358(NH), 2850-3250(br, OH), 1717, 1652(C=O); MS *m/z* (%): 237(M⁺, 2), 121(100). HRMS Calcd. for C₁₂H₁₅NO₄: 237.100109. Found: 237.09736.

(R)-2-(3'-chlorobenzyl)-2-methyl-malonamic acid 5g. m.p. 111.1-112.1°C; $[\alpha]_D^{25}$ -9.63(c 1.25, MeOH), 95%ee; ¹H NMR (300 MHz, [D₆]acetone): δ 12.2(br s, 1H, OH), 7.32-7.18(m, 5H, ArH, NH), 6.90(br s, 1H, NH), 3.24(s, 2H, CH₂), 1.42(s, 3H, CH₃); IR(KBr): ν 3454, 3331(NH), 3084(br, OH), 1749, 1670(C=O); MS *m/z* (%): 243(M⁺+2, 3), 241(M⁺,

11), 44(100); Anal. Calcd. for $C_{11}H_{12}NO_3Cl$: C, 54.67; H, 5.00; N, 5.80; Cl, 14.67. Found: C, 54.95; H, 5.07; N, 5.71; Cl, 14.76.

(R)-2-(2'-chlorobenzyl)-2-methyl-malonamic acid 5h. m.p. 104.6-105.6°C; $[\alpha]_D^{25}$ -16.93 (c 1.14, MeOH), 97%ee; 1H NMR (300 MHz, $[D_6]$ acetone): δ 12.0 (br s, 1H, OH), 7.42-7.18(m, 5H, ArH, NH), 6.85(br s, 1H, NH), 3.47(s, 2H, CH_2), 1.41(s, 3H, CH_3); IR (KBr): ν 3475, 3325 (NH), 3000-2850 (br OH), 1698(C=O); MS m/z (%): 206 (M^+-Cl , 73), 162(100); Anal. Calcd. for $C_{11}H_{12}NO_3Cl$: C, 54.67; H, 5.00; N, 5.80; Cl, 14.67. Found: C, 54.71; H, 5.11; N, 5.74; Cl, 14.59.

(R)-2-phenylethyl-2-methyl-malonamic acid 5i. m.p. 104.5-105.5°C; $[\alpha]_D^{15}$ -4.72(c 0.924, $CHCl_3$), >99%ee; 1H NMR (300 MHz, $[D_6]$ acetone): δ 12.5(br s, 1H, OH), 7.39-7.05(m, 5H, ArH), 2.62-2.56(m, 2H, CH_2), 2.21-2.17(m, 2H, CH_2), 1.53(s, 3H, CH_3); IR(KBr): ν 3449, 3313, 3250(NH), 2500-3250(br OH), 1710, 1650(C=O); MS m/z (%): 222(M^++1 , 11), 178(M^+-CONH_2 , 32), 73(100); Anal. Calcd. for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.16; H, 6.83; N, 6.16.

Synthesis of amino acid

(R)-2-cyano-2-methyl-3-phenylpropionic acid ethyl ester 6. To a solution of (*R*)-2-benzyl-2-methyl-malonamic acid **5a** (33mg, 0.16mmol) in dry DMF (0.3ml), ethyl bromide (0.32mmol) and solid K_2CO_3 (0.32mmol) were added at room temperature. After 24 h, the resulting mixture was poured into water and extracted with ether, washed and dried on Na_2SO_4 . The solvent was removed under reduced pressure and the resulting product was purified by flash chromatograph (98% yield). The product was dissolved in dry toluene (2ml). Phosphorus pentoxide (0.3mmol) was added. The resulting mixture was refluxed for 4h, cooled to room temperature, poured into water, and extracted with ethyl acetate. The solvent was removed under reduced pressure and the resulting product was purified by flash chromatograph to afford (*R*)-**6** (93% yield). Oil; $[\alpha]_D^{25}$ -23.8 (c 0.51, $CHCl_3$); Enantiomeric excess was determined by HPLC on a Chiralcel OJ column with hexane/2-propanol mixtures 9:1; 1H NMR (300 MHz, $CDCl_3$): δ 7.39-7.23(m, 5H, ArH), 4.19(q, 2H, $J=7.2$ Hz, OCH_2), 3.23(d, 1H, $J=13.5$ Hz, CH), 3.08(d, 1H, $J=13.5$ Hz, CH), 1.62(s, 3H, CH_3), 1.24(t, 3H, $J=7.1$ Hz, CH_3); IR(film): ν 2245(CN), 1743(C=O); MS m/z (%): 217(M^+ , 3), 144($M^+-COOEt$, 5), 91(100).

(R)-2-methoxycarbonylamino-2-methyl-3-phenylpropanonitrile 7. To a solution of (*R*)-2-cyano-2-methyl-3-phenylpropionic acid ethyl ester **6** (0.27mmol) in THF (1.5ml) was added 3N NaOH (1.5ml). The mixture was stirred at room temperature for 30 min, then acidified, extracted with ethyl acetate and dried on $MgSO_4$. After evaporation, the residue was purified by flash chromatograph. To the product was added $SOCl_2$ (0.3ml) and the reaction mixture was stirred at 40°C for 3 h. The excess $SOCl_2$ was removed at reduced pressure. The residue was dissolved in cyclohexane and evaporated again. The acid chloride was then cooled to room temperature and dissolved in dry acetone (1ml). Then a solution of sodium azide (26mg, 0.4mmol) in water(0.2ml) was added and the stirring was continued for 1h. The mixture was poured into water, extracted with ether and dried on $MgSO_4$. After filtration dry methanol (1ml) was added. The solution was stirred at 80°C for 2 h and evaporated for flash chromatograph to afford (*R*)-**7** (94% yield). Lit.² m.p. 81°C; $[\alpha]_D^{22}$ -46.6(c 0.798, $CHCl_3$), {Lit.² $[\alpha]_D$ -46.1(c 2, $CHCl_3$), S}; Enantiomeric excess was

determined by HPLC on a Chiralcel OJ column with hexane/2-propanol mixtures 9:1; ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.34(m, 3H, ArH), 7.30-7.27(m, 2H, ArH), 4.94(brs, 1H, NH), 3.74(s, 3H, OMe), 3.29(d, 1H, J=13.8Hz, CH), 3.19(d, 1H, J=13.5Hz, CH), 1.66(s, 3H, CH₃); IR(KBr): ν 3325(NH), 2240(CN), 1699(C=O); MS *m/z* (%): 219(M⁺+1, 8), 218(M⁺, 15), 192(M⁺-CN, 99), 91(100).

(S)-2-ethoxycarbonylamino-2-methyl-3-phenylpropanoic acid ethyl ester 8. To a solution of (*R*)-2-benzyl-2-methyl-malonamic acid **5a** (33mg, 0.16mmol) in dry DMF (0.3ml), ethyl bromide (0.32mmol) and solid K₂CO₃ (0.32mmol) were added at room temperature. After 24 h, the resulting mixture was poured into water and extracted with ether, washed and dried on Na₂SO₄. The solvent was removed under reduced pressure and the resulting product was purified by flash chromatograph (98% yield). The product was dissolved in dry DMF (1.5ml). Hg(OAc)₂ (61mg, 0.19mmol), dry ethanol (220mg, 5mmol) and NBS (37mg, 0.21mmol) were added at room temperature. After 16 h, the resulting mixture was poured into water and extracted with ether, washed and dried on Na₂SO₄. The solvent was removed under reduced pressure and the resulting product was purified by flash chromatograph to afford (*S*)-**8** (96% yield). Oil; [α]_D¹⁸ +31.02(c 2.15, CHCl₃); Enantiomeric excess was determined by HPLC on a Chiralcel OD column with hexane/2-propanol mixtures 8:2; ¹H NMR (300 MHz, CDCl₃): δ 7.30-7.23(m, 3H, Ar-H), 7.08-7.05(m, 2H, Ar-H), 5.37(brs, 1H, NH), 4.27-4.10(m, 4H, 2OCH₂), 3.43(d, 1H, J=13.8Hz, CH), 3.18(d, 1H, J=13.2Hz, CH), 1.64(s, 3H, CH₃), 1.33-1.23(m, 6H, 2CH₃); IR(film): ν 3358(NH), 1721(C=O), 704; MS *m/z* (%): 279(M⁺, 0.8), 234(2.5), 206(M⁺-COOEt, 43.3), 190(28.0), 188(100), 142(53.5), 116(53.6), 91(58.8), 88(35.7), 42(43.0); HRMS: Calcd. for (C₁₅H₂₁NO₄)⁺ 279.14706, Found: 279.15017

(S) and (R)-α-methylphenylalanine 9. (*R*)-2-Methoxycarbonylamino-2-methyl-3-phenylpropanonitrile **7** or (*S*)-2-ethoxycarbonylamino-2-methyl-3-phenylpropanoic acid ethyl ester **8** (0.13mmol) was hydrolyzed by refluxing for 3h with 20% aqueous hydrochloric acid (3ml). The solution was evaporated under *vacuo*. To the residue was added distilled water (3ml) and evaporated again. The residue was purified by Dowex 50×2-400 ion-exchange resin (Acros), yield 95%. [α]_D¹⁷ -22.0(c 0.61, H₂O), *S*; [α]_D¹⁷ +21.8(c 0.73, H₂O), *R*; {Lit.⁵ [α]_D -22(c 1, H₂O), *S*}; ¹H NMR (300 MHz, D₂O): δ 7.22-7.19(m, 3H, ArH), 7.10-7.07(m, 2H, ArH), 3.11(d, 1H, J=14.4Hz, CH), 2.79(d, 1H, J=14.4Hz, CH), 1.37(s, 3H, CH₃); IR(KBr): 2500-3300, 1650(C=O); MS (ESI) 202([M+Na]⁺), 180([M+H]⁺).

1 J. C. Westfahl and T. L. Gresham, *J. Am. Chem. Soc.* 1955, **77**, 936.

2 C. Cativiela, M. D. Diaz-de-Villegas and J. A. Galvez, *Tetrahedron: Asymmetry*, 1994, **5**, 261.

3 H. Meyer, *Monatsh. Chem.* 1906, **27**, 1093.

4 M. Lee, Y. Jin and D. H. Kim, *Bioorg. Med. Chem.* 1999, **7**, 1755.

5 W. H. Kruizinga, J. Bolster, R. M. Kellogg, J. Kampuis, W. H. J. Boesten, E. M. Meijer and H. E. Schoemaker, *J. Org. Chem.* 1988, **53**, 1826.