Lipase-catalyzed Remote Kinetic Resolution of Quaternary Carbon-containing Alcohols and Determination of Their Absolute Configuration

Dai Sig Im,† Chan Seong Cheong,* and So Ha Lee

Life Sciences Division, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea
**Computer-aided Molecular Design & Bioscience Research Co., Ltd. 464-5, Jung-ri, Kusong-myon, Yongin-shi, Kyonggi-do 449-916, Korea*
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The quaternary carbon-containing alcohols (**1-6**) were resolved enantioselectively by various lipases such as PFL (*Pseudomonas fluorescens* lipase), LAK (*Pseudomonas fluorescens* lipase), CRL (*Candida rugosa* lipase) and PCL (*Pseudomonas cepacia* lipase). The enzymatic resolution of racemic alcohol (±)-**2** gave the excellent enantioselectivity in favor of (*S*)-**2d** in 99% ee, while those of the racemic alcohols (**1**, **3**, **4**, **5** and **6**) gave the resolved alcohols with moderate to good enantioselectivity. Also, their absolute configurations were determined by chemical transformation to the known compounds.

Key Words : Chiral tertiary nitriles, Lipase, Enantioselective, Resolution

Introduction

Chiral tertiary benzylic nitriles[1] are valuable building blocks in organic synthesis, as this unique motif can be used to create quaternary chiral centers present in numerous biologically active natural products and pharmaceutical compounds.[2-6] One of the methods for constructing of the quaternary stereogenic centers is chemo-enzymatic synthesis. Both whole-cell system and isolated enzymes from these cells are widely used as biocatalyst for this purpose.[4-6]

In the present report, the efficacies of various lipases from PCL, PFL, LAK and CRL to enantioselectively resolve racemic primary alcohols containing tertiary nitriles (**1-6**) are presented. Enzymatic enantioselective transesterification of racemic primary alcohols (**1-6**) using vinyl acetate as an acyl donor was readily accomplished. The absolute configuration of primary alcohols was determined by chemical method.

Experimental Section

General. ^{1}H NMR (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Varian Gemini 300 MHz spectrometer with TMS as an internal reference. Optical rotation was measured on Autopol® III polarimeter (Rudolph Research Co.). Low EI resolution mass spectra were determined on HP GC 5972 (column: Hewlett-Packard fused silica capillary column HP-5 cross-linked 5% phenyl methyl silicone, column ID 0.20 mm, film thickness: 0.11 μm, length: 25 m, detector : mass selective detector, 280 °C, injector 280 °C, program: initial temp 70 °C (2 min), 20 °C/min, final temp. 300 °C) and HP MS 5988A system at 70 eV. Analytical HPLC works were carried out on Varian 9010 solvent delivery system, Varian 9050 variable wavelength UV-Vis detector, and Varian 4400 integrator using the chiral columns such as Chiralcel OD, Chiralcel OJ, Chiralpak AD and OB (250 × 4.6 mm, Daicel) for substrate alcohols (**1-6**).

Materials. Column chromatography was performed on Merck Silica Gel 60 (230-400 mesh). TLC was carried out using glass sheets precoated with silica gel 60 F_{254} prepared by E. Merck. All the commercially available reagents were obtained from Aldrich, Fluka and Tokyo Kasei Chemical and generally used without further purification. Solvents were distilled over appropriate drying materials before use. PCL (lipase from *Pseudomonas cepacia* lipase, 30,000 u/g) and Lipase AK (*Pseudomonas fluorescens* lipase, >20,000 u/g) were purchased from Amano enzyme Co., Ltd. CRL (*Candida rugosa* lipase, 860 u/mg) and PFL (*Pseudomonas fluorescens* lipase, 42.5 u/mg) were purchased from Sigma and Aldrich, respectively.

(±)-4-Cyano-4-(4-nitrophenyl)-1-hexanol (1) and its derivatives

(±)-2-(4-Nitrophenyl)-5-(tetrahydropyran-2-yloxy)pentanenitrile (1b). To a stirred suspension of 60% sodium hydride (1.92 g, 48.0 mmol) in dry DMF (70 mL) was added dropwise 4-nitrophenylacetonitrile (**1a**) (6.62 g, 40.0 mmol) for 30 min at 0 °C. After the mixture was stirred for 30 min, 3-tetrahydropyranyloxypropyl bromide (10.0 g, 45.0 mmol) was added and the mixtures were further stirred at 15 °C for 12 h. The reaction medium was quenched with cold ice water (50 mL) and extracted with diethyl ether (2 × 70 mL). The organic extracts were washed with saturated aqueous $NaHCO_3$ solution, brine, dried over anhydrous $MgSO_4$ and concentrated to furnish the cyanide **1b**. The cyanide **1b** was purified by column chromatography (*n*-hexane/EtOAc = 10/1, v/v).

Yield (9.7 g) 80%; R_f 0.48 (benzene/EtOAc, 5/1, v/v); GC/MSD retention time (min) 13.01, m/z 56, 67, 77, 85 (100), 101, 115, 130, 142, 157, 174, 185, 204, 218, 231, 246, 259, 269, 277, 287, 304 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43-1.49 (m, 4H), 1.55-2.00 (m, 5H), 2.37-2.45 (m, 2H), 3.39-3.44 (m, 2H), 3.72-3.77 (m, 2H), 4.50 (t, J = 3.4 Hz, 1H), 8.05 (d, J = 8.9 Hz, 2H), 8.22 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 20.0, 25.6, 31.0, 36.2, 62.3,

65.2, 99.2, 119.9, 124.0, 139.4, 141.8, 150.5.

(±)-2-Ethyl-2-(4-nitrophenyl)-5-(tetrahydropyran-2-yloxy)pentanenitrile (1c). Compound **1c** was synthesized by the same method as compound **1b** using compound **1b** (4.0 g, 13.1 mmol) and iodoethane (1.26 mL, 15.8 mmol).

Yield (4.15 g) 95%; R_f 0.33 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 13.23, m/z 56, 67, 76, 85 (100), 101, 115, 128, 143, 155, 170, 189, 204, 214, 231, 260, 274, 287, 305, 315, 332 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H), 1.30-1.51 (m, 5H), 1.60-1.81 (m, 3H), 1.91-2.50 (m, 5H), 3.33-3.46 (m, 2H), 3.65-3.79 (m, 2H), 4.43-4.50 (m, 1H), 7.57 (d, J = 8.9 Hz, 2H), 8.23 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.9, 19.1, 25.7, 26.1, 30.9, 34.4, 37.5, 49.4, 62.8, 66.7, 99.2, 121.5, 124.4, 127.6, 146.0, 147.7.

(±)-4-Cyano-4-(4-nitrophenyl)-1-hexanol (1). To a solution of compound **1c** (3.2 g, 9.63 mmol) in methanol (40 mL) was added 1N methanolic HCl (10 mL), and stirred for 10 h at 25 °C. The reaction medium was quenched with cold ice water (50 mL) and extracted with diethyl ether (100 mL). The organic extracts were washed with saturated aqueous $NaHCO_3$ solution, brine, dried over anhydrous $MgSO_4$ and concentrated to furnish the cyanide **1**. Compound **1** was purified by column chromatography (*n*-hexane/EtOAc = 4/1, v/v).

Yield (2.1 g) 88%; mp 94-97 °C; R_f 0.10 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 11.88, m/z 51, 63, 77, 89, 102, 128, 140, 155, 175, 190 (100), 201, 218, 230, 248 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.3 Hz, 3H), 1.23-1.37 (m, 1H), 1.62-1.78 (m, 1H), 1.92-2.14 (m, 4H), 2.24 (s, 1H), 3.57 (t, J = 6.1 Hz, 2H), 7.64 (d, J = 9.0 Hz, 2H), 8.20 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.9, 28.7, 34.4, 37.2, 49.4, 62.0, 121.6, 124.5, 127.6, 141.0, 147.8; Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.6; H, 6.57; N, 10.8; HPLC analysis (Chiralcel OD column, *n*-hexane/IPA, 8/2, v/v), retention time (min) 10.18 (*S*) and 11.74 (*R*).

(±)-4-Cyano-4-(4-nitrophenyl)hexyl acetate (1d). To a solution of (±)-**1** (1.0 g, 4.0 mmol) was added 4-(dimethylamino)pyridine (10 mg), Et_3N (1.68 mL, 12.0 mmol) and acetic anhydride (1.1 mL, 8.0 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at 25 °C for 5 h. The reaction mixture was neutralized with 2% aqueous HCl solution and extracted with CH_2Cl_2 (50 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ solution, brine, dried over anhydrous $MgSO_4$ and concentrated to afford (±)-**1d** which was purified by column chromatography (*n*-hexane/ethyl acetate, 10/1, v/v).

Yield (1.0 g) 86%; R_f 0.34 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 11.86, (m/z) 50, 63, 73, 89, 101 (100), 115, 128, 143, 155, 175, 190, 202, 212, 221, 230, 248, 273, 290 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, J = 7.4 Hz, 3H), 1.38-1.44 (m, 1H), 1.80-1.85 (m, 1H), 1.95-2.18 (m, 4H), 2.04 (s, 3H), 4.03 (t, J = 6.2 Hz, 2H), 7.62 (d, J = 11.3 Hz, 2H), 8.30 (d, J = 11.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 25.1, 34.6, 37.5, 49.4, 63.6, 121.3, 124.6, 127.5, 145.5, 171.2; HPLC analysis (Chiralcel OD column, *n*-hexane/IPA, 8/2, v/v), retention time (min) 13.63 (*S*) and 15.57 (*R*).

(±)-4-Cyano-4-phenyl-1-octanol (3) and its derivatives

(±)-4-Cyano-4-phenyl-1-octanol (3). Compound **3** was synthesized by the same method as compound **1** using comound **3c** (3.8 g, 12.0 mmol).

Yield (1.78 g) 62%; R_f 0.38 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 9.62, (m/z) 51, 57, 77, 91, 103, 116, 129 (100), 142, 156, 172, 189, 204, 231 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 7.3 Hz, 3H), 1.03-1.20 (m, 1H), 1.22-1.48 (m, 4H), 1.50 (s, 1H), 1.63-1.78 (m, 1H), 1.84-2.17 (m, 4H), 3.59 (t, J = 6.2 Hz, 2H), 7.30-7.40 (m, 5H); Anal. Calcd for $C_{15}H_{21}NO$: C, 77.88; H, 9.15; N, 6.05. Found: C, 77.1; H, 9.18; N, 5.94; HPLC analysis (Chiralcel OD, *n*-hexane/IPA, 95/5, v/v), retention time (min), 29.22 (*R*) and 31.25 (*S*); HPLC analysis (Chiralcel OJ, *n*-hexane/IPA, 94/6, v/v), retention time (min), 16.48 (*S*) and 21.12 (*R*); (*R*)-(-)-**3** (>99% ee) $[\alpha]_D^{23}$ −8.34 (*c* 0.20, MeOH) (*S*)-(+)-**3** (>99% ee) $[\alpha]_D^{23}$ + 8.33 (*c* 0.20, MeOH).

(±)-4-Cyano-4-phenyloctyl acetate (3d). Compound **3d** was synthesized by the same method as compound **1d** using compound **3** (0.7 g, 3.0 mmol).

Yield (0.79 g) 96%; R_f 0.78 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 10.51, m/z 51, 77, 101, 116, 129 (100), 145, 156, 175, 187, 213, 230, 246, 273 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 7.24 Hz, 3H), 0.95-1.18 (m, 1H), 1.25-1.32 (m, 2H), 1.39-1.43 (m, 2H), 1.75-2.10 (m, 5H), 2.05 (s, 3H), 4.00 (t, J = 6.19 Hz, 2H), 7.28-7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 21.2, 22.8, 25.0, 27.6, 37.9, 41.2, 47.9, 63.9, 122.2, 127.7, 129.5, 134.1, 137.1, 171.3; HPLC analysis (Chiralcel OD, *n*-hexane/IPA, 95/5, v/v), retention time (min), 14.41 (*S*) and 19.15 (*R*); (*S*)-(+)-**3d** (>56% ee) $[\alpha]_D^{23}$ +4.1 (*c* 1.0, MeOH).

(±)-4-(4-Chlorophenyl)-4-cyano-1-octanol (4) and its derivatives

(±)-4-(4-Chlorophenyl)-4-cyano-1-octanol (4). Compound **4** was synthesized by the same method as compound **1** using compound **4c**.

Yield (0.3 g) 79%; R_f 0.38 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 10.83, (m/z) 57, 77, 85, 101, 115, 128, 150 163 (100), 179, 190, 206, 238, 247, 265 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 7.2 Hz, 3H), 1.03-1.20 (m, 1H), 1.25-1.35 (m, 4H), 1.40-1.48 (m, 1H), 1.52-1.89 (m, 4H), 2.11 (s, 1H), 3.55 (t, J = 6.0 Hz, 2H), 7.30-7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.8, 27.7, 28.7, 37.7, 41.2, 48.0, 62.2, 122.5, 127.7, 129.4, 133.9, 137.4; Anal. Calcd for $C_{15}H_{20}ClNO$: C, 67.79; H, 7.58; N, 5.27. Found: C, 67.8; H, 7.73; N, 5.29; HPLC analysis (Chiralcel OD, *n*-hexane/IPA, 95/5, v/v), retention time (min), 39.58 (*S*) and 44.18 (*R*).

(±)-4-(4-Chlorophenyl)-4-cyanooctyl acetate (4d). Compound **4d** was synthesized by the same method as compound **1d** using compound **4** (0.8 g, 3.0 mmol).

Yield (0.83 g) 90%; R_f 0.78 (*n*-hexane/EtOAc, 2/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 7.2 Hz, 3H), 0.95-1.18 (m, 1H), 1.25-1.32 (m, 2H), 1.39-1.43 (m, 2H), 1.75-2.10 (m, 5H), 2.05 (s, 3H), 4.00 (t, J = 6.19 Hz, 2H), 7.28-

7.38 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 21.2, 22.8, 25.0, 27.6, 37.9, 41.2, 47.9, 63.9, 122.2, 127.7, 129.5, 134.1, 137.1, 171.3; HPLC analysis (Chiralcel OD, *n*-hexane/IPA, 95/5, v/v), retention time (min), 14.41 (*S*) and 19.15 (*R*).

(±)-4-Cyano-4-cyclohexyl-4-phenyl-1-butanol (5) and its derivatives

(±)-2-Phenyl-5-(tetrahydro-pyran-2-yloxy)pentanenitrile (5b). To a stirred suspension of 60% sodium hydride (1.2 g, 29.0 mmol) in dry DMF (50 mL) was added dropwise benzyl cyanide (3.1 mL, 26.4 mmol) for 30 min at 0 °C. After the mixture was stirred for 30 min, 3-tetrahydropyranyloxypropyl bromide (5.89 g, 26.4 mmol) was added for 30 min and the mixtures were further stirred at 15 °C for 12 h. The reaction medium was quenched with cold ice water (50 mL) and extracted with diethyl ether (2 × 50 mL). The organic extracts were washed with saturated aqueous $NaHCO_3$ solution, brine, dried over anhydrous $MgSO_4$ and concentrated to furnish the cyanide **5b**. The compound **5b** was purified by column chromatography (*n*-hexane/ethyl acetate = 4/1, v/v).

Yield (5.82 g) 85%; R_f 0.52 (*n*-hexane/EtOAc, 2/1, v/v); GC/MSD retention time (min) 13.32, m/z 55, 67, 85 (100), 101, 129, 143, 156, 189, 198, 216, 234, 259 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48-1.53 (m, 4H), 1.56-1.92 (m, 4H), 1.98-2.08 (m, 2H), 3.38-3.56 (m, 2H), 3.75-3.83 (m, 2H), 3.87 (t, J = 7.4 Hz, 1H), 4.52-4.57 (m, 1H), 7.28-7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.0, 25.8, 27.5, 31.0, 33.4, 37.5, 62.8, 66.8, 99.3, 121.2, 127.7, 128.4, 129.4, 136.2.

(±)-2-Cyclohexyl-2-phenyl-5-(tetrahydropyran-2-yloxy)-pentanenitrile (5c). The reaction was progressed by the same method as compound **1c** using compound **5b** (2.6 g, 10.02 mmol) and bromocyclohexane (1.23 mL, 10.02 mmol).

Yield (3.0 g) 82%; R_f 0.49 (*n*-hexane/EtOAc, 4/1, v/v); GC/MSD retention time (min) 13.60, m/z 55, 85 (100), 101, 129, 150, 175, 213, 240, 259, 283, 323, 341 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06-1.12 (m, 2H), 1.20-1.30 (m, 4H), 1.50-2.03 (m, 13H), 2.05-2.11 (m, 1H), 2.15-2.30 (m, 1H), 3.29-3.35 (m, 1H), 3.45-3.49 (m, 1H), 3.63-3.67 (m, 1H), 3.74-3.78 (m, 1H), 4.45-4.51 (m, 1H), 7.27-7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.0, 26.1, 26.3, 26.7, 28.9, 29.1, 31.0, 34.3, 47.4, 53.3, 62.7, 62.8, 67.2, 99.1, 122.0, 126.9, 127.8, 129.0, 138.3.

(±)-4-Cyano-4-cyclohexyl-4-phenyl-1-butanol (5). Compound **5** was synthesized by the same method as compound **1** using compound **5c** (3 g, 8.78 mmol).

Yield (2.1 g) 91%; GC/MSD retention time (min) 11.76, m/z 55, 73, 89, 103, 115, 129 (100), 142, 158, 175, 221, 237, 257 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05-1.27 (m, 6H), 1.56-1.73 (m, 4H), 1.83-2.25 (m, 4H), 2.03 (s, 1H), 3.46-3.55 (m, 2H), 4.07-4.12 (m, 1H), 7.28-7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 21.4, 26.3, 28.9, 29.1, 34.0, 47.5, 53.3, 60.8, 62.5, 122.1, 126.8, 129.1, 138.1; Anal. Calcd for $C_{17}H_{23}NO$: C, 79.33; H, 9.01; N, 5.44. Found: C, 79.4; H, 8.98; N, 5.41; HPLC analysis (Chiralcel OD, *n*-hexane/*iso*-PrOH, 95/5, v/v), retention time (min), 50.64 (*S*) and 57.39 (*R*); (*R*) form $[\alpha]_D^{24}$ +11.4 (c = 0.25, MeOH) 99% ee.

(±)-4-Cyano-4-cyclohexyl-4-phenylbutyl acetate (5d). Compound **5d** was synthesized by the same method as compound **1d** using compound **5** (0.8 g, 3.1 mmol).

Yield (0.85 g) 91%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06-1.28 (m, 6H), 1.53-1.76 (m, 4H), 1.83-2.25 (m, 4H), 2.36 (s, 3H), 3.44-3.56 (m, 2H), 4.06-4.11 (m, 1H), 7.29-7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 21.7, 26.8, 28.8, 29.3, 34.4, 47.5, 53.3, 60.8, 62.5, 122.1, 126.8, 129.1, 138.1, 172.3; HPLC analysis (Chiralcel OD, *n*-hexane/*iso*-PrOH, 95/5, v/v), retention time (min), 10.73 (*S*) and 13.91 (*R*); (*S*) form $[\alpha]_D^{24}$ −15.1 (c = 1.8, MeOH) 99% ee.

(±)-5-Cyano-5-phenyl-1-nonanol (6) and its derivatives

(±)-5-Cyano-5-phenyl-1-nonanol (6). Compound **6** was synthesized by the same method as compound **1** using compound **6c** (**6c**) (2.90 g, 8.8 mmol).

Yield (1.81 g) 83%; R_f 0.29 (*n*-hexane/EtOAc, 1/1, v/v); GC/MSD retention time (min) 10.42, m/z 55, 65, 77, 91, 103, 116, 129, 143, 158, 173 (100), 189, 202, 218, 227, 245 (M^-); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 7.3 Hz, 3H), 1.01-1.38 (m, 5H), 1.39-1.60 (m, 3H), 1.50 (s, 1H), 1.81-2.09 (m, 4H), 3.58 (t, J = 6.2 Hz, 2H), 7.27-7.39 (m, 5H); HPLC analysis (Chiralpak AD, *n*-hexane/IPA, 94/6, v/v), retention time (min), 31.34 (*R*) and 36.78 (*S*).

(±)-5-Cyano-5-phenylnonyl acetate (6d). Compound **6d** was synthesized by the same method as compound **1d** using compound **6** (0.4 g, 1.64 mmol).

Yield (0.4 g) 88%; R_f 0.52 (*n*-hexane/EtOAc, 1/1, v/v); GC/MSD retention time (min) 10.90, (m/z) 55, 61, 91, 103, 115, 123 (100), 145, 173, 189, 200, 227, 244, 260, 287 (M^+); HPLC analysis (Chiralpak AD, *n*-hexane/IPA, 94/6, v/v), retention time (min), 8.50 (*S*) and 9.40 (*R*).

General procedure for the enzymatic kinetic transesterification of racemic alcohols (1-6) using several lipases. To a stirred solution of the primary racemic alcohols (**1-6**) (1.0 mmol) in anhydrous *n*-hexane or the mixed solvent of *n*-hexane and ethyl acetate was added any lipase in PCL (half mass), PFL (10% mass), LAK (half mass), and CRL (equivalent mass) and vinyl acetate (89 mg, 1.0 mmol) as an acyl donor at 32-34 °C and the progress of the reaction was monitored by chiral column of HPLC. The reaction mixture was diluted with diethyl ether and the enzyme was removed by filtration and the organic solvent was evaporated under reduced pressure. The reaction residue was chromatographed on silica-gel column with the mixed solvent of *n*-hexane and ethyl acetate to give the reacted acetates of each alcohol and unreacted alcohol. The isolated acetate was hydrolyzed with 1 N methanolic KOH solution to afford the corresponding alcohol.

Syntheses of the compounds for the determination of absolute configuration of the resolved alcohol (*R*)-3

(*S*)-2-Formyl-2-phenylhexanenitrile ((*S*)-8). To a solution of (*S*)-2-cyano-2-phenyl-1-hexanol (7) (0.47 g, 2.32 mmol) in methylene chloride (20 mL) was added PCC (0.6 g, 2.78 mmol) and stirred at rt for 5 h. The reaction mixture was concentrated and chromatography (*n*-hexane/EtOAc, 4/1, v/v) of the residue gave the pure (*S*)-**8**.

Yield (0.4 g) 86%; R_f 0.52 (*n*-hexane/EtOAc, 4/1, v/v);

GC/MSD retention time (min) 7.69, (m/z) 51, 63, 77, 89, 103 (100), 117, 130, 145, 158, 173, 201 245 (M^-); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.3 Hz, 3H), 1.18-1.45 (m, 4H), 1.87-2.11 (m, 2H), 7.35-7.47 (m, 5H), 8.03 (s, 1H).

***trans* (*R*)-4-Cyano-4-phenyloct-2-enoic acid methyl ester ((*R*)-9).** To a solution of (*R*)-**8** (0.12g, 0.6 mmol) in methylene chloride (15 mL) was added methyl (triphenylphosphoranylidene)acetate (0.23 g, 0.70 mmol) and stirred at rt for 2 h. The mixture was concentrated and chromatography (*n*-hexane/EtOAc, 10/1, v/v) of the residue gave the pure (*R*)-**9**.

Yield (0.13 g) 85%; R_f 0.37 (*n*-hexane/EtOAc, 4/1, v/v); GC/MSD retention time (min) 10.11, (m/z) 51, 57, 77, 89, 115, 128, 140, 158, 169 (100), 186, 201, 226, 257 (M^-); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.2 Hz, 3H), 1.32-1.41 (m, 4H), 2.06-2.14 (m, 2H), 3.76 (s, 3H), 6.29 (d, J = 15.5 Hz, 1H), 6.98 (d, J = 15.5 Hz, 1H), 7.35-7.47 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.8, 27.7, 39.7, 49.9, 52.3, 120.1, 122.4, 126.5, 128.8, 129.6, 137.4, 146.3, 166.3; (*R*)-(+)-**9** (90% ee) $[\alpha]_D^{24}$ +8.0 (*c* 0.50, MeOH).

(*R*)-(-)-4-Cyano-4-phenyloctanoic acid methyl ester ((*R*)-(-)-10). To a solution of (*R*)-**9** (0.13 g, 0.51 mmol, 90% ee) in methanol (8 mL) was added 10% Pd/C (10 mg) and charged with hydrogen by hydrogen-contained balloon. After 1h, the reaction mixture was filtered by Celite 545 and the filtrate was concentrated. The crude was purified by column chromatography (*n*-hexane/EtOAc, 10/1, v/v) to give (*R*)-**10**.

Yield (0.13 g) 95%; R_f 0.57 (*n*-hexane/EtOAc, 5/1, v/v); GC/MSD retention time (min) 9.98, (m/z) 55, 59, 77, 91, 115, 129 (100), 142, 158, 171, 185, 203, 216, 228, 244, 259 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 7.2Hz, 3H), 1.02-1.11 (m, 1H), 1.15-1.29 (m, 2H), 1.30-1.45 (m, 1H), 1.81-1.96 (m, 2H), 2.00-2.11 (m, 1H), 2.21-2.35 (m, 2H), 2.38-2.47 (m, 1H), 3.59 (s, 3H), 7.28-7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.8, 27.7, 30.5, 36.1, 41.2, 47.9, 52.1, 122.2, 126.4, 128.3, 129.4, 137.8, 173.1; (*R*)-(-)-**10** (90% ee) $[\alpha]_D^{24}$ −17.3 (*c* 1.0, MeOH).

(*R*)-(-)-4-Cyano-4-phenyl-1-octanol ((*R*)-(-)-3). To a solution of (*R*)-**10** (0.13 g, 0.48 mmol, 90% ee) in methanol (8 mL) was added $NaBH_4$ (0.18 g, 4.8 mmol) and refluxed for 5 h. The reaction mixture was quenched by 1 N aqueous HCl solution in ice bath and extracted by diethyl ether (2 × 10 mL). The organic layer was purified by column chromatography (*n*-hexane/EtOAc, 4/1, v/v) to give (*R*)-**3**. (*R*)-(-)-**3** (90% ee) $[\alpha]_D^{30}$ −6.29 (*c* 0.35, MeOH).

Syntheses of compounds for the determination of absolute configuration of the resolved alcohol (*S*)-4

(*S*)-2-(4-Chlorophenyl)-2-formylhexanenitrile ((*S*)-12). (*S*)-**12** was synthesized by the same method as (*S*)-**8** using compound **11** (0.15 g, 0.63 mmol, 95% ee) and PCC (0.27 g, 1.27 mmol).

Yield (0.13 g) 87%; R_f 0.26 (*n*-hexane/ EtOAc, 4/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.3 Hz, 3H), 1.17-1.46 (m, 4H), 1.98-2.15 (m, 1H), 2.17-2.30 (m, 1H), 7.35-7.47 (m, 5H), 9.42 (s, 1H).

(*S*)-4-(4-Chlorophenyl)-4-cyanooct-2-enoic acid methyl ester ((*S*)-13). (*S*)-**13** was synthesized by the same method as (*R*)-**9** using (*S*)-**12** (70 mg, 0.3 mmol) and methyl (triphenylphosphoranylidene)acetate (0.11 g, 0.34 mmol).

Yield (72 mg) 83%; R_f 0.46 (*n*-hexane/EtOAc, 4/1, v/v); GC/MSD retention time (min) 11.51, (m/z) 57, 65, 75, 88, 101, 113, 128, 140, 149, 163, 174, 185, 193, 203 (100), 212, 220, 235, 248, 260, 276, 291 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3H), 1.28-1.39 (m, 4H), 2.02-2.08 (m, 2H), 3.76 (s, 3H), 6.27 (d, J = 15.5 Hz, 1H), 6.91 (d, J = 15.5 Hz, 1H), 7.25-7.38 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.7, 27.7, 39.7, 49.4, 52.4, 119.7, 122.8, 128.0, 129.7, 134.9, 136.0, 145.7, 166.1.

(*S*)-4-Cyano-4-(4-chlorophenyl)octan-1-ol ((*S*)-4). (*S*)-**4** was synthesized by the same method as (*R*)-**3** using (*S*)-**14** (54 mg, 0.18 mmol) and $NaBH_4$ (70 mg, 1.80 mmol).

Yield (35 mg) 73%; HPLC analysis (Chiralcel OD, *n*-hexane/IPA, 95/5, v/v), retention time (min), 39.58 (*S*) and 44.18 (*R*).

Syntheses of compounds for the determination of absolute configuration of the resolved alcohol (*S*)-6

(*S*)-2-(3-Oxopropyl)-2-phenylhexanenitrile ((*S*)-15). (*S*)-**15** was synthesized by the same method as (*S*)-**8** using (*S*)-**3** (0.11 g, 0.46 mmol, 73% ee) and PCC (0.15 g, 0.69 mmol).

Yield (83 mg) 79%; R_f 0.26 (*n*-hexane/EtOAc, 4/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 7.2 Hz, 3H), 1.03-1.19 (m, 1H), 1.25-1.50 (m, 3H), 1.88-2.10 (m, 3H), 2.25-2.43 (m, 2H), 2.63-2.77 (m, 1H), 7.32-7.44 (m, 5H), 9.68 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.8, 27.8, 33.2, 40.5, 41.3, 47.8, 122.2, 126.2, 128.4, 129.5, 137.8, 200.5.

***cis*- and *trans*-(*S*)-2-Butyl-6-methoxy-2-phenylhex-5-enenitrile ((*S*)-16).** To a solution of (methoxymethyl)triphenylphosphonium chloride (0.15 g, 0.43 mmol) in THF (10 mL) was added dropwise *n*-butyl lithium (0.27 mL, 0.43 mmol, 1.6 M in *n*-hexane) at 78 °C and stirred for 10 min. (*S*)-**15** (83 mg, 0.36 mmol) was added to reaction mixture dropwise at 78 °C at 30 min and the mixture was stirred for 10 h at rt. After the reaction mixture was quenched by ice water (5 mL) and extracted by diethyl ether (20 mL). The organic layer was concentrated and purified by column chromatography (*n*-hexane/EtOAc, 10/1, v/v) to give (*S*)-**16**.

Yield (72 mg) 77%; R_f 0.56 (*n*-hexane/EtOAc, 4/1, v/v); GC/MSD retention time (min) 9.95 and 10.29, (m/z) 55, 63, 71 (100), 78, 85, 92, 103, 117, 129, 136, 143, 154, 166, 173, 186, 193, 201, 215, 222, 228, 242, 250, 257 (M^+).

(*S*)-(+)-2-Butyl-6-methoxy-2-phenylhexanenitrile ((*S*)-(+)-17). (*S*)-**17** was synthesized by the same method as (*R*)-**9** using (*S*)-**16** (72 mg, 0.28 mmol).

Yield (65 mg) 90%; R_f 0.42 (*n*-hexane/EtOAc, 4/1, v/v); GC/MSD retention time (min) 10.02, (m/z) 55, 65, 77, 87 (100), 103, 116, 129, 143, 158, 173, 186, 203, 216, 232, 244, 259 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 7.2 Hz, 3H), 1.00-1.17 (m, 1H), 1.20-1.34 (m, 2H), 1.38-1.60 (m, 4H), 1.82-2.03 (m, 4H), 3.28 (s, 3H), 3.29-3.41 (m, 2H), 7.29-7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.5, 22.9, 27.7, 29.8, 41.2, 48.6, 58.9, 72.7, 122.9, 126.2, 127.9, 129.2, 139.0; HPLC analysis (Chiralcel OJ, *n*-hexane/IPA, 97/3, v/v), retention time (min), 10.78 (*S*) and 15.87 (*R*); (*S*)-(+)-**17** (74% ee) $[\alpha]_D^{23}$ + 4.56 (c 0.36, MeOH).

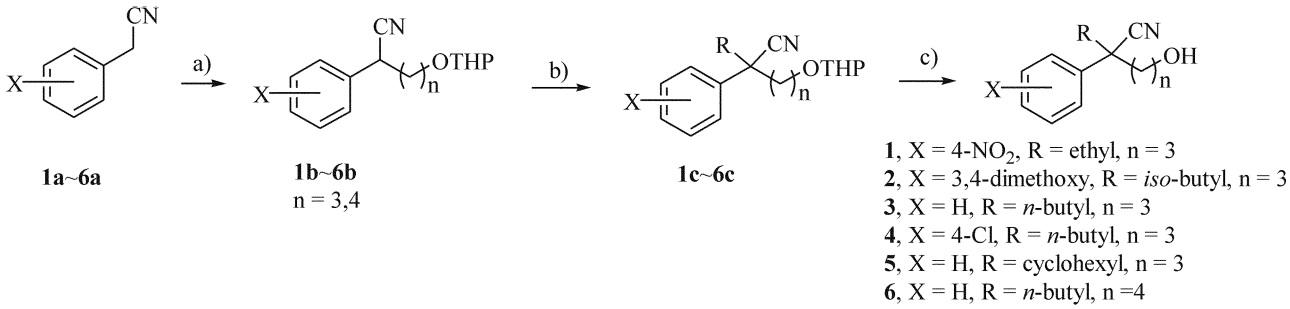
Results and Discussion

The racemic alcohols (**1-6**) were obtained in high yield from the reaction of nitriles **1a-6a** by successively reacting with THP-protected alkyl bromide and alkyl bromide. The THP-protected ether was cleaved by 1 N methanolic HCl to release racemic alcohols (**1-6**) as shown in Scheme 1.

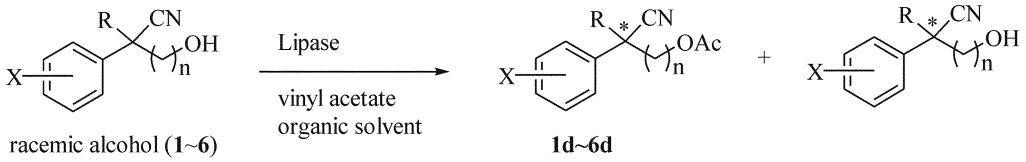
Various lipases from PFL, LAK, CRL and PCL were screened for resolving the racemic alcohols (**1-6**) by enantioselective transesterification using vinyl acetate as an acyl donor in organic solvents. Results of enzymatic reaction of the alcohols (**1-6**) with different lipases are summarized in Table 1.

In the transesterification of substrate alcohol (+)-**1**, using PFL lipase, (*R*)-**1** was the faster reacting enantiomer, and thus (*S*)-**1** remained unreacted. However, the resolved (*R*)-**1** showed low ee value. The enzymatic reaction of (+)-**2** by LAK lipase in the presence of pyridine as additive gave an excellent enantioselectivity in 99% ee over 34% conversion, but that of (±)-**2** by LAK lipase in the absence of additive did not give good enantioselectivity. Addition of additives in a lipase-catalyzed reaction is known to significantly enhance both enantioselectivity and reaction efficiency.[7] In the enzymatic reaction of racemic alcohol **2**, the addition of pyridine or 18-crown-6 as additives enhanced enantioselectivity surprisingly. In the enzymatic reaction of (+)-**3**, (*S*)-**3** was the faster reacting enantiomer, yielding (*S*)-**3d** in moderate ee, and leaving (*R*)-**3** as an enantiomerically enriched unreacted enantiomer. Also the enzymatic reaction of (±)-**4** gave excellent enantiometrically enriched unreacted enantiomer (*S*)-**4**.

In the kinetic resolution of substrate alcohol (+)-**5**, using LAK lipase, (*S*)-**5** was the faster reacting enantiomer. It gave unreacted enantiomer (*R*)-**5** in 26% ee for a reaction time of 1.5 h, and it gave the unreacted enantiomer (*R*)-**5** in 99% ee for a reaction time of 8 h. The enzymatic reaction of (+)-**6** by



Scheme 1. Syntheses of the racemic alcohols (**1-6**).



Scheme 2. Lipase-catalyzed reaction of the racemic alcohols (**1-6**).

Table 1. Results of lipase-catalyzed transesterification of racemic primary alcohols (**1-6**) using vinyl acetate as an acyl donor

Substrate alcohol	Lipase (mass equiv.)	Additives	Time (h)	Solvent	Conv. (%)[a]	ee (%)[b]		E[a]
						Reacted acetate	Residue alcohol	
1	PFL(0.1)	–	1	*n*-hexane/EtOAc (9/1)	52	40 (*R*)	44 (*S*)	3.5
2	LAK(0.5)	–	2	*n*-hexane/EtOAc (9/1)	50	76 (*S*)	76 (*R*)	16
2	LAK(0.5)	pyridine	3	*n*-hexane/EtOAc (9/1)	34	99 (*S*)	52 (*R*)	335
2	LAK(0.5)	18-crown-6	1.5	*n*-hexane/EtOAc (9/1)	50	88 (*S*)	89 (*R*)	47
3	PFL(0.1)	–	0.5	*n*-hexane	57	73 (*S*)	97 (*R*)	26
4	PFL(0.1)	–	1.5	*n*-hexane	74	34 (*R*)	99 (*S*)	8.8
5	LAK(0.5)	–	2	*n*-hexane	34	50 (*S*)	26 (*R*)	3.8
5	LAK(0.5)	–	6	*n*-hexane	61	42 (*S*)	66 (*R*)	4.6
5	LAK(0.5)		8	*n*-hexane	75	33 (*S*)	99 (*R*)	8.6
6	CRL(1)	–	1.5	*n*-hexane	40	30 (*S*)	20 (*R*)	2.2
6	PCL(0.5)	–	1.5	*n*-hexane	60	28 (*S*)	42 (*R*)	2.6

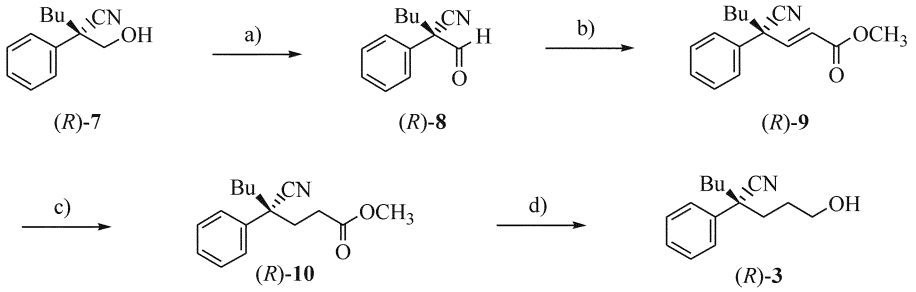
[a]Conversion and E were calculated from $ee_{substrate\ alcohol}$ and $ee_{product\ acetate}$, respectively.[8,9] [b]Measured by HPLC and GC and see experiment part.

enzymes did not give good enantioselectivity.

The absolute configuration of the resolved alcohol (*R*)-**1** was determined by the reaction of the resolved alcohol (*R*)-**1** with Jones reagent, followed by treatment with concentrated sulphuric acid to give (*R*)-(−)-3-ethyl-3-(4-nitrophenyl)-piperidine-2,6-dione ($[\alpha]_D^{20}$ +55.4 (c 0.2, MeOH, 48% ee, lit.[10] $[\alpha]_D^{20}$ +129 (c 1.0, MeOH)) as reported in the literature. (*R*)-2-Cyano-2-phenyl-1-hexanol ((*R*)-**7**)[6] was reacted with pyridinium chlorochromate (PCC) to give (*R*)-**8**, which on Wittig olefination with methyl (triphenylphosphoranylidene)acetate gave (*R*)-**9**. (*R*)-**9** was reduced by 10% palladium on charcoal, followed by sodium borohydride to give (*R*)-**3**. The optical rotation of the resolved alcohol (*R*)-**3** was favorably compared with that of the chiral compound **3** synthesized as shown in Scheme 3.

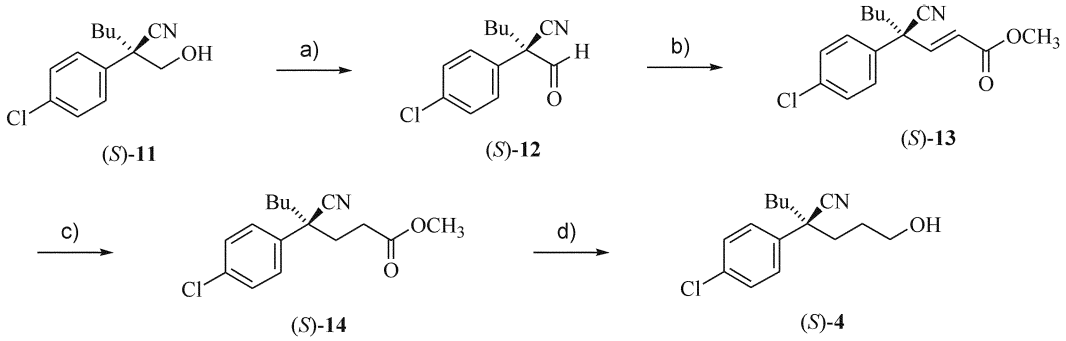
The absolute configuration of the resolved alcohol (*S*)-**4** was determined by the chemical transformation of (*S*)-2-(4-chlorophenyl)-2-cyano-2-phenyl-1-hexanol ((*S*)-**11**)[6,11] to (*S*)-**4** as shown in Scheme 4.

Also the absolute configuration of the resolved alcohol



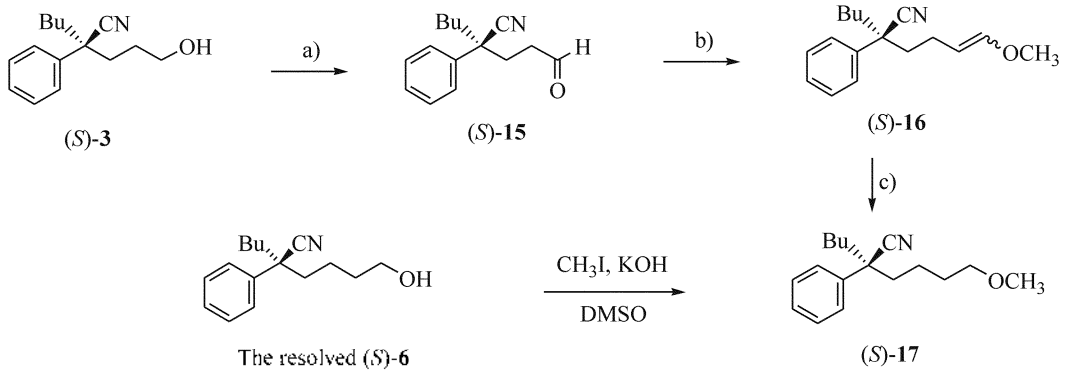
a) PCC, CH_2Cl_2, rt, 86%, b) $Ph_3PCHCO_2CH_3$, CH_2Cl_2, 90%
c) 10% Pd/C, CH_3OH, rt, 95%, d) $NaBH_4$, CH_3OH, reflux, 90%

Scheme 3. Synthesis of authentic (*R*)-**3**.



a) PCC, CH_2Cl_2, rt, 87%, b) $Ph_3PCHCO_2CH_3$, CH_2Cl_2, 83%
c) 10% Pd/C, CH_3OH, rt 90%, d) $NaBH_4$, CH_3OH, reflux, 73%

Scheme 4. Synthesis of authentic (*S*)-**4**.



a) PCC, CH_2Cl_2, rt, 79%, b) $Ph_3PCHOCH_3Cl$, *n*-BuLi, -78 °C to rt, THF, 77%.
c) 10% Pd/C, CH_3OH, rt, 90%

Scheme 5. Synthesis of authentic (*S*)-**6**.

(*S*)-**6** was determined by the chemical transformation of (*S*)-**3** to (*S*)-**17** as shown in Scheme 5. The resolved alcohol (*S*)-**6** reacted with methyl iodide to give (*S*)-**17** and the optical value of (*S*)-**17** was compared with that of (*S*)-**17** synthesized.

In conclusion, the racemic alcohols (**1-6**) were resolved by enzymes and their absolute configurations were determined. Also the resolved alcohols (**1-6**) can be further employed to synthesize various derivatives such as bicyclic amidines, lactones, primary amines, aldehydes, amides, carboxylic acid and pyridine.

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