Enzymatic Preparation of Enantiomerically Pure (1*R*,2*R*)- and (1*S*,2*S*)-2-Aminocyclohexanols

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Synopsis. The *Pseudomonas cepacia* lipase-catalyzed enantioselective hydrolysis of *trans*-2-(*t*-butoxycarbonyl)-aminocyclohexyl acetate was readily accomplished to provide a practical method for the industrial preparation of enantiomerically pure (1*R*,2*R*)- and (1*S*,2*S*)-2-aminocyclohexanols.

A number of approaches to the optical resolution of *trans*-2-aminocyclohexanol and its derivatives have been reported by several groups.[1] Faber et al. reported the lipase-catalyzed resolution of (1*R*,2*R*)- and (1*S*,2*S*)-2-aminocyclohexanols; however their method resulted in a low yield (34—35%).[2] Herein, we describe a practical enzymatic resolution of (±)-*trans*-2-aminocyclohexanol in high chemical and optical yield (97—>99%ee). The substrates **1a**—**e** were synthesized by standard methods (Fig. 1).

The enzymatic hydrolyses of **1a**—**e** using several available lipases[3] were studied. Although lipase-catalyzed hydrolyses of **1b**—**e** furnished no reaction, two lipases, *Pseudomonas cepacia* lipase and Lipase AMANO A6, gave satisfactory results in the case of **1a**.

Next, a multigram preparation was attempted by using 10 g of **1a** in 200 cm^3 of a 0.1 M (1 M=1 mol dm^{-3}) phosphate buffer (pH 7.0) and 1.0 g of enzyme at 30 °C (Table 1). The reaction was monitored by HPLC. After the reaction was completed a mixture of hydrolyzed product **3** and remaining acetate **6** was purified by column chromatography on silica gel and the corresponding *O*,*N*-protections were easily removed to afford **4** and **7**[4] in quantitative yields (Scheme 1). The optical purities of **4** and **7** were determined by HPLC using a chiral column after conversion into *N*-(2,4-dinitrophenyl)derivatives **5** and **8**.

As shown in Table 1, *Pseudomonas cepacia* lipase afforded the best results in terms of the optical purity and chemical yield, affording **3** (48%) of >99% ee and **6** (50%) of 97% ee. Furthermore, it is noteworthy that this method was also shown to be applicable to kilogram-scale reactions for industrial production.

Experimental

Proton NMR spectra were obtained on a JEOL-EX-270 (270 MHz) in $CDCl_3$ or DMSO-d_6 using TMS as an internal standard. The IR spectra were taken on a Hitachi 260-10 infrared spectrophotometer. The MS measurements were carried out with a JEOL-SX-102 mass spectrometer. Elemental analyses were performed using a Yanagimoto-MT-3-CHN-corder; they agreed with the calculated values within ±0.3%. The melting points were determined in open capillary tubes in a Büchi 510 circulating oil melting-point apparatus and were uncorrected. Optical rotations were measured with a PM-101 (Union giken).

1 (1*R*/*S*,2*R*/*S*)

1a : R_1=H, R_2=BOC **1d** : R_1,R_2=Phthaloyl
1b : R_1=H, R_2=Ac **1e** : R_1=H, R_2=H
1c : R_1=H, R_2=Z

Fig. 1.

***trans*-2-(*t*-Butoxycarbonylamino)cyclohexyl Acetate (1a).** To a solution of *trans*-2-aminocyclohexanol (100 g, 865 mmol) in water (300 cm^3) and 1,4-dioxane (300 cm^3), di-*t*-butyl dicarbonate (198 g, 907 mmol) was added. After stirring the reaction mixture for 3 h at room temperature water (1.0 L) was added to the mixture. The separated crystals were collected and dried in vacuo to give the desired carbamate. Then acetic anhydride (195 cm^3, 2.1 mol) was added dropwise under a nitrogen atmosphere at room temperature to a stirred suspension of the above-mentioned carbamate in triethylamine (290 cm^3). The reaction mixture was stirred at room temperature for 18 h, and then poured into cold water. Stirring the mixture gave crystals which were collected and dried in vacuo to give acetate **1a** (176 g, 2 steps 79%). Mp 92—93 °C; ^{1}H NMR ($CDCl_3$) δ=1.16—1.54 (4H, m, AcOCHCH$_2$C$\underline{H}_2$C$\underline{H}_2$), 1.43 (9H, s, BOC), 1.69—1.76 (2H, m, AcOCHC$\underline{H}_2$), 1.94—2.07 (2H, m, BOCHNCHC$\underline{H}_2$), 2.05 (3H, s, OAc), 4.54—4.64 (2H, m, C$\underline{H}$NH and C$\underline{H}$OAc); IR (KBr) 3310, 1725, 1670 cm^{-1} ($\nu_{C=O}$); FABMS *m*/*z* 258 (MH^+). Found: C, 60.50; H, 9.27; N, 5.44%. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44%.

(1*R*,2*R*)-2-(*t*-Butoxycarbonylamino)cyclohexanol (3) and (1*S*,2*S*)-2-(*t*-Butoxycarbonylamino)cyclohexyl Acetate (6). *Pseudomonas cepacia* lipase (10 g) was added to a rapidly stirred suspension of acetate **1a** (10 g, 39 mmol) in a 0.1 M phosphate buffer (200 cm^3) of pH 7.0. After stirring for 30 h at 40 °C, the mixture was extracted with chloroform (200 cm^3). The extract was filtered and the filtrate was dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by flush column chromatography (ethyl acetate/hexane=1/4), to give alcohol **3** (4.0 g, 48%) and acetate **6** (5.0 g, 50%).

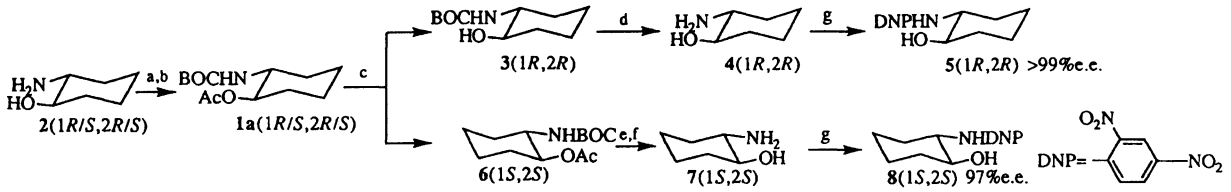
3; mp 110—111 °C; ^{1}H NMR ($CDCl_3$) δ=1.11—1.33 (4H, m, HOCHCH$_2$C$\underline{H}_2$C$\underline{H}_2$), 1.45 (9H, s, BOC), 1.67—1.73 (2H, m, HOCHC$\underline{H}_2$ or BOCHNCHC$\underline{H}_2$), 1.94—2.06 (2H, m, HOCHC$\underline{H}_2$ or BOCHNCHC$\underline{H}_2$), 3.28—3.30 (2H, m, C$\underline{H}$NH and C$\underline{H}$OH); IR (KBr) 3310, 1670 cm^{-1} ($\nu_{C=O}$); FABMS *m*/*z* 216 (MH^+). Found: C, 61.23; H, 10.12; N, 6.57%. Calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51%.

6; mp 106—107 °C. Found: C, 60.50; H, 9.27; N, 5.44%. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44%. The

Table 1. Lipase-Catalyzed Hydrolyses of **1a**

Lipase	Lipase (mg)/Substrate (mg)	Time	Hydrolyzed alcohol **4**	**5**-%ee[a)]	**8**-%ee[a)]
		h	Conv. (%)[a)]		
Pseudomonas cepacia lipase	0.1	30	48	>99	97
Lipase AMANO A6	0.1	173	33	94	67
Lipase AMANO A6	1.0	28	51	90	96

a) Determined by HPLC.



(a) $(t\text{-BuOCO})_2O$, H_2O, dioxane. (b) NEt_3, Ac_2O, 2steps 79% .(c)*Pseudomonas cepacia* lipase, 0.1M phosphate buffer, **3**(48%) and **6**(50%). (d) HCl, dioxane, 97%. (e)1N NaOHaq, MeOH. (f) HCl, dioxane, 2steps 92%. (g) 2,4-dinitrofluorobenzene, NEt_3, $CHCl_3$.

Scheme 1.

^{1}H NMR, IR, FABMS data of **6** were identical to those of **1a**.

Hydrochloride of (1*R*,2*R*)-2-Aminocyclohexanol (4). To a solution of alcohol **3** (850 mg, 3.9 mmol) in 1,4-dioxane (8.5 cm^3), 4 M HCl in 1,4-dioxane (3.0 cm^3, 11.8 mmol) was added dropwise with stirring at 60 °C; the stirring was continued for 2.5 h. The resulting crystals were collected and dried in vacuo to give amine **4** (583 mg, 97%). ^{1}H NMR (DMSO-d_6) δ=1.20—1.44 (4H, m, $HOCHCH_2C\underline{H}_2C\underline{H}_2$), 1.62—1.65 (2H, m, $HOCHC\underline{H}_2$), 1.87—2.01 (2H, m, $H_2NCHC\underline{H}_2$), 2.68 (1H, br s, $C\underline{H}NH_2$), 3.37 (1H, brs, $C\underline{H}OH$), 5.42 (1H, d, J=4.6 Hz, OH), 8.10 (3H, br s, NH_2HCl); IR (KBr) 2950, 1510 cm^{-1}. Found: m/z 115.1002. Calcd for $C_6H_{13}ON$: M, 115.0997. $[\alpha]_D^{22}$ −36.8° (c 0.4, H_2O).

Hydrochloride of (1*S*,2*S*)-2-Aminocyclohexanol (7). To a solution of alcohol **6** (850 mg, 3.9 mmol) in methanol (10 cm^3), a 1 M NaOH soln (5.1 cm^3, 5.1 mmol) was added. After stirring for 1.5 h at 60 °C the mixture was extracted with dichloromethane (50 cm^3). The extract was dried over $MgSO_4$ and then evaporated. To a solution of the residue (846 mg) in 1,4-dioxane (8.5 cm^3), 4 M HCl in 1,4-dioxane (3.0 cm^3, 11.8 mmol) was added dropwise with stirring at 60 °C. Stirring was continued for 2.5 h to give crystals, which were collected and dried in vacuo to give amine **7** (540 mg, 2 steps 97%). The ^{1}H NMR, IR data of **7** were identical to those of **4**. Found: m/z 115.1013. Calcd for $C_6H_{13}ON$: M, 115.0998. $[\alpha]_D^{22}$ +33.1° (c 0.4, H_2O).

Conversion into *N*-(2,4-Dinitrophenyl) Derivatives 5 and 8. To a solution of amine **4** or **7** (5 mg, 33 μmol) in chloroform (0.5 cm^3), triethylamine (10 μl, 72 μmol) and 2, 4-dinitro fluorobenzene (63 μl, 0.5 mmol) were added. After stirring for 1 h at 60 °C the mixture was dried in vacuo. The residue was dilluted with ethanol (5.0 cm^3). The enantiomeric excesses of **5** and **8** were determined by HPLC using CHIRALPAK AS (Daicel Chem. Ind., Ltd.), using hexane/ethanol=94/6 as the mobile phase. The retention times of **5** and **8** were 20.8 and 27.2 min, respectively, at a flow rate of 1.5 cm^3 min^{-1}.

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References

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2) K. Faber, H. Hönig, and P. Seufer-Wasserthal, *Tetrahedron Lett.*, **29**, 1903 (1988).

3) *Pseudomonas cepacia* lipase (Lipase AMANO PS), Lipase AMANO A6, Lipase AMANO M, Lipase AMANO F, Lipase AMANO AY, Lipase AMANO PGE.

4) Absolute configuration of **3** and **6** was determined based on the specific rotation. **4** (>99% ee) $[\alpha]_D^{22}$ −36.8° (c 0.4, H_2O), **7** (97% ee) $[\alpha]_D^{22}$ +33.1° (c 0.4, H_2O), lit,[1a)] for (1*R*,2*R*)-**4** $[\alpha]_D$ −36.8° for (1*S*,2*S*)-**7** $[\alpha]_D$ +31.2°.