Journal of Molecular Catalysis B: Enzymatic 64 (2010) 96–100

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Elaboration on the access to (*S*)-4-(4-methylbenzyl)oxy-3-hydroxybutanenitrile, a key intermediate for statins, by combining the kinetic resolution of racemate and the recycle of undesired enantiomer

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ARTICLE INFO

Article history:
Received 24 January 2010
Received in revised form 14 February 2010
Accepted 15 February 2010
Available online 21 February 2010

Keywords:
Lipase
Hydrolysis
Kinetic resolution
Statin
Asymmetric reduction
Yeast

ABSTRACT

High enantioselectivity (*E* 94) was observed in *Candida antarctica* lipase B-catalyzed hydrolysis of the corresponding acetate of racemic title compound. The reaction rate of the slow (*S*)-isomer was effectively suppressed by lowering the reaction temperature from 25 °C to 5 °C, to allow a five times increase of the enantioselectivity. The ee of the (*S*)-isomer, reached 97.8% at the reasonable conversion (52%) as the unreacted recovery, and the repetition of the enzymatic reaction provided pure enantiomer. The undesired (*R*)-isomer was oxidized with IBX and reduced with whole-cell biocatalysis with *Candida floricola* JCM 9439 to (*S*)-isomer (63.2% ee), which serves as the enantiomerically enriched substrate for further lipase-catalyzed resolution. The combination of total processes provided over 50% yield of the pure (*S*)-isomer, exceeding the theoretical limit for the enantiomeric resolution of racemate.

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1. Introduction

The preparation of hydroxy-oxoester (*S*)-**1a** and its precursor, hydroxynitrile (*S*)-**2a**, as the synthetic intermediates to statins, had so far been developed [1] (Fig. 1). The enzyme-catalyzed enantiomeric resolution of **2a** is promising, as the corresponding racemate is easily available in large scale by nucleophilic ring opening of glycidyl ether with cyanide ion. To date, *Artgribacter* lipase-mediated enantioselective acetylation of racemic alcohol **2a** had been reported [2]. The reaction proceeded with $E = 40$, and the ee of desired "slow-reacting isomer" reached 97.0% at $c = 54\%$.

Especially in the case that the desired enantiomer is slow-reacting isomer in kinetic resolution, the conversion as high as possible is expected to provide enantiomerically pure product, in addition to the higher enantioselectivity. We turned our attention to the lipase-catalyzed hydrolysis under aqueous conditions for two reasons. First, in aqueous conditions, enzymes work more efficiently, and the ability fits the purpose to remove "fast-reacting isomer". Second, during the progress of lipase-catalyzed transesterification, equilibrium always exists between the fast-reacting isomers, the alcohol (substrate) and the ester (product). Even by applying highly active acyl donor such as vinyl acetate which works in irreversible manner, the above-mentioned equilibrium is problematic to suppress the forward reaction for the removal of final trace of "fast-reacting isomer".

2. Experimental

^{1}H NMR and ^{13}C NMR spectra were measured at 400 MHz and 100 MHz, respectively, on a VARIAN 400-MR spectrometer. IR spectra were measured as thin films for oils or ATR for solid on a Jeol FT-IR SPX60 spectrometer. HPLC data were recorded on Jasco MD-2010 and SHIMADZU SPD-M20A multi-channel detectors at 215 nm. Optical rotation values were recorded on a Jasco P-1010 polarimeter. Silica gel 60 N (spherical, neutral, 63–210 μm, 37565-79) of Kanto Chemical Co. was used for column chromatography. Preparative TLC was performed with Merck Silica Gel 60 F_{254} plates (0.5 mm thickness, No. 5744). Yeast strains are attributed to Japan Collection of Microorganisms; Riken Bioresource Center, Planning Section, Research Promotion Division, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan, and to NITE Biological Resource Center; Department of Biotechnology, National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan.

2.1. (±)-4-(4-Methylbenzyl)oxy-3-hydroxybutanenitrile (2a)

According to the reported procedure [3], 1-(4-methylbenzyl)oxy-2,3-epoxypropane [2] was treated with NaCN in aqueous buffer solution. To a mixture of the epoxide (2.0 g,

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doi:10.1016/j.molcatb.2010.02.008

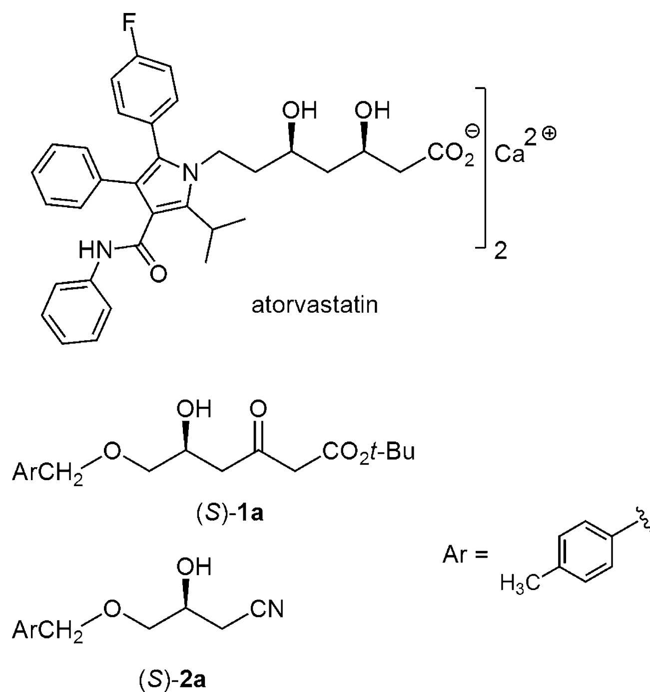


Fig. 1. Representative statin and its precursors.

11.2 mmol) and Tris buffer (50 mM, 20 mL, pH 8), NaCN (708 mg, 14.4 mmol, 1.3 equiv.) was added with stirring. The mixture was stirred for 5 days at room temperature and extracted three times with AcOEt. The combined organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (70 g). Elution with hexane/AcOEt = 4:3 afforded (±)-**2a** (1.7 g, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.33 (s, 3H, Me), 2.54 (dd, $J_{2a,3}$ = 6.4 Hz, $J_{2a,2b}$ = 16.6 Hz, 1H, H2a), 2.59 (dd, $J_{2b,3}$ = 6.0 Hz, 1H, H2b), 3.49 (dd, $J_{3,4a}$ = 5.6 Hz, $J_{4a,4b}$ = 9.6 Hz, 1H, H4a), 3.55 (dd, $J_{3,4b}$ = 4.0 Hz, 1H, H4b), 4.07 (dddd, 1H, H3), 4.51 (s, 2H, Bn-C*H*$_2$), 7.16 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.20 (d, 2H, Ar-*H*). Its NMR spectrum was identical with that reported previously [2]. HPLC [column, Daicel CHIRALCEL® AD-H, 0.46 cm × 25 cm; hexane/EtOH = 95:5; flow rate 1.0 mL/min]: t_R (min) = 34.8, 38.9.

2.2. (±)-1-Cyanomethyl-[2-(4-methylbenzyl)oxy]ethyl acetate (2b)

This was prepared from (±)-**2a** by acetylation in a conventional manner. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3H, Ac), 2.33 (s, 3H, Me), 2.74 (dd, $J_{1,1'a}$ = 5.7 Hz, $J_{1'a,1'b}$ = 17.0 Hz, 1H, H1′a), 2.78 (dd, $J_{1,1'b}$ = 5.5 Hz, 1H, H1′b), 3.56 (dd, $J_{1,2a}$ = 6.0 Hz, $J_{2a,2b}$ = 10.2 Hz, 1H, H2a), 3.63 (dd, $J_{1,2b}$ = 4.7 Hz, 1H, H2b), 4.48 (d, *J* = 11.8 Hz, 1H, Bn-C*H*), 4.52 (d, 1H, Bn-C*H*), 5.10 (dddd, 1H, H1), 7.15 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.19 (d, 2H, Ar-*H*). Its NMR spectrum was identical with that reported previously [2]. HPLC [column, CHIRALCEL® AD-H, 0.46 cm × 25 cm; hexane/EtOH = 95:5; flow rate 1.0 mL/min]: t_R (min) = 14.7, 16.0.

2.3. C. antarctica lipase B-catalyzed hydrolysis of (±)-2b

To a mixture of acetate (±)-**2b** (10.9 g, 44.3 mmol) and phosphate buffer (0.2 M, 90 mL, pH 7.0) was added *C. antarctica* lipase B (Novozym 435, 8.8 g). The mixture was stirred for 24 h at 5 °C. The mixture was filtered through a Celite pad and extracted three times with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100 g). Elution with hexane/AcOEt = 3:1 afforded (*S*)-**2b** (5.2 g, 48%, 97.8% ee) and (*R*)-**2a** (4.1 g, 45%, 90.8% ee). The ees were determined by the HPLC analysis as described for racemic **2a** and **2b**. (*S*)-**2b**: t_R (min) = 14.7 (1.1%), 16.0 (98.9%). (*R*)-**2a**: t_R (min) = 34.8 (95.4%), 38.9 (4.6%); $[\alpha]_D^{26}$ +1.3 (*c* 3.0, $CHCl_3$) [lit. [2] $[\alpha]_D^{25}$ −2.6 (*c* 3.0, $CHCl_3$), for (*S*)-**2a**]. The conversion and *E* value were calculated to be 52% and 94%, respectively.

2.4. Approach to enantiomerically pure (S)-2a

In the similar manner as described for the hydrolysis of (±)-**2b**, treatment of (*S*)-**2b** (5.2 g, 97.8% ee) with *C. antarctica* lipase B (4.8 g) for 24 h at 5 °C gave (*S*)-**2b** (4.8 g, 93%, 100% ee) as unreacted recovery. HPLC: t_R (min) = 16.0 (single peak); $[\alpha]_D^{26}$ −4.6 (*c* 3.0, $CHCl_3$) [lit. [2] $[\alpha]_D^{20}$ +4.0 (*c* 3.0, $CHCl_3$), for (*R*)-**2b**]. ^{1}H NMR spectrum was identical with that of racemic sample. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.8, 20.6, 20.9, 67.3, 68.5, 73.2, 116.1, 127.7, 128.9, 134.0, 137.5, 169.7; the signal 127.7 and 128.9 included totally four carbons; IR: 2921, 2863, 1733, 1375, 1232 cm^{-1}. This was hydrolyzed by treatment with K_2CO_3 in methanol to give (*S*)-**2a** (3.7 g, 95%). HPLC: t_R (min) = 38.9 (single peak); $[\alpha]_D^{24}$ −1.3 (*c* 3.0, $CHCl_3$) [lit. [2] $[\alpha]_D^{25}$ −2.6 (*c* 3.0, $CHCl_3$)]. ^{1}H NMR spectrum was identical with that of racemic sample. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.1, 22.4, 66.5, 71.9, 73.5, 117.2, 128.0, 129.2, 134.1, 137.9; the signal 128.0 and 129.2 included totally four carbons; IR: 3450, 2924, 2868, 2254, 1103 cm^{-1}. Its ^{13}C NMR and IR spectra were in good accordance with those reported previously [2].

2.5. C. antarctica lipase B-catalyzed transesterification of (±)-2b

To a solution of (±)-**2b** (11.5 mg, 46.6 μmol) in cyclopentanol (100 μL) was added *C. antarctica* lipase B (46 mg), and the mixture was stirred for 7 h at 5 °C. After removal of insoluble materials, the filtrate was concentrated *in vacuo* to give a mixture of (*S*)-**2b** and (*R*)-**2a** (total 12.4 mg). The mixture was analyzed by HPLC: t_R (min) = 14.7 (4.7%) [(*R*)-**2b**], 16.0 (44.4%) [(*S*)-**2b**], 34.8 (45.0%) [(*R*)-**2a**], 38.9 (5.9%) [(*S*)-**2a**]. The conversion and *E* value were calculated to be 51% and 19, respectively.

2.6. tert-Butyl (S)-5-hydroxy-6-(4-methylbenzyl)oxy-3-oxohexanoate (1a)

To a solution of $MeSO_3H$ [1] (0.3 μL, 4.87 μmol, 0.01 equiv.) in THF (100 μL) was added pre-treated Zn powder [4] (320 mg, 4.87 mmol, 10 equiv.), and the resulting mixture was stirred for 30 min under the ultrasonic vibration (200 W) for further activation. A solution of (*S*)-**2a** (100 mg, 487 μmol) in THF (2 mL) was added dropwise over 30 min under ultrasonic vibration. Then *tert*-butyl bromoacetate (950 mg, 4.87 mmol, 10 equiv.) was also added dropwise over 30 min while applying the ultrasonic vibration. The mixture was further stirred for 3 h at 50 °C. After cooling, the mixture was filtered through a Celite pad, and to the filtrate was added hydrochloric acid (1 M) to pH 2 and the mixture was stirred for 30 min at 0 °C. The mixture was extracted three times with AcOEt, and the combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (4.5 g). Elution with hexane/AcOEt = 5:1 afforded (*S*)-**1a** (118 mg, 75%) as a yellow oil. $[\alpha]_D^{26}$ −8.6 (*c* 2.0, $CHCl_3$) [lit. [1] $[\alpha]_D^{20}$ −13.0 (*c* 2.5, $CHCl_3$)]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, *tert*-butyl), 2.32 (s, 3H, Me), 2.72 (d, $J_{4,5}$ = 6.9 Hz, 2H, H4), 2.84 (br s, 1H, OH), 3.36 (s, 2H, H2), 3.41 (dd, $J_{5,6a}$ = 6.2 Hz, $J_{6a,6b}$ = 9.5 Hz, 1H, H6a), 3.46 (dd, $J_{5,6b}$ = 4.5 Hz, 1H, H6b), 4.26 (ddt, 1H, H5), 4.49 (s, 2H, Bn-C*H*$_2$), 7.13 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.19 (d, 2H, Ar-*H*); ^{13}C

Table 1
Lipase-catalyzed hydrolysis and transesterification of (±)-**2b**.

Entry	Method	Lipase	Temp. (°C)	Sub. conc. (M)	Conv. (%)	ee (*S*) (%)	*E* value
1	A	*B. cepacia* (Amano PS-IM)	5	0.5	58	11.0	1.3
2	A	*C. antarctica* B (Novo, Novozym 435)	25	0.1	48	73.4	18
3	A	Novozym 435	5	0.1	45	79.1	105
4	A	Novozym 435	5	0.5	52	97.8	94
5	B	Amano PS-IM	25	0.1	72	69.4	3
6	B	Novozym 435	25	0.1	42	50.8	10
7	B	Novozym 435	5	0.1	43	59.6	17
8	B	Novozym 435	5	0.5	51	80.9	19

A: hydrolysis in buffer solution; B: transesterification in cyclopentanol. For detail, see Sections 2.3 and 2.5.

NMR ($CDCl_3$, 100 MHz): δ 21.1, 27.9, 46.1, 51.1, 66.6, 72.9, 73.2, 82.0, 127.8, 129.0, 134.7, 137.4, 166.1, 202.9; the signal 127.8 and 129.0 included totally four carbons, and the signal 27.9 included totally three carbons; IR: 3437, 2976, 2929, 2866, 1711, 1146, 1093 cm^{-1}. Its NMR and IR spectrum were in good accordance with those reported previously [1].

For the confirmation of enantiomeric purity, this was converted to the dihydroxy ester **3**. To a solution of (*S*)-**1a** (31.0 mg, 96.2 μmol) in methanol (300 μL) was added $NaBH_4$ (10.9 mg, 288 μmol, 3 equiv.), and the mixture was stirred for 2.5 h at 0 °C. The reaction was quenched with mannitol (15.6 mg), and extracted three times with AcOEt. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by preparative TLC with hexane/AcOEt = 4:3 to give a mixture of (3*R*,5*S*)- and (3*S*,5*S*)-**3** (20.7 mg, 66%) as a colorless oil. The dihydroxy ester **3** was analyzed by HPLC [column, CHIRALCEL® AD-H, 0.46 cm × 25 cm; hexane/*i*-PrOH = 10:1; flow rate 0.5 mL/min]: t_R (min) = 23.0 [59.6%, (3*R*,5*S*)-**3**], 35.0 [40.4%, (3*S*,5*S*)-**3**].

On the other hand, for providing an authentic mixture of stereoisomers, a solution of (±)-**1a** (30.8 mg, 95.5 μmol) in MeOH (300 μL) was treated with $NaBH_4$ (10.8 mg, 286 μmol, 3 equiv.), to give **3** (20.2 mg, 65%) as a colorless oil. HPLC analysis was performed in the same manner: t_R (min) = 23.0 [28.0%, (3*R*,5*S*)-**3**], 27.0 [28.0%, (3*S*,5*R*)-**3**], 27.0 [22.0%, (3*R*,5*R*)-**3**], 35.0 [22.0%, (3*S*,5*S*)-**3**].

2.7. 4-(4-Methylbenzyl)oxy-3-oxobutanenitrile (4)

To a solution of (*R*)-**2a** (410 mg, 2.00 mmol) in acetonitrile (8.2 mL) was added IBX (1.12 g, 4.00 mmol, 2 equiv.) with stirring. The progress of the reaction was monitored by silica gel TLC, developed with hexane/AcOEt = 4:3, R_f for **2a**: 0.34; **4**: 0.50. After stirring for 8 h at 70 °C, the mixture was filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 g), to remove contaminated 4-methylbenzaldehyde (R_f: 0.70). Elution with hexane/AcOEt = 3:1 afforded **4** (204 mg, 50%) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H, Me), 3.63 (s, 2H, H2), 4.09 (s, 2H, H4), 4.55 (s, 2H, Bn-CH_2), 7.17 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 7.20 (d, 2H, Ar-*H*). This was employed for the next step without further purification.

2.8. Screening of microorganisms

The screening was performed as follows. The microorganisms from stock culture samples were incubated in glucose medium [containing glucose (200 mg), peptone (80 mg), yeast extract (20 mg), KH_2PO_4 (12 mg), K_2HPO_4 (8 mg), at pH 6.5, total volume of 4 mL, in the test tube] for 2 days at 30 °C. Then ketone **4** (20 mg) and glucose (200 mg) were added, and the mixture was shaken on a reciprocal shaker (200 cpm) for 1 day at 30 °C. Each mixture was filtered through a Celite pad and extracted three times with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by preparative TLC with hexane/AcOEt = 4:3 to afford **2a** as a colorless oil. Product **2a** was analyzed by HPLC under the conditions in Section 2.1.

2.9. Pre-incubation of C. floricola JCM 9439

A small portion of yeast cells of *C. floricola* JCM 9439 grown on the agar-plate culture was aseptically inoculated to a glucose medium [containing glucose (600 mg), peptone (240 mg), yeast extract (60 mg), KH_2PO_4 (36 mg), K_2HPO_4 (24 mg), at pH 6.5, total volume of 12 mL] in a test tube and then shaken on a reciprocal shaker (180 cpm) for 2 days at 30 °C.

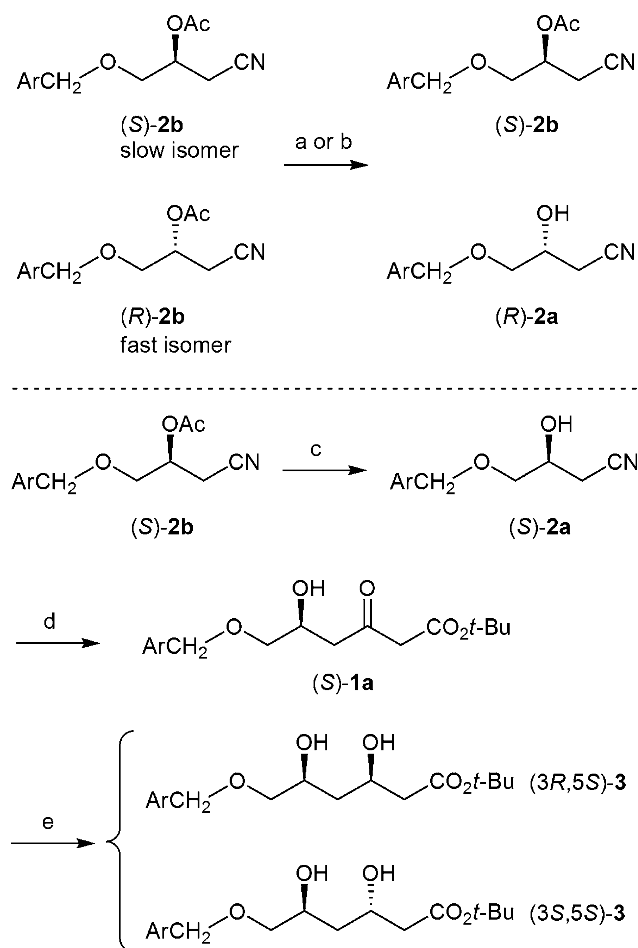
2.10. Reduction of 4 by C. floricola JCM 9439

To a mixture of **4** (32.5 mg, 160 μmol) and a glucose medium incubated as described above (10 mL), was added glucose (500 mg) in a test tube. The mixture was shaken on a reciprocal shaker (180 cpm) for 1 day at 30 °C, and then was saturated with NaCl and added AcOEt. The mixture was stirred for 10 min and filtered through a Celite pad. The organic layer of the filtrate was separated and aqueous layer was further extracted three times with AcOEt. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by preparative TLC with hexane/AcOEt = 4:3 to afford (*S*)-**2a** (63.2% ee). The ee was determined by HPLC: t_R (min) = 34.8 (18.4%), 38.9 (81.6%). The yield of (*S*)-**2a** was estimated to be 75%, at the stage just before the final purification, based on ^1H NMR with an internal standard [(±)-(1*R**,2*R**,3*R**,6*R**,7*S**)-2-iodo-4,8-dioxatricyclo[4.2.1.0$^{3.7}$]nonan-5-one, whose purity is guaranteed by its crystalline nature [5], by comparing with its standard signal at δ = 5.10 (d, *J* = 5.2 Hz, 1H)].

3. Results and discussion

To our disappointment, our first trial by applying commercially available lipases was miserable; *E* values only as low as 1.3 (lipase PS-IM, *Burkholderia cepacia*, Amano) and 18 (lipase B Novozym 435, *C. antarctica*, Novozymes Japan) were observed. The reactivity of "slow" isomer toward *C. antarctica* lipase B, however, turned out to be surprisingly sensitive to reaction temperature, and became very slow at low temperature. *E* value was raised to 105 (5 °C) from 18 (25 °C), as shown in Table 1 (Scheme 1).

Aiming more practical resolution conditions, the higher substrate concentration was attempted from 0.1 M to 0.5 M. The longer reaction time was effective to provide (*S*)-**2b** with 97.8% ee at the conversion of 52%. The high enantioselectivity (*E* = 94) was retained under these conditions. By the repetition of the same lipase-catalyzed reaction, enentiomerically pure (*S*)-**2b** became available. For the second run, conversion only as low as 5% was enough, so that the undesired (*R*)-**2b** was completely removed by hydrolysis.



Scheme 1. Reagents and conditions: (a) *C. antarctica* lipase B (Novozym 435), phosphate buffer (0.2 M, pH 7.0); (b) Novozym 435, cyclopentanol; (c) K_2CO_3, MeOH (95%); (d) Zn, *tert*-butyl bromoacetate, $MeSO_3H$, THF (75%); (e) $NaBH_4$, MeOH (66%).

The larger difference between the reaction rates of enantiomers of **2b** was observed under hydrolysis in buffer solution than the transesterification in organic solvent. In latter case, *E* 17–19 were shown even under at 5 °C, by applying cyclopentanol as nucleophile as well as solvent [6]. A similar temperature-dependent increase of enentioselectivity was observed, but not comprehensive: *E* 17 at 5 °C from 10 at 25 °C.

The removal of acetyl group, on rather labile β-hydroxy nitrile under basic conditions, worked well by applying K_2CO_3 in methanol with the entire retention of enantiomeric purity.

The ee of an advanced intermediate (*S*)-**1a** obtained by two carbon elongation with Blaise reaction conditions [1,4] was also guaranteed to be 100% in the following manner. The reduction of (*S*)-**1a** with $NaBH_4$ in methanol provided an inseparable mixture of dihydroxy esters, (3*R*,5*S*)-**3** (*syn* isomer) and (3*S*,5*S*)-**3** (*anti* isomer). HPLC analysis of this mixture with CHIRALCEL® AD-H, two peaks with retention times of 23.0 min and 35.0 min appeared in 3:2 ratio. The assignment of the former was (3*R*,5*S*)-**3** and of the latter was (3*S*,5*S*)-**3**, respectively, by combining those NMR spectra, after derivation to the stereochemically established corresponding acetonides of diol moiety [1]. Each diol **3** was revealed to be enentiomerically pure, as there were no (3*S*,5*R*)- and (3*R*,5*R*)-isomers both of which appeared at the retention time at 27.0 min.

The next and very important task was the utilization of undesired (*R*)-**2a**, merging into (*S*)-isomer. In this case, the dynamic kinetic resolution [7,8] is not available, as the desirable (*S*)-isomer is slow-reacting isomer [2,9,10]. We decided to examine an oxido-reductive recycling of (*R*)-**2a**. Unfortunately, the first oxidation turned out to be hard task because of facile β-elimination during the course of dehydrogenation of β-hydroxynitrile (*R*)-**2a**. After several experiments, only the treatment with IBX in acetonitrile was found to be successful, to give the ketone **4** in 50% yield. As the substrate became in hand, the enantioselective reduction by means of incubated whole cells of yeasts was examined [11,12]. The results for the screening of yeast strains are summarized in Table 2 (Scheme 2).

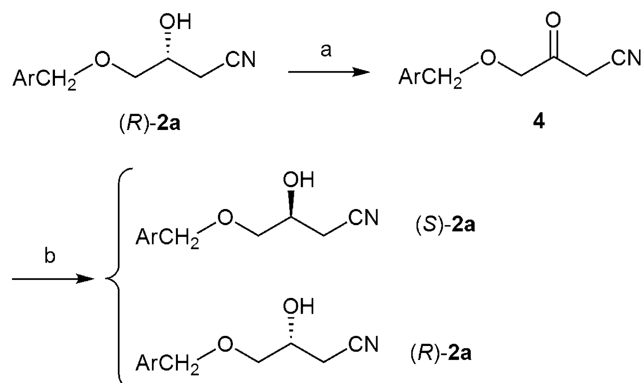
Table 2
Whole-cell microorganism-catalyzed reduction of **4**.

Entry	Microorganisms	JCM No.	Abs. config.	ee (%)
1	*Candida floricola*	9439	*S*	63.9
2	*Trichosporon cutaneum*	1534	*S*	61.1
3	*Yamadazyma farinosa*	10896[a]	*S*	59.9
4	*Geotrichum candidum*	5767[a]	*S*	58.5
5	*Williopsis californica*	3600	*S*	57.8
6	*Candida kefyr*	21874	*S*	45.2
7	*Pichia finlandica*	3639	*S*	35.7
8	*Pichia henricii*	3611	*S*	31.0
9	*Bullera alba*	6139	*S*	23.0
10	*Saccharomyces cerevisiae*	2214	*S*	14.9
11	*Candida nitratophila*	9856	*S*	8.7
12	*Rhodotorula auranitiaca*	3771	*R*	44.1
13	*Yarrowia lipolytica*	21884	*R*	12.6
14	*Rhodotorula rubra*	21966	*R*	11.4
15	*Rhodotorula minuta*	8105	*R*	7.8
16	*Toluraspora delbrueckii*	10921[a]	*R*	3.4

For incubation and reaction conditions, see Section 2.8.
[a] NBRC number.

C. floricola JCM 9439, producing the desired (*S*)-**2a** was selected by the enantioselectivity as the index, and the incubation and the reaction conditions were further elaborated. A deleterious, but not negligible side reaction was the rather strange formation of 4-methylbenzyl alcohol. This was produced from 4-methylbenzaldehyde under yeast-mediated reduction conditions. That aldehyde was caused by an overoxidation of ketone **4** in the precedent, IBX oxidation of alcohol **2a**. As the reduction of this aldehyde predominated over that of **4**, the aldehyde seemed to have an inhibitory effect for the reduction of ketones. To avoid this, the suppression of the contaminating aldehyde in **4** to a minimal amount was necessary.

The next task was the enhancement of the activity of carbonyl reductase in *C. floricola* JCM 9439. In the particular case for the reduction of **4**, it was revealed that pre-incubated whole broth without any isolation of cells worked well in a reproducible manner. The enantiomerically enriched (63.2% ee) of (*S*)-**2a** was produced in 75% yield, which can be further recycled as the start-



Scheme 2. Reagents and conditions: (a) IBX, acetonitrile (50%); (b) whole-cell microorgnisms.

ing material for kinetic resolution after acetylation. This result was obtained under aerobic, weakly acidic (around pH 4, with no external control), and high glucose (5%) conditions. When the pre-incubated cells in stationary phase were once harvested by centrifugation and subsequently applied in a buffer to the substrate **4**, both of the ee (58.6%) and the yield (64%) of **2a** were lower. Such change was in good accordance with our previous experiences, that the enantioselectivity and the efficiency highly depended upon the incubation and reaction conditions, growth phase and the supply of oxygen in *C. floricola* JCM 9439 [11].

4. Conclusion

High enantioselectivity as well as the proper conversion (*E* 94, conv. 52%) was achieved, at low reaction temperature (5 °C) in *C. antarctica* lipase B-catalyzed hydrolysis of (±)-**2b**. The repetition of the hydrolysis yielded desired enantiomerically pure (*S*)-**2b** in 44% yield of the starting racemate. In the oxido-reductive recycle of undesired (*R*)-**2a**, an asymmetric reduction with *C. floricola* JCM 9439 provided (*S*)-**2a** (63.2% ee) in total 37.5% from (*R*)-**2a**. By another lipase-catalyzed resolution, this enantiomerically enriched substrate **2b** provided pure (*S*)-**2b** in 13% yield, based on the starting racemate at the beginning of the total scheme. The combined yield of (*S*)-**2b** through the total process exceeded over 50%, which is the limit for the enantiomeric resolution, especially under the circumstance that the dynamic kinetic resolution is not available for the desired slow-reacting isomer.

Acknowledgements

The authors thank Dr. Yoshihiko Hirose of Amano Enzyme Inc. for generous gift of lipase PS-IM and Dr. Yoichi Suzuki of Novozymes Japan for Novozym 435. M.S. thanks "Program for Enhancing Systematic Education in Graduate Schools" from the Japanese Society for the Promotion of Science. This work was supported both by a Grant-in-Aid for Scientific Research (No. 18580106) and "High-Tech Research Center" Project for Private Universities: matching fund subsidy 2006–2011 from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and acknowledged with thanks.

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