Enzymatic Resolution of Racemic Secondary Alcohols by Lipase B from *Candida antarctica*[1]

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ABSTRACT: Chiral intermediates *S*-(+)-2-pentanol and *S*-(+)-2-heptanol were prepared by a lipase-catalyzed enzymatic resolution process. Among various lipases evaluated for the stereoselective acylation of racemic alcohols, lipase B from *Candida antarctica* catalyzed the acylation of the undesired enantiomer of racemic alcohols leaving the desired *S*-(+)-alcohols unreacted. A reaction yield of 43–45% and an enantiomeric excess (e.e.) of >99% were obtained for *S*-(+)-2-pentanol or *S*-(+)-2-heptanol when the reaction was carried out using vinyl acetate or succnic anhydride as acylating agent. In an alternative process, an enantioselective hydrolysis of 2-pentyl acetate was demonstrated using lipase B giving *S*-(+)-2-pentyl acetate and *R*-(–)-2-pentanol. A reaction yield of 45% and an e.e. of 98.6% were obtained for *S*-(+)-2-pentyl acetate.

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KEY WORDS: Anti-Alzheimer drugs, *Candida antarctica,* chiral intermediates, enzymatic resolution, lipase, secondary alcohols.

The current interest in enzymatic production of chiral compounds lies in the preparation of intermediates for pharmaceutical synthesis (1–10). *S*-(+)-2-pentanol is a key chiral intermediate required for synthesis of anti-Alzheimer drugs that inhibit β-amyloid peptide release and/or its synthesis (11–13).

Chiral secondary alcohols have been pepared by microbial reduction of ketones (14–18). Resolution of secondary alcohols by lipase- and acylase-catalyzed reactions has been demonstrated (19–27), but most of the reports were on the resolution of compounds having bulky groups such as phenyl groups. In this report we describe an enzymatic process for the resolution of the aliphatic compounds such as racemic 2-pentanol and 2-heptanol by lipase B from *Candida antarctica*. By using succinic anhydride as acylating agent, the unreacted desired chiral alcohols can be easily separated from the product. We have scaled up the resolution process and have used substrates (racemic 2-pentanol and 2-heptanol) as a solvent in enzyme-catalyzed reactions. We are also describing acetylation of secondary alcohols using vinyl acetate and the hydrolysis of 2-pentyl acetate.

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MATERIALS AND METHODS

Materials. Racemic 2-pentanol, racemic 2-heptanol, *S*-(+)-2-pentanol, *S*-(+)-2-heptanol, *R*-(–)-2-pentanol, *R*-(–)-2-heptanol, and succinic anhydride were purchased from Aldrich Chemicals (Milwaukee, WI). *Candida antarctica* lipase B (Chirazyme L-2) and other lipases (lipases L-3–L-8) were obtained from Boehringer Mannheim (Indianapolis, IN); lipase PS-30, lipase FAP-15, lipase AK, lipase MAP-10, and lipase R were purchased from Amano International Enzyme Company (Troy, VA); porcine pancreatic lipase (PPL) was purchased from Sigma Chemicals (St. Louis, MO); and Lipomod 200 I was purchased from Biocatalysts Ltd., (Pontypridd, Wales, UK).

Analytical methods. Gas chromatography (GC) was performed on a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA) by using a cross-bonded 100% polydimethyl-polysiloxane capillary GC column (15 m × 0.32 µm, Rtx-1, Restek Corporation, Bellefonte, PA) with helium as carrier gas at 8.5 mL/min and a flame-ionization detector.

Method 1: The injector temperature was 150°C and the detector temperature was 200°C. The initial column temperature of 30°C was maintained for 10 min and then increased at the rate of 5°C/min to 50°C and kept at 50°C for an additional 10 min.

Method 2: The injector temperature was 200°C and the detector temperature was 250°C. The initial column temperature of 100°C was maintained for 10 min and then increased at the rate of 10°C/min to 200°C and kept at 200°C for an additional 2 min.

Method 3: Separation of enantiomers of racemic 2-pentanol and 2-heptanol was carried out by GC. Samples from reaction mixtures were extracted with ethyl acetate and dried over anhydrous magnesium sulfate. Analyses of samples were carried out using a Hewlett-Packard 5800 GC with flame-ionization detector. Astec Chiraldex G-TA (Whippany, NJ) (gamma cyclodextrin, 20 m × 0.25 mm × 0.125 mm thickness) was used at 28°C (for 2-pentanol) or at 44°C (for 2-heptanol). Helium was used as carrier gas at 22 mL/min. The injector temperature was 150°C, and the detector temper-

ature was 200°C. Initial oven temperature was 28°C for 15 min, and then increased at the rate of 5°C/min to 50°C and held at 50°C for 5 min for 2-pentanol analysis. Under the above conditions the retention times for *S*-2-pentanol and *R*-2-pentanol were 10.6 and 11.6 min, respectively. The retention times for *S*-2-heptanol and *R*-2-heptanol were 11.2 and 11.8 min, respectively.

The nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ solution using a JEOL (Tokyo, Japan) NMR spectrophotometer operating at 399.8 MHz for ^1H and 100.5 MHz for ^{13}C.

Lipase screening for resolution of 2-pentanol. Screening of various lipases for the resolution of racemic 2-pentanol was carried out in 10 mL of hexane containing 50 mg of racemic 2-pentanol, 200 mg of vinyl acetate, and 50 mg of enzyme. Reactions were carried out at 30°C, 150 rpm for 72 h on a rotary shaker. Samples were analyzed by GC method 1.

Preparative scale acetylations of racemic 2-pentanol were carried out in heptane. The reaction mixture in 1 L of heptane contained 100 g of racemic 2-pentanol, 1.02 mole equivalent of vinyl acetate, and 1 g of lipase B. The reaction was carried out at 35°C and 150 rpm.

Preparative scale acylation of racemic 2-pentanol was also carried out in racemic 2-pentanol or 2-heptanol as a solvent. The reaction mixture contained 1 kg of racemic alcohol, 0.68 mole equivalent of succinic anhydride, and 13 g of lipase B. The reaction was carried out at 38°C and 150 rpm.

Preparation of RS-*acetate*. To a solution of *RS*-alcohol (2 mL) in pyridine (5 mL), acetic anhydride (5 mL) was added and the mixture kept at room temperature for 16 h. The mixture was poured in ice cold water and extracted with methyl tertiary butyl ether (MTBE, 3 × 20 mL). The MTBE extract was washed with water (2 × 10 mL), 1 N HCl (2 × 10 mL) and finally with water (3 × 10 mL), dried over Na_2SO_4, filtered, and solvent was removed on a water bath maintained at 70–80°C. The retention times of the acetates and alcohols by GC method 1 were 2-pentanol 1.7 min, 2-pentyl acetate 5.6 min, 2-heptanol 9.5 min, and 2-heptyl acetate 19.9 min.

2-Pentyl acetate. ^1H NMR: δ (ppm) 0.83 (3H, *t*, *J* = 7 Hz, 5-CH_3), 1.12 (3H, *d*, *J* = 6 Hz, 1-CH_3), 1.2–1.5 (4H, *m*, 3- and 4-CH_2), 1.93 (3H, *s*, 2′-CH_3), 4.83 (1H, *m*, 2-CH). ^{13}C NMR: δ (ppm) 14.55 (C-5), 19.29 (C-2′), 20.57 (C-1), 21.92 (C-4), 38.57 (C-3), 70.96 (C-2), 170.11 (C-1′).

2-Heptyl acetate. ^1H NMR: δ (ppm) 0.82 (3H, *t*, *J* = 7 Hz, 7-CH_3), 1.14 (3H, *d*, *J* = 6 Hz, 1-CH_3), 1.2–1.6 (8H, *m*, 3,4,5,6-CH_2), 4.84 (1H, *m*, 2-CH). ^{13}C NMR: δ (ppm) 14.68 (C-7), 20.60 (C-2′), 21.98 (C-1), 23.19 (C-6), 25.70 (C-4), 32.21 (C-3), 36.42 (C-5), 71.26 (C-2), 170.16 (C-1′).

Enzymatic acetylation of 2-pentanol and 2-heptanol. Identification of acetate. Enzymatic acetylation was carried out with 500 mg of *RS*-alcohol in 10 mL heptane, 1.02 mole equivalent of vinyl acetate, and 5 mg of lipase B. The reaction was carried out at 35°C and 150 rpm for 4 h. The reaction was stopped by filtration of the enzyme. The enzymatic reaction products were analyzed by GC method 1 as described above.

Enzymatic acylation of 2-pentanol and 2-heptanol with succinic anhydride: Isolation of hemisuccinate. Enzymatic acylation was carried out with neat racemic alcohol, 0.68 molar equivalent succinic anhydride, and 13 mg lipase B/g of racemic alcohol. The reaction was carried out at 35°C and 150 rpm for 48 h. The reaction was stopped by filtration. The hemisuccinate was isolated as follows.

A portion of the filtered reaction mixture was dissolved in MTBE (5 vol). The MTBE solution was extracted with 5% $NaHCO_3$ to extract the hemisuccinate in base. The MTBE extract contained unreacted alcohol. The 5% $NaHCO_3$ extract was washed with MTBE until free from alcohol. The hemisuccinate was liberated by slow acidification of the 5% $NaHCO_3$ layer with 1 N HCl to pH 4. The hemisuccinate was extracted in MTBE from the acidic solution. The final MTBE solution was washed with 5% NaCl until neutral pH was attained, dried over anhydrous Na_2SO_4, filtered, and the solvent was removed on a water bath maintained at 70–80°C. The hemisuccinates obtained were analyzed by GC method 2 as described in analytical methods. The retention times for 2-pentyl hemisuccinate and 2-heptyl hemisuccinate were 5.9 and 8.7 min, respectively.

2-Pentyl hemisuccinate. ^1H NMR: δ (ppm) 0.81 (3H, *t*, *J* = 7 Hz, 5-CH_3), 1.11 (3H, *d*, *J* = 6 Hz, 1-CH_3), 1.2–1.5 (4H, *m*, 3- and 4-CH_2), 2.52 (4H, AB quartet, 2′ and 3′-CH_2), 4.84 (1H, *m*, 2-CH). ^{13}C NMR: δ (ppm) 14.52 (C-5), 19.21 (C-4), 20.49 (C-1), 29.57 (C-2′), 29.86 (C-3′), 38.49 (C-3), 71.54 (C-2), 171.31 (C-1′), 176.6 (C-4′).

2-Heptyl hemisuccinate. ^1H NMR: δ (ppm) 0.86 (3H, *t*, *J* = 7 Hz, 7-CH_3), 1.18 (3H, *d*, *J* = 6 Hz, 1-CH_3), 1.2–1.6 (8H, *m*, 3,4,5,6-CH_2), 2.6 (4H, AB quartet, 2′ and 3′ CH_2), 4.91 (1H, *m*, 2-CH). ^{13}C NMR: δ (ppm) 14.75 (C-7), 20.59 (C-1), 23.21 (C-6), 25.67 (C-4), 29.7 (C-2′), 29.86 (C-3′), 32.22 (C-5), 36.39 (C-3), 72.02 (C-2), 171.27 (C-1′), 177.84 (C-4′).

Isolation of S-*(+)-2-pentanol.* A neat reaction mixture from acylation of racemic 2-pentanol (1.2 L) containing 399 g (4.526 moles, 44.3 M%) of *S*-(+)-2-pentanol was used to develop the recovery process. A portion of the reaction mixture (600 mL) containing *S*-(+)-2-pentanol (193.3 g, 2.193 moles) was cooled to –12–14°C and the precipitates were removed by filtration. The clarified crude oil was distilled, with the *S*-(+)-2-pentanol distilling at 51 to 67°C under vacuum (20–25 mm Hg) to yield 165 g of product [1.866 moles, 98% enantiomeric excess (e.e.), GC homogeneity index 99.5%].

Isolation of S-*(+)-2-heptanol*. A neat reaction mixture from acylation of racemic 2-heptanol (67.5 mL) containing 22 g of *S*-(+)-2-heptanol was used to develop a recovery process. A portion of the reaction mixture (62 mL) containing *S*-(+)-2-heptanol (20.13 g, 0.173 moles) was cooled to –12–14°C, and the precipitates were removed by filtration. The clarified crude oil was distilled, with the *S*-(+)-2-heptanol distilling at 60 to 62°C under vacuum (6.0 mm Hg) to yield 17.17 g of product (0.148 moles, 98% e.e., GC homogeneity index 98.3%).

RESULTS AND DISCUSSION

Commercially available lipases from *C. rugosa* (lipase L-3),

TABLE 1
Screening of Enzymes for Resolution of Racemic 2-Pentanol[a]

Enzymes	2-Pentanol (g/L)	2-Pentylacetate (g/L)	Yield of 2-pentanol (%)	e.e. of *S*-2-Pentanol (%)
Candida antarctica lipase B (L-2)	2.48	3.5	49.6	99
C. rugosa lipase L-3	1.06	5.6	21.2	92
BM lipase L-7	3.65	1.97	73	30
BM lipase L-8	5	0	100	0
PPL	5	0	100	0
Lipomod 200 I	2.57	3.4	51.4	0
Lipase PS-30	3.89	1.56	77.8	20
Lipase FAP-15	5	0	100	0
Lipase AK	4.9	0.2	98	0
Lipase MAP-10	0	7.5	0	—

[a]Reaction mixture in 10 mL of hexane contained 50 mg of racemic 2-pentanol, 200 mg of vinyl acetate, and 50 mg of enzyme as indicated. Reactions were carried out on a rotary shaker at 150 rpm for 72 h. Suppliers: L-2, L-3, L-7, L-8 (Boehringer Mannheim, Indianapolis, IN); PS-30, FAP-15, AK, MAP-10 (Amano International Enzyme Co., Troy, VA), porcine pancreatic lipase (Sigma Chemical, St. Louis, MO); Lepomod 200 I (Biocatalysts Ltd., Pontypridd, Wales, United Kingdom). Abbreviations: e.e., enantiomeric excess; PPL, porcine pancreatic lipase.

C. antarctica (lipase B), *Penicillium* sp. (lipase R), *Mucor* sp. (lipase MAP-10), porcine pancreatic lipase, *Pseudomonas cepacia* (lipase PS-30), *Pseudomonas* sp. (lipase AK), *Geotrichum candidum* (lipase G), and Boehringer Mannheim (BM) lipases L-4, L-5, L-6, L-7, and L-8 were screened for the stereoselective acetylation of racemic 2-pentanol. *Candida antarctica* lipase B and *C. rugosa* lipase efficiently catalyzed the enantioselective acetylation. The reaction yields and enantiomeric excess (e.e.) of *S*-(+)-2-pentanol obtained with various enzymes are shown in Table 1. A reaction yield of 49.6% and an e.e. of 99% were obtained using *C. antarctica* lipase B. BM lipases L-4, L-5, L-6, lipase MAP-10, and lipase R acetylated both enantiomers of racemic 2-pentanol. BM lipase L-8, immobilized Lipomod-200, lipase FAP-15, and lipase AK were inactive in the acetylation reaction. Lower reaction yields and e.e. of *S*-(+)-2-pentanol were obtained when other enzymes were used.

Since the *R*-isomer of racemic 2-pentanol is the substrate for *C. antarctica* lipase B catalyzed acylations and the desired *S*-2-pentanol remained unreacted in the reaction mixture, we concentrated our efforts only on the isolation of the desired *S*-(+)-2-pentanol. The formation of 2-pentyl acetate by acetylation of racemic 2-pentanol with vinyl acetate was confirmed by GC comparison with the corresponding acetates prepared by conventional chemical acetylation. Preparative scale enzymatic acetylation of racemic 2-pentanol was carried out in heptane. Kinetics of the acetylation reaction are shown in Table 2. At the end of the reaction, 44.5 g of *S*-(+)-2-pentanol was obtained with an e.e. of 98%. The enantiomeric ratio E value (28) of 37 was obtained.

Among acylating agents tested, succinic anhydride was found to be the best choice due to easy recovery of *S*-(+)-2-pentanol at the end of the reaction (Scheme 1). The products 2-pentyl and 2-heptyl hemisuccinates were isolated from a portion of the enzymatic reaction mixture, and their structures were confirmed by NMR. Since the desired (*S*)-alcohols remained in the reaction mixture, we focused only on the isolation of the desired (*S*)-alcohols. The enzymatic acylation reactions of 2-pentanol were carried out using racemic 2-pentanol as solvent as well as substrate. A reaction yield of 43 M% (theoretical maximum 50%) and e.e. of 98.8–99.3% were obtained for *S*-(+)-2-pentanol. Results from three preparative batches are as shown in Table 3. The E value of 48 was obtained. From the reaction mixture *S*-(+)-2-pentanol was isolated in overall 36% yield (theoretical maximum 50%).

As described above, the resolution of 2-heptanol was also carried out using lipase B. A reaction yield of 44 M% (theoretical maximum 50%) and e.e. of 99.3% were obtained for *S*-(+)-2-heptanol. Results from two preparative batches are as shown in Table 4. The E value of 49 was obtained. From the reaction mixture *S*-(+)-2-heptanol was isolated in overall 40% yield (theoretical maximum 50%).

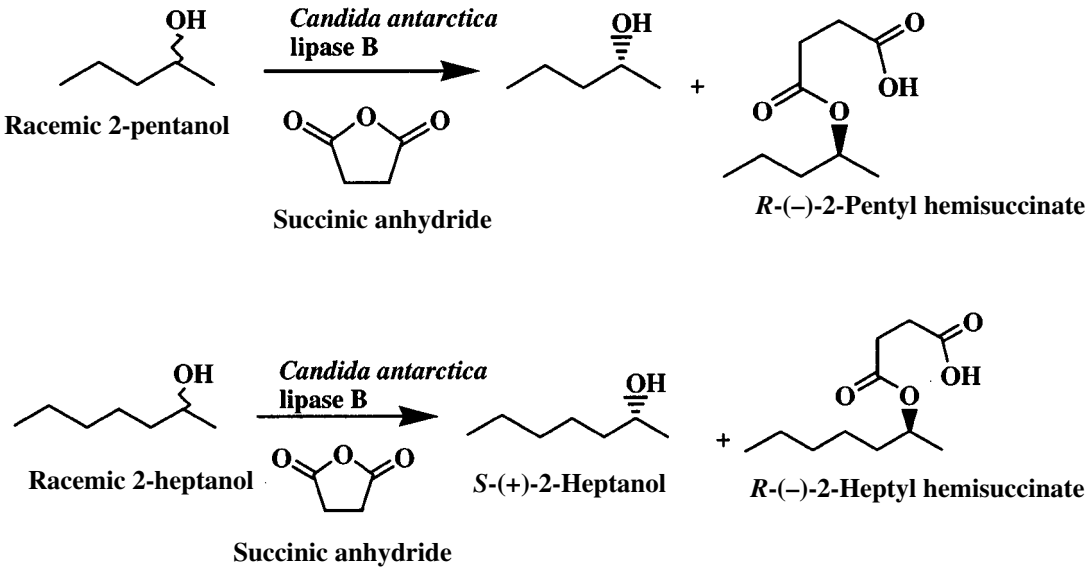
To prepare *R*-(–)-2-pentanol, hydrolysis of racemic 2-pentyl acetate was carried out using lipase B. *S*-(+)-2-Pentyl acetate was obtained in 45% yield with an e.e. of 98.6%. *R*-(–)-2-pentanol was obtained in 48 M% yield with an e.e. of 96%. The E value of 47 was obtained. The kinetics of the reaction are shown in Table 5.

Various research groups have used different solvents and different acylating agents to resolve a variety of complex secondary alcohols using lipases. Most of the reports were on the resolution of compounds having bulky group such as phenyl. Ema

TABLE 2
Preparative Scale Enzymatic Acetylation of Racemic 2-Pentanol Using Lipase B from *Candida antarctica*[a]

Reaction time (h)	2-Pentanol (g/L)	e.e. of *S*-2-Pentanol (%)
0	100	0
2	70	32
4	56	60
6	44.52	98

[a]Reaction mixture in 1 L of heptane contained 100 g of racemic 2-pentanol, 1.02 mole equivalent of vinyl acetate, and 1 g of lipase B from *C. antarctica*.The reaction was carried out at 35°C and 150 rpm. For supplier and abbreviations see Table 1.



SCHEME 1

et al. (22) has demonstrated the resolution of a large secondary alcohol, 5-[4-(1-hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin by lipase-catalyzed transesterification using vinyl acetate. Lipase PS-30 from *P. cepacia* and *C. antarctica* lipase B in dry diisopropyl ether as a solvent demonstrated high selectivity and gave the (*S*)-alcohol in 98% e.e. 1-Ethoxyvinyl esters were used as acyl donors for enzymatic resolution of secondary alcohols using *C. rugosa* lipase. The 1-ethoxyvinyl esters generate ethyl acetate as the co-product, which does not affect the enzyme activity, whereas the acetaldehyde liberated from the vinyl esters inactivates some lipases (21). Bevinakatti *et al.* (28) resolved sterically hindered secondary alcohols by lipase-catalyzed enantioselective transesterification of their *O*-acetyl esters with primary alcohols in diisopropyl ether. Mandelic acid esters, mandelonitrile, 2-chloro-1-phenylethanol, and pentolactone were resolved using *C. rugosa* lipase. (*S*)-Propanolol was prepared by lipoprotein lipase-catalyzed resolution of racemic alcohol. XAD-8 immobilized enzyme was used in tert-butyl ether as solvent with vinyl acetate as acylating agent (29). Suginaka *et al.* (25) have demonstrated the resolution of 1-(1-naphthyl)ethanol, 1-(2-naphthyl)ethanol, 1-phenyl-2-propanol, and 1,2,3,4-tetrahydro-1-naphthol by lipase-catalyzed acylation reaction using diketene as acylating agent in isopropyl ether. 1-Phenylethanol and a variety of secondary alcohols were resolved by PPL-catalyzed transesterification reactions in alkylcarbonate as solvent. Addition of molecular sieves (4Å) in the reaction mixture improved the e.e. of chiral alcohols (27).

Resolution of 3-hydroxy-1-undecyne, 3-hydroxy-1-nonene, 3-nonanol, and 1-chloro-2-octanol was demonstrated by Orrenius *et al.* (26) using lipase B from *C. antarctica* and *S*-ethyl thiooctanoate as acylating agent. Enzymatic resolution of secondary alcohols under substrate racemizing conditions was studied by Persson *et al.* (30) using an immobilized lipase from *C. antarctica* in the presence of a ruthenium catalyst. A specifically designed acyl donor, 4-chlorophenyl acetate, was used with toluene or hexane as a solvent. With this process, a variety of secondary alcohols were converted to the corresponding enantiomerically pure acetates with >99% e.e.

In this paper we have reported the lipase-catalyzed resolution of aliphatic compounds such as secondary alcohols lacking bulky groups. In the resolution process, substrates were used as solvent, giving about 450 g of resolved (*S*)-secondary alcohol from a 1-L preparative batch. Succinic anhydride was used as an acylating agent, providing easy recovery of the chiral alcohol at the end of the reaction.

TABLE 3
Enzymatic Acylation of Racemic 2-Pentanol Using Succinic Anhydride and Lipase B from *Candida antarctica*[a]

Batch number	2-Pentanol input (g)	2-Pentanol remaining (g)	e.e. of *S*-2-Pentanol (%)
132	500	210	99.2
133	500	222	98.8
136	900	400	99.3

[a]Reaction mixture contained racemic 2-pentanol as solvent and substrate, 0.68 mole equivalent of succinic anhydride, and 13 g of lipase B per kg of substrate input. The reaction was carried out at 38°C and 150 rpm. For supplier and abbreviations see Table 1.

TABLE 4
Enzymatic Acylation of Racemic 2-Heptanol Using Succinic Anhydride and Lipase B from *Candida antarctica*[a]

Batch number	2-Heptanol input (g)	2-Heptanol remaining (g)	e.e. of *S*-2-Heptanol (%)
140	100	43	99.3
141	500	222	99.4

[a]Reaction mixture contained racemic 2-pentanol as solvent and substrate, 0.68 mole equivalent of succinic anhydride, and 13 g of lipase B per kg of substrate input. The reaction was carried out at 38°C and 150 rpm. For supplier and abbreviations see Table 1.

TABLE 5
Enantioselective Enzymatic Hydrolysis of 2-Pentyl Acetate by Lipase B from *Candida antarctica*[a]

Reaction time (mm)	2-Pentyl acetate (g/L)	2-Pentanol (g)	e.e. of *S*-2-pentanol acetate (%)
9	100	0	0
15	78	15.6	28
30	67	23	46
90	48	37	83
150	45.2	39	98.6

[a]Reaction mixture in 1 L of 50 mM phosphate buffer (pH 7.0) contained 100 g of racemic 2-pentyl acetate and 0.6 g of lipase B. The pH was maintained at 7.0 with 5 N NaOH during reaction.The reaction was carried out at 32°C, 150 rpm. For abbreviation see Table 1.

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