Organic Process Research & Development **2001**, *5*, 415–420

Chemical and Enzymatic Resolution of (*R*,*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxymethylpiperidine

Animesh Goswami,* Jeffrey M. Howell, Edward Y. Hua, K. David Mirfakhrae, Maxime C. Soumeillant, Shankar Swaminathan, Xinhua Qian, Fernando A. Quiroz, Truc C. Vu, Xuebao Wang, Bin Zheng, David R. Kronenthal, and Ramesh N. Patel

Process Research and Development, Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, New Jersey 08903

Abstract:

(*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxymethylpiperidine 1 was made from (*R*,*S*)-3-hydroxymethylpiperidine 2 via fractional crystallization of the corresponding L(−)-dibenzoyl tartarate salt 3 followed by hydrolysis and acylation. Lipase from *Pseudomonas cepacia* was found to be the best enzyme for the stereospecific resolution of (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine 4. (*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxymethylpiperidine 1 was obtained in 16% yield and >95% enantiomeric excess (ee) by hydrolysis of (*R*,*S*)-acetate 5 by lipase PS from *Pseudomonas cepacia*. Lipase PS-catalyzed esterification of the (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine 4 with succinic anhydride provided the *S*-hemisuccinate ester 6, which could be easily separated and hydrolyzed by base to the (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine 1. The yield and ee could be improved greatly by repetition of the process. Using the repeated esterification procedure (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine 1 was obtained in 32% yield (maximum theoretical yield 50%) and 98.9% ee.

Introduction

S-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1** is a key intermediate in the synthesis of a potent tryptase inhibitor.[1] Previous reports on the enzymatic resolution of (*R*,*S*)-3-(hydroxymethyl)piperidine **2** by pig liver esterase and acylase showed no[2] and only marginal[3] enantiospecificity, respectively. Enzymatic resolution of (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **4** by lipase P from *Pseudomonas fluorescens* was reported for the preparation of *R*-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **7**.[4] This reported large-scale preparation involved hydrolysis of the corresponding (*R*,*S*)-butyrate **8** at 6% concentration in an aqueous system and separation of the product *R*-alcohol **7** from the unreacted *S*-acetate by chromatography. A process with low substrate concentration and chromatographic separation will have limited practical use in large-scale industrial application.

The present work describes two alternate methods for the preparation of (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1** by either resolution of the L-(−)-dibenzoyl tartaric acid salt of (*R*,*S*)-3-(hydroxymethyl)piperidine **2** or by an efficient and simple lipase-catalyzed resolution of (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **4**.

Results and Discussion

Fractional crystallization of the salt of (*R*,*S*)-3-(hydroxymethyl)piperidine **2** with L-(−)-dibenzoyl tartaric acid resulted in the separation of the salt of *S*-3-(hydroxymethyl) piperidine **3** in good yield (Figure 1) which was then converted directly to (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1** by hydrolysis and acylation in situ. One equivalent of L-(−)-dibenzoyl tartaric acid was needed to obtain an excellent enantiomeric excess (ee) and a good yield in the resolution of **1**. Utilization of less than one equivalent of the dibenzoyl tartaric acid gave **3** with low ee. Choice of ethanol was important for the success of this resolution. Absolute ethanol gave the best results. Denatured ethanol (SDA Formula 1 or 3A) containing small quantities of other solvents gave **2** with lower ee even after four and five successive crystallizations. The ee value of the resolved salt **3** after each crystallization was determined by measuring the ee of **1**. In general, the ee of **1** was about 50% after the first crystallization. The ee increased by about 15% with each crystallization thereafter. After four crystallizations, the ee of **1** usually reached 97%, and a fifth crystallization gave **1** with ee > 99% in > 30% yield.

Enzymes from many different sources were screened for the stereospecific hydrolysis of the esters. The enzymes showing promising results are shown in Table 1. The lipase of *Pseudomonas cepacia* from various commercial sources, especially the lipase PS from Amano, was found to be the best enzyme for the stereospecific hydrolysis of the esters of (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **4** to the (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1**. Increasing the chain length from acetate **5** to butyrate **8** resulted in no significant difference in either the conversion or ee. The *E*-value was found to be between 40 and 60 for both acetate **5** and butyrate **8**. The ee of the *S*-alcohol **1** was ≥95% only at very low (10−15%) conversion. *S*-alcohol **1** with an ee of ≥90% was obtained at 43% conversion (theoretical maximum 50%). The *n*-octanoyl ester **9**, however, showed a lower *E*-value of about 30. The benzoate ester **10** was not hydrolyzed at all by lipase PS. A small-scale hydrolysis of (*R*,*S*)-acetate **5** with lipase PS was stopped at 5 h when the HPLC showed 44% alcohol with

(1) Bisacchi, G.; Slusarchyk, W. A.; Treuner, U.; Sutton, J. C.; Zahler, R.; Seiler, S.; Kronenthal, D. R.; Randazzo, M. E.; Xu, Z.; Shi, Z.; Schwinden, M. D. Pat. Appl.: WO 99US13811 99/67215.
(2) Toone, E. J.; Jones, J. B. *Can. J. Chem.* **1987**, *65*, 2722−2726.
(3) Herradon, B.; Valverde, S. *Synlett* **1995**, 599−602.
(4) Wirz, B.; Walther, W. *Tetrahedron: Asym*metry **1992**, *3*, 1049−1054.

10.1021/op010210b CCC: $20.00 © 2001 American Chemical Society and The Royal Society of Chemistry
Published on Web 06/15/2001

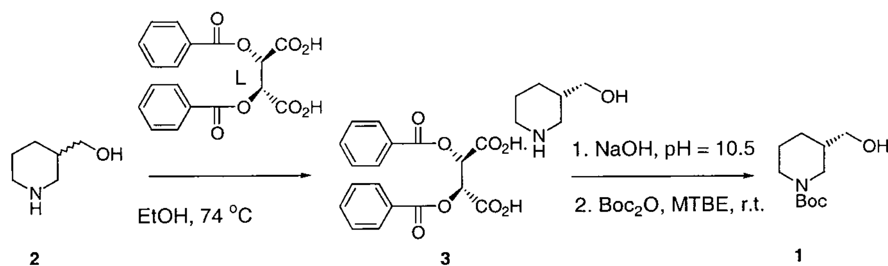

Figure 1.

Table 1. **Results of screening enzymes for the stereospecific hydrolysis of esters**

enzyme	vendor	source/microorganism	ester screened	area ratio by HPLC: alcohol (%)	area ratio by HPLC: ester (%)	ee of *S*-alcohol (%)
lipase PS	Amano	*Pseudomonas cepacia*	*n*-butyrate	45	55	70.7
lipase PS/PP, immobilized	Amano	*Pseudomonas cepacia*	*n*-butyrate	59	41	45.5
lipase P	Amano	*Pseudomonas fluorescens*	*n*-butyrate	50	50	71.1
lipase AK	Amano	*Pseudomonas fluorescens*	*n*-butyrate	38	62	38.1
lipase AK Amano 20	Amano	*Pseudomonas fluorescens*	*n*-butyrate	30	70	40.2
lipase	Biocatalyst	*Pseudomonas fluorescens*	*n*-butyrate	54	46	60.7
lipase AY 30	Amano	*Candida rugosa*	*n*-butyrate	100	0	0.4
chirazyme L-3	Boehringer	*Candida rugosa*	*n*-butyrate	100	0	0.4
chirazyme l-2, immobilized	Boehringer	*Candida antarctica*	*n*-butyrate	83	17	12.0
lipase MAP-10	Amano	*Mucor javanicus*	*n*-butyrate	42	58	61.5
lipase FAP 15	Amano	*Rhizopus oryzae*	*n*-butyrate	77	23	17.8
lipase	Biocatalyst	*Aspegillus niger*	*n*-butyrate	13	87	21.0
lipase	Biocatalyst	*Geotrichum candidum*	*n*-butyrate	11	89	−12.1
porcine pancreatic lipase, type II	Sigma	porcine pancreas	*n*-butyrate	9	91	48.7
lipase	Biocatalyst	*Penicillium cyclopium*	acetate	14	86	42.9
LIP F13	Enzymatrix	*Humicola lanuginosa*	acetate	41	59	62.3
protease, type IV, bacterial	Sigma	*Streptomyces griseus*	acetate	27	73	87.6
protease, type XVIII, fungal	Sigma	*Rhizopus sp.*	acetate	19	81	88.9
protease, type VIII	Sigma	*Bacillus licheniformis*	acetate	17	83	51.1

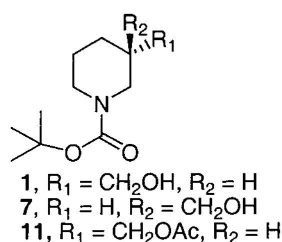

Figure 2.

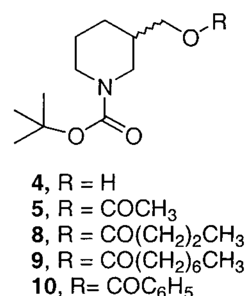

Figure 3.

an ee of 87.5% for the *S*-alcohol. The solid *S*-alcohol **1** was isolated in 16% yield with ee > 95% by extraction of the reaction mixture and dissolution in hexane. Although hexane provided direct isolation of some *S*-alcohol **1**, a large amount of the alcohol remained mixed with the acetate in hexane, and as reported by previous workers,[4] a chromatographic separation would be necessary to improve the yield (See Figures 2 and 3).

Enzyme-catalyzed acetylation of (*R*,*S*)-alcohol **4** by vinyl acetate with lipase PS immobilized on polypropylene, Accurel PP[5] (referred henceforth as "lipase PS/PP") proceeded well in either toluene or MTBE. The reaction was very slow with isopropenyl acetate as the acyl donor. The unreacted *R*-alcohol **7** was obtained directly in 46% yield with an ee of 99.4%. This process could be used if the desired alcohol would have been the *R*-alcohol **7**. The mother liquor after removal of the solid still contained considerable *R*-alcohol **7** and the product *S*-acetate **11**. Separation of the desired *S*-acetate **11** from the remaining *R*-alcohol **7** could only be carried out by chromatography as was reported previously.[4]

To develop a process for enzymatic resolution followed by easy separation, lipase PS/PP enzyme-catalyzed esterification of (*R*,*S*)-alcohol **4** by succinic anhydride was investigated. Attempts to use succinic anhydride to separate the alcohol and acetate after enzymatic acetylation were reported by previous authors;[4] however, they did not carry out the enzyme-catalyzed esterification with succinic anhydride as the acylating agent for resolution. Our process involved lipase PS/PP enzyme-catalyzed esterification of (*R*,*S*)-alcohol **4** with succinic anhydride (Figure 4) followed

(5) Patel, R. N.; Banerjee, A.; Ko, R. Y.; Howell, J. M.; Li, W.-S.; Comezoglu, F. T.; Partyka, R. A.; Szarka, L. J. *Biotechnol. Appl. Biochem.* **1994**, *20*, 23−33.

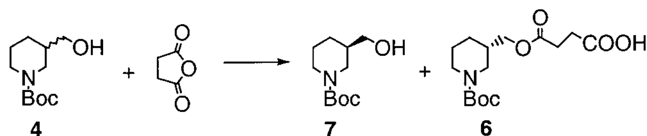

Figure 4.

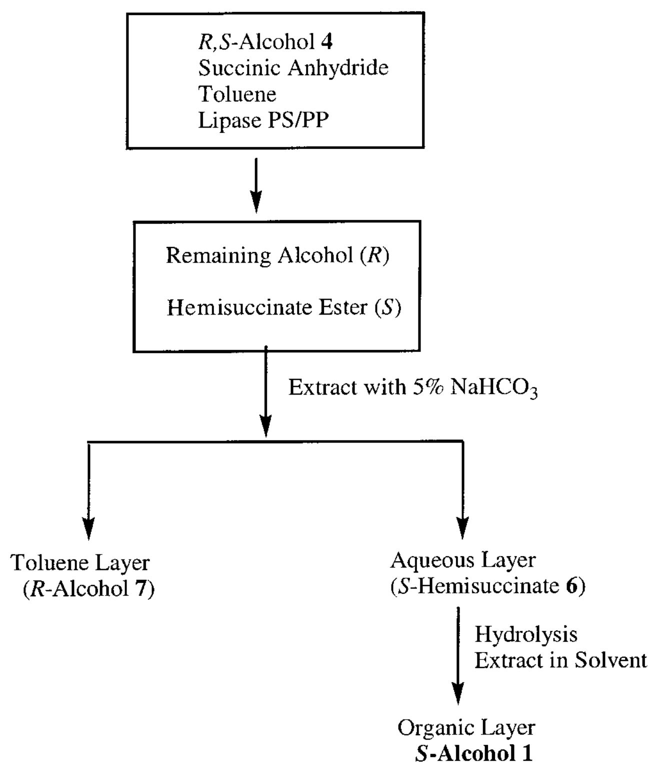

Figure 5.

by separation of the unreacted *R*-alcohol **7** from the *S*-hemisuccinate **6** by extraction with base. After investigation of the use of different bases, 5% $NaHCO_3$ was selected as the base for separating the alcohol and hemisuccinate. Subsequent hydrolysis of the *S*-hemisuccinate **6** with NaOH provided the desired *S*-alcohol **1**. The separation process is summarized in Figure 5. By using toluene as solvent and following the separation scheme, the *S*-alcohol **1** was isolated in 23% yield (maximum theoretical yield 50%) and ee ≥ 95%. The *E*-value was found to be 65−70. The esterification proceeded at a much faster rate in MTBE compared to toluene at the same enzyme-to-substrate ratio. The reaction also showed lower enantiospecificity in MTBE (*E*-value 32−47) compared to toluene. The reaction rate was also increased by increasing the amount of succinic anhydride (Figure 6). Thus the reaction can be controlled by choice of solvent, amount of succinic anhydride, and amount of enzyme. The intermediate formation of hemisuccinate **6** was confirmed by isolating hemisuccinate **6** and establishing its structure.

A Celite immobilized form, lipase PS-C, is available from Amano. The esterification of (*R*,*S*)-alcohol **4** with succinic anhydride, catalyzed by lipase PS−C, provided the *S*-alcohol **1** with an ee of only 72.2% at 49% conversion, suggesting an *E*-value of only 9. Thus, lipase PS-C is not as good as lipase PS/PP for this process.

The enzyme-catalyzed succinylation process provided *S*-alcohol **1** with 95% ee at about 30% conversion and lower ee at higher conversion. To improve the ee and also the yield, repeated esterification of the enriched alcohol was pursued.[6] The objective was to carry out the first esterification of the (*R*,*S*)-alcohol **4** to a higher conversion and even a slightly lower ee, isolate the product ester, hydrolyze to the *S*-alcohol **1**, and then re-esterify the enriched *S*-alcohol **1** a second time to obtain finally the *S*-alcohol **1** with high (>98%) ee and in high yield.

In a typical experiment, the first reaction was stopped at an HPLC area ratio of 53% unreacted alcohol and 47% hemisuccinate ester. Separation of the alcohol from the ester followed by hydrolysis of the ester by base gave the *S*-alcohol **1** in 47.8% yield (maximum theoretical yield 50%) with an ee of 85.7%. This enriched *S*-alcohol **1** was used for the second reaction. The second reaction was stopped when the area ratio of remaining alcohol and hemisuccinate ester reached 25:75. After repetition of the separation and base hydrolysis, the *S*-alcohol **1** was finally obtained in 32% overall yield (maximum theoretical yield 50%) with an ee of 98.9%. Although this process involves repetition, the unit operations are simple and do not involve any chromatographic separations.

Thus, the fractional crystallization of the dibenzoate salt and repeated enzyme catalyzed succinylation provided two alternate simple nonchromatographic methods for the preparation of (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1** in high yield and high enantiospecificity.

Experimental Section

Chemicals and Enzymes. Chemicals were purchased from Aldrich. Lipase PS was purchased from Amano. Lipase PS was immobilized on polypropylene (Accurel PP) as described in a previous report from this laboratory.[5]

Analytical Methods. The amounts of alcohol and different esters (acetate, butyrate, octanoate, and benzoate) were determined by HPLC method 1 on a reversed phase column (Zorbax Eclipse XDB C-8 column, 5 μm, 0.46 × 15 cm, Hewlett-Packard) at 40 °C with gradient elution using two solvents: A (0.2% H_3PO_4) and B (acetonitrile:0.2% H_3PO_4, 90:10) as follows: 0 to 15 min from A:B (95:5) to A:B (0:100), 15 to 20 min A:B (0:100) followed by reequilibration. The flow rate was 1 mL/min, and detection was by UV at 210 nm. The different compounds were eluted at the following retention times: alcohol **4**, 9.7 min; acetate **5**, 12.6 min; *n*-butyrate **8**, 14.8 min; *n*-octanoate **9**, 17.6 min; benzoate **10**, 15.2 min.

The alcohol **4** and hemisuccinate **6** were analyzed by HPLC method 2 using the same system as that of method 1 except that the gradient elution was carried out as follows: 0 to 10 min from A:B (60:40) to A:B (0:100), followed by reequilibration. The alcohol **4** and the hemisuccinate **6** eluted at 4.2 and 5.2 min, respectively.

The enantiomeric excess (ee) of the alcohol **4** was determined by HPLC method 3 on a Chiralpak AD column (0.46 × 25 cm, 10 μm, Daicel Chemical Industries Ltd.) and elution with a mixture of hexane:2-propanol:trifluoro-

(6) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

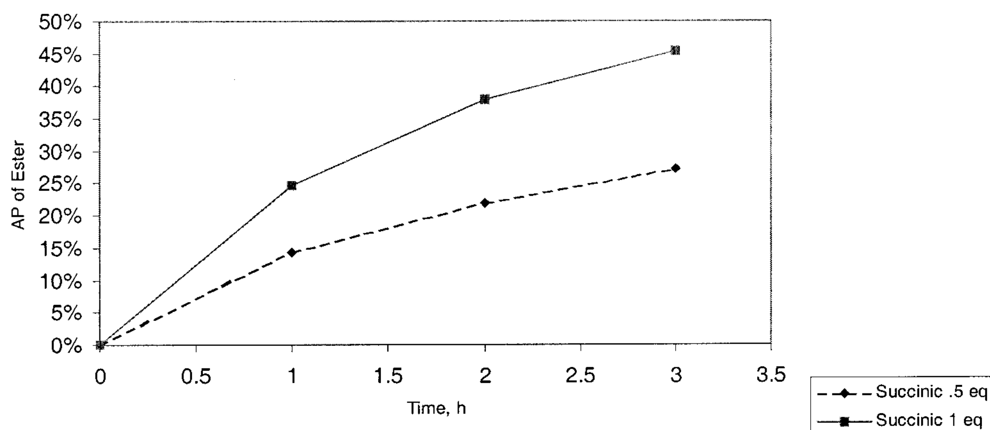

Figure 6.

acetic acid (97.9:2:0.1) at a flow rate of 1 mL/min and detection by UV at 210 nm. The enantiomers of (*R*,*S*)-alcohol **4** eluted at 21.3 (*R*, **7**) and 24.5 (*S*, **1**) min. The enantiomers of (*R*,*S*)-acetate **5** also showed two peaks with some separation and eluted at 6.8 (*S*) and 7.4 (*R*) min. The *E*-values were determined from the conversion and the ee of substrate.[6] For succinylation reactions, the ee of the *S*-alcohol **1** obtained by hydrolysis of hemisuccinate **6** was used as the ee of the product and that of the unreacted *R*-alcohol **7** as that of the substrate to calculate the *E*-values.[6]

NMR spectra were recorded in $CDCl_3$ solution at 300 MHz for ^{1}H and 125 MHz for ^{13}C, and δ values are reported.

Preparation of (*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxymethylpiperidine 1. *A. Preparation and Fractional Crystallization of Dibenzoate Salt **3**.* To a 12-L, round-bottomed flask equipped with a heating mantle, a condenser, a thermocouple, and an overhead stirrer were added **1** (210 g) and L-(−)-dibenzoyl-tartaric acid (686 g) followed by ethanol (absolute, 3.76 L) at ambient temperature. The temperature of the mixture increased from 17.6 to 32.8 °C during the addition. The solids were dissolved in ethanol by heating to 74 °C with stirring. The clear solution was then allowed to cool to room temperature and was kept stirring overnight. The white crystalline solid was collected by filtration and was subsequently washed with a small amount of ethanol. The solid was crystallized a second time by dissolving in ethanol (2.13 L) at 74 °C and cooling to room temperature. The crystallization was repeated two more times with 1.81 and 1.60 L of ethanol, respectively. After drying in a vacuum oven for 50 h at 40 °C, the final product **3** was obtained as a white crystalline solid (350 g).

*B. Hydrolysis of Dibenzoate Salt **3** and Esterification to (S)-N-(tert-Butoxycarbonyl)-3-hydroxymethylpiperidine **1**.* To a 2-L, round-bottomed flask equipped with an overhead stirrer were added **3** (100 g) and water (450 mL). The pH of the milky suspension was 2.54. The pH was adjusted to 10 by addition of a solution of 10 N NaOH while keeping the temperature below 10 °C. The resulting solution was stirred at 10 °C for 10 min followed by the addition of a solution of di-*tert*-butyl dicarbonate (96.8 g) in MTBE (300 mL) within 5 min. The reaction mixture was allowed to warm to ambient temperature. The pH value of the mixture was controlled between 10 and 10.5 by adding 10 N NaOH solution. The reaction was stirred overnight at ambient temperature. The aqueous layer was separated and subsequently extracted with MTBE (3 × 100 mL). The combined organic layer was washed with 0.5 N NaOH (100 mL), water (2 × 100 mL), and brine (150 mL), separated, dried over $MgSO_4$, filtered, and evaporated to give crude (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1** as a white solid. After being crystallized from hexanes (100 mL) and dried in a vacuum oven, (*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **1** was obtained as a white crystalline solid: 42.3 g, mp 90 °C, ee > 99%, $[\alpha]^{20}_D = +12.55°$ (c 1.07, $CHCl_3$), $[\alpha]_{365} = +40.21°$ (c 1.07, $CHCl_3$) [literature[4] for the enantiomer with 95% ee: $[\alpha]_D = -11.1°$ (c 1.48, $CHCl_3$), $[\alpha]_{365} = -36.2°$ (c 1.48, $CHCl_3$)]. ^{1}H NMR: δ 3.73 (m, br, 2H), 3.49 (t, J = 5.9 Hz, 2H), 3.30−2.60 (m, br, 2H), 1.80−1.20 (m, br, 5H), 1.42 (s, 9H). ^{13}C NMR: δ 155.1, 79.4, 64.3, 46.2, 45.0, 38.0, 28.3, 26.9, 23.9. HRMS: calcd for $C_{11}H_{21}NO_3$ (M^+ + H) 216.1600, found 216.1604.

Preparation of (*R*,*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxymethylpiperidine 4. To a 50-L three-necked round-bottomed flask equipped with an overhead stirrer, thermocouple, addition funnel, and cooling bath were added (*R*,*S*)-3-hydroxymethyl piperidine (**2**, 2.946 kg), MTBE (9 L), and water (3.5 L). The mixture was cooled to 10 °C followed by the addition of a solution of di-*tert*-butyldicarbonate (6.141 kg) in MTBE (6 L) over 2h. The reaction temperature was controlled between 10 and 18 °C during the addition of the di-*tert*-butyldicarbonate solution. After stirring for 5 h at 10−18 °C, the reaction was stirred overnight at room temperature. The aqueous phase was separated, and the organic phase was washed with 10% NaCl solution (3.5 L). The combined aqueous phase was extracted with MTBE (2.5 L) and mixed with the organic phase. Evaporation of the combined organic phase produced crude product as a light-yellow oil (6.02 kg). The oil was combined with heptane (5.6 L) and stirred overnight at room temperature to provide the solid product. The solid product was filtered, washed with cold heptane (4 L), and dried to give (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **4** (4.459 kg) as a white solid.

General Method of Hydrolysis of Esters with Enzymes. To a weighed amount of enzyme (1 to 5 mg) was added 1 mL of 100 mM phosphate buffer, followed by 20 μL (approximately 15 mg) of the ester. The mixtures were stirred for 20 h at room temperature. The reaction mixture was extracted with 2 mL of MTBE. A portion (1 mL) of the MTBE extract was evaporated, dissolved in 1 mL of acetonitrile, and analyzed by HPLC method 1 to determine the extent of hydrolysis. The remainder of the MTBE extract was evaporated and dissolved in hexane:IPA (1:1, 1 mL) and analyzed by HPLC method 3 to determine the ee of the alcohol produced.

Hydrolysis of Esters by Lipase PS. To 40 mL of 100 mM phosphate buffer, pH 7 (in a constant pH-maintaining system (Dosimat, Brinkman)), maintained at 40 °C, was added 10 mg of lipase PS-30, followed by 200 mg of *n*-butyrate ester. The pH was maintained at 7, and the progress of the hydrolysis reaction was monitored by following the consumption of 0.1 N NaOH solution. Consumption of NaOH started immediately, and the rate of addition was initially very fast. At 30 min intervals (1 h at the end), 4 mL of sample was withdrawn and extracted with 8 mL of MTBE. The MTBE extract was analyzed by HPLC to determine the conversion and ee as described before.

Hydrolysis of Acetate by Lipase PS. A solution of lipase PS-30 (50 mg) in 500 mL of 100 mM phosphate buffer, pH 7, was placed in a jacketed, three-necked, 1-L reaction vessel. The mixture was stirred magnetically, and the temperature was maintained at 40 °C throughout the reaction. (*R*,*S*)-acetate **5** (2.5 g) was added, and the pH was maintained at 7 by automatic addition of 0.1 N NaOH. The progress of the reaction was monitored by following the consumption of the NaOH solution. The theoretical amounts of 0.1 N NaOH for 100% and 50% hydrolysis are 97.28 and 48.64 mL, respectively. Consumption of NaOH started immediately. The reaction was terminated at 4.75 h when 41.585 mL of NaOH was consumed suggesting 43% conversion. The reaction mixture was extracted with MTBE (2 × 500 mL). Samples from the MTBE extract were evaporated and analyzed by HPLC as before. HPLC showed 44% alcohol with an ee of 87.5% for the *S*-alcohol. The MTBE extract was evaporated, and the residual oil was crystallized from hexane to provide 340 mg of solid *S*-alcohol **1** (16% yield from acetate) with ee > 95%.

Acetylation of *(R,S)*-Alcohol with Lipase PS/PP and Vinyl Acetate. In a vial, (*R*,*S*)-alcohol **4** (500 mg) was dissolved in 10 mL of toluene. Lipase PS/PP (10 mg) was added to the vial. Vinyl acetate (250 μL) was added to each vial. The contents were gently stirred by magnetic stirrer at room temperature. At 0.5 1, 1.5, 2, 2.5, 3, and 4 h, samples (50 μL each) were withdrawn and analyzed by HPLC as described before. After 4 h, the mixture was filtered to separate the enzyme, and the toluene solvent was evaporated in a rotary evaporator. The residue was crystallized from hexane. The *R*-alcohol **7** was obtained as a white solid (135 mg). The mother liquor was analyzed by HPLC and found to contain both the alcohol and acetate.

Esterification of (*R*,*S*)-Alcohol 4 with Succinic Anhydride Catalyzed by Lipase PS/PP. To a solution of (*R*,*S*)-alcohol **4** (2.4 g) in toluene (120 mL) in a 500-mL, round-bottomed flask fitted with a Drierite guard tube was added succinic anhydride (600 mg), and the mixture was stirred by magnetic stirrer. Lipase PS/PP (60 mg) was added. Samples were withdrawn at various intervals and analyzed by HPLC methods 2 and 3 to determine the conversion and ee. At 5 h the AP ratio showed unreacted alcohol 67% and hemisuccinate 33%, and 100 mL (equivalent to 2 g alcohol) of the mixture was taken out and filtered to remove the insoluble enzyme. Further work on the filtrate from the 5-h reaction is described below.

The remainder of the reaction was allowed to continue to 7 h and then was filtered. The AP ratio showed unreacted alcohol 59% and hemisuccinate 41%. Further work on the filtrate from the 7-h reaction is described below.

A. Filtrate from the 5-h Reaction. The filtrate (100 mL) from the 5-hour reaction was extracted with 5% $NaHCO_3$ (2 × 50 mL). The toluene layer containing the unreacted alcohol was washed with water (3 × 100 mL). HPLC showed that the ee of the unreacted *R*-alcohol was 50.4%.

The aqueous layer was washed with toluene (3 × 100 mL). HPLC showed that the 5% $NaHCO_3$ extract contained only the product hemisuccinate. To regenerate the *S*-alcohol from the hemisuccinate, 100 mL of 2 N NaOH was added to the 5% $NaHCO_3$ solution, and the mixture was stirred at room temperature for 30 min. The aqueous layer was extracted with toluene (2 × 50 mL). The toluene extract was washed with water (3 × 50 mL). HPLC showed that the hemisuccinate was completely hydrolyzed, and only *S*-alcohol with an ee of 95% was present. Removal of the toluene at the rotary evaporator provided 600 mg of oil (30% yield from 2 g of alcohol). This was dissolved in hexane (5 mL) and was left in the freezer overnight to crystallize, giving the *S*-alcohol (463 mg, 23% yield from 2 g of alcohol) with ee 95.8%.

B. Filtrate from the 7-h Reaction. The filtrate from the 7-h reaction was worked up in the same way to provide the *S*-alcohol (73.4 mg, 18% yield from 400 mg alcohol) with ee 94.9%. The ee of the unreacted *R*-alcohol was 72.7%.

Isolation of Hemisuccinate 6. To a solution of (*R*,*S*)-alcohol **4** (2 g) in toluene (20 mL) were added succinic anhydride (1 g) and lipase PS/PP (50 mg), and the mixture was stirred by magnetic stirrer at room temperature for 16 h. The reaction mixture was filtered to separate the insoluble enzyme and succinic anhydride. The filtrate was extracted with 5% $NaHCO_3$ (2 × 20 mL). The 5% $NaHCO_3$ layer was washed with toluene (3 × 10 mL) to remove any unreacted alcohol **7**. HPLC showed that the 5% $NaHCO_3$ layer contained only the hemisuccinate **6**.

To isolate the hemisuccinate **6**, the 5% $NaHCO_3$ layer was carefully adjusted to pH 4.5 by slow addition of 1 N HCl. After acidification, the aqueous layer was extracted with MTBE (3 × 10 mL). The MTBE extract was washed with 10% NaCl (3 × 10 mL) until the washing was neutral. Removal of MTBE gave the hemisuccinate ester as an oil. On mixing with 2-propanol−heptane and scratching, the oil

turned to a solid. The hemisuccinate **6**, obtained as white solid (1 g), showed only one peak at 5.2 min by HPLC method 2. ^{13}C NMR: δ 24.3, 27.1, 28.3 (3C), 28.9, 29.0, 35.3, 44.4, 48.8, 66.4, 79.7, 154.9, 171.9, 176.4.

Resolution of (*R*,*S*)-Alcohol 4 by Repeated Esterification with Succinic Anhydride. *A. First Esterification.* To a solution of (*R*,*S*)-alcohol **4** (255 g) in toluene (2.55 L) were added succinic anhydride (100 g) and lipase PS/PP (6.4 g), and the mixture was stirred by magnetic stirrer. At 2 h, the HPLC area ratio showed 53% unreacted alcohol and 47% hemisuccinate. The reaction was stopped by filtering out the enzyme. The filtrate was extracted with 5% $NaHCO_3$ (4 × 1 L). The toluene layer was separated from the aqueous layer.

The 5% $NaHCO_3$ layer was washed with toluene (3 × 1 L) to remove any unreacted alcohol. To regenerate the *S*-alcohol **1** from hemisuccinate **6**, the hydrolysis was carried out in the presence of toluene to aid extraction of the alcohol as it formed. Toluene (1 L) was added to the 5% $NaHCO_3$ solution of the hemisuccinate. The mixture was stirred, and 800 mL of 5 N NaOH was added slowly. The mixture was stirred at room temperature for 30 min. The two layers were separated, and the aqueous layer was extracted with more toluene (2 × 1 L). The toluene extract was washed with 10% NaCl solution (2 × 1 L). Removal of toluene gave 122 g of *S*-alcohol **1** with an ee of 85.7%, which was used for the second esterification below.

B. Second Esterification. The enriched *S*-alcohol **1** (122 g from the first reaction above) and succinic anhydride (42.3 g) were dissolved in toluene (1.112 L). Lipase PS/PP (3.7 g) was added, and the reaction was carried out as before. At 4.5 h, HPLC showed the area ratio of remaining alcohol and hemisuccinate to be 25:75, and the reaction was stopped by filtering the enzyme. The unreacted alcohol and hemisuccinate were separated as before by extracting the filtrate with 5% $NaHCO_3$ (1 L, 500 mL, 500 mL).

The 5% $NaHCO_3$ layer was washed with toluene (1 L, 500 mL, 500 mL). HPLC showed that the 5% $NaHCO_3$ contained only the product hemisuccinate **6**. The hydrolysis of hemisuccinate **6** was carried out in the presence of toluene (1 L) and with slow addition of 5 N NaOH (400 mL). The mixture was stirred at room temperature for 30 min. The two layers were separated, and the aqueous layer was extracted with more toluene (2 × 500 mL). The toluene extract was washed with 10% NaCl (2 × 1 L). Removal of toluene gave an oil, which solidified on cooling to room temperature. The yield of solid *S*-alcohol **1** was 82 g (32% overall yield from 255 g, maximum theoretical yield 50%) with an ee of 98.9%.

Acknowledgment

We thank Dr. Richard Mueller for reviewing the manuscript and providing valuable suggestions.

Received for review March 23, 2001.

OP010210B