Kinetic Resolution of 2-Substituted Esters Catalyzed by a Lipase Ex. *Pseudomonas fluorescens*

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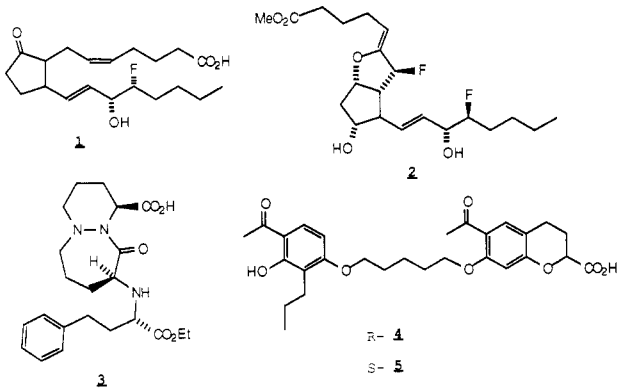
Enantioselective hydrolyses of a variety of 2-substituted racemic esters catalyzed by a bacterial lipase have been effected in high enantiomeric excess. These enzyme-catalyzed kinetic resolutions constitute an economical method of preparing enantiomerically pure 2-substituted esters and acids on a large scale. Several of the resolved enantiomers are important intermediates in the synthesis of bioactive compounds in the areas of prostanoids, angiotensin-converting enzyme inhibitors, and leukotriene antagonists.

In recent years there has been an increasing interest in the use of enzymes and microorganisms to produce optically active compounds either by means of a kinetic resolution or stereospecific chemical transformations (e.g. reductions, oxidations, epoxidations, hydroxylations, etc.).[1] Hydrolases in general have been used to effect kinetic resolutions of racemic esters. In particular, esterases have been applied primarily in enantioselective hydrolyses of mono- and diesters with chiral centers on the carboxylate part of the molecule.[2] Lipases, which are widely used in food chemistry and processes, have been used to effect kinetic resolutions of chiral alcohols via enantioselective hydrolysis of their esters.[3] However, it has become apparent recently that lipases are also effective in catalyzing enantioselective hydrolysis of racemic esters with the chirality on the carboxylate part of the molecule.[4-16] The extent of such application has been limited so far to 2-chloro- and 2-bromopropanoates,[4-7,13] certain 2-methyl-substituted esters,[8-11] 2-benzyloxy esters,[12] some carbocyclic esters,[13-15] and an indoline-2-carboxylate.[16] In addition, 2-bromopentanoate has been resolved with lipase MY (*C. cylindracea*) in low enantiomeric excess.[4] It is known from the available data that some lipases display *R* specificity and some *S* specificity toward certain substrates. The availability and low cost of lipases render them a very attractive class of catalysts for effecting kinetic resolutions in industrial applications.

This report describes the use of a bacterial lipase ex. *Pseudomonas fluorescens* (P-30 Amano) to effect kinetic resolution of a variety of 2-substituted esters. The selection of these substrates was based on their utility in enantiospecific syntheses of the bioactive target molecules **1-5**. This commercially available and inexpensive enzyme has shown specificity for the *S* enantiomer of all 2-substituted esters tested and has a broad spectrum of substrate specificity. Its application to the preparation of optically pure alcohols required for other synthesis projects in these laboratories has been highly successful.[17]

Results

The bacterial lipase ex. *Pseudomonas fluorescens* (P-30 Amano) catalyzed enantioselectively the hydrolysis of several 2-substituted hexanoic acid esters. 2-Bromo-, 2-hydroxy- and 2-fluorohexanoates were resolved kinetically in high enantiomeric excess. The results are summarized in Table I. (*R*)- and (*S*)-ethyl-2-fluorohexanoate (**9**, **15**) are important intermediates in the synthesis of 16-fluoroprostaglandins (e.g. the antihypertensive (16*R*)-**1**[18])



and 16-fluoroprostacyclins (e.g. the vasodilator and platelet aggregation inhibitor (7*S*,16*S*)-**2**[19]). The enantioselective

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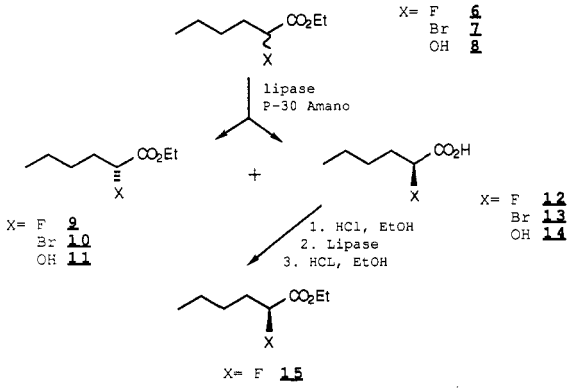
Table I. Hydrolysis of 2-Substituted Hexanoates with Bacterial Lipase from *Pseudomonas*

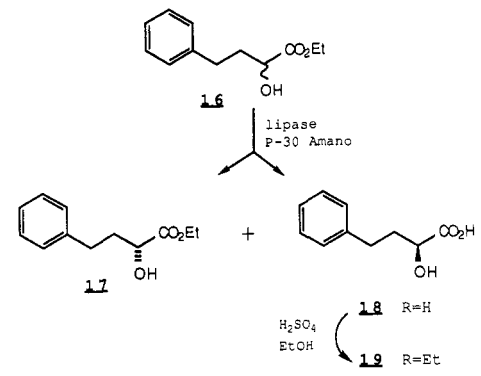
			ester		acid	
X	R	% conversion[a]	% yield[b]	% ee[c]	% yield[b]	% ee[c]
F	Et	50	46 (92)	84[d]	–	–
F	Et	60	37 (93)	99.9	53 (88)	68.5
OH	Et	50	34 (68)	97	40 (80)	73[d]
OH	Et	50	43 (86)	95	48 (96)	79
Br	Me	60	30 (75)	93.5[d]	40 (67)	93[d]
Br	*n*-Bu	50	49 (98)	72	44.5 (89)	–
Br	Et	50	40 (80)	72.5	47.5 (95)	69.4
CF_3	Et	no reaction				
Et	Et	no reaction				

[a] % conversion was based on the amount of base added. [b] Numbers in parentheses represent % of theoretical yield based on % conversion. [c] % ee determined by GC of the diastereomeric Mosher's ester derivatives. [d] The enantiomeric purity of these compounds was based on optical rotation.[7,26]

Scheme I



Scheme II



hydrolysis of these substrates with lipase P-30 Amano was accomplished in a buffered aqueous medium at pH 7–8 without any cosolvents. The fast rate and high enantioselectivity provided an efficient route to enantiomerically pure 2-substituted hexanoic acid esters. In contrast to the 2-halo- and 2-hydroxyhexanoates, 2-(trifluoromethyl)- and 2-ethylhexanoate were not hydrolyzed by lipase P-30 Amano. When the hydrolysis of the above substrates was allowed to proceed to 50% conversion, the unreacted 2*R*-substituted esters were isolated by a simple extraction in 72–91% ee. (2*R*)-Ethyl 2-fluorohexanoate (**9**) was obtained in enantiomerically pure form by simply allowing the hydrolysis to proceed to 60% conversion (Scheme I). The preparation of enantiomerically pure (2*S*)-ethyl 2-fluorohexanoate (**15**) required two successive hydrolyses with the same enzyme.[20] This method was still efficient because the additional steps were operationally simple and high yielding. A more efficient way would involve the use of an enzyme specific for the *R* enantiomer of this substrate. The undesired enantiomers were easily racemized by stirring an ethereal solution of these compounds in the presence of catalytic amount of sodium hydride at ambient temperature overnight.

The enantiomeric excess (% ee) of the 2-halo hexanoates was determined by reduction to the corresponding alcohols with DIBAL or LAH and esterification with 100% excess of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Mosher's reagent)[21] in (1:1) pyridine–carbon tetrachloride for 18 h. The diastereomeric ratio of these derivatives was determined by isothermal gas chromatography on a capillary OV-17 column at 160 °C. Similarly, the diastereomeric ratio of the derivatives of the enantiomers of 2-hydroxyhexanoate, prepared by reacting it directly with Mosher's reagent, was determined by isothermal gas chromatography on a capillary OV-17 column at 200 °C.

(2*R*)-Ethyl 2-hydroxy-4-phenylbutanoate (**17**) is a useful intermediate in the synthesis of several angiotensin converting enzyme (ACE) inhibitors such as cilazapril[22] (**3**). The bacterial lipase ex. *Pseudomonas fluorescens* (P-30 Amano) also catalyzed the enantioselective hydrolysis of the racemic ester **16** to produce the enantiomerically pure 2*R* ester (**17**) and optically enriched[23] 2*S* acid (**18**) (Scheme II). The rate of hydrolysis of this substrate was slower than that observed with the 2-substituted hexanoates, but it was sufficiently fast to be of synthetic utility. The enantiomeric ratio of this compound was determined via the Mosher's reagent derivative as described above.

It has also been found that the broad spectrum of substrates for lipase ex. *Pseudomonas fluorescens* (P-30 Amano) includes chromans such as the 3,4-dihydro-7-hydroxybenzo[*b*]pyran-2-carboxylic acid ethyl ester (**22**).[24]

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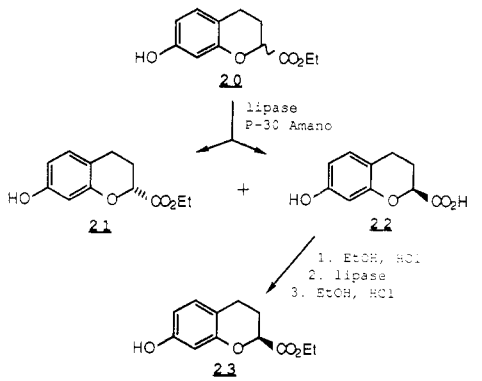
Scheme III



The *R* and *S* enantiomers of this compound (**21** and **23**) are important intermediates in the synthesis of the enantiomerically pure compounds **4** and **5**, which comprise the racemic leukotriene antagonist Ro 23-3544 currently being clinically evaluated. In contrast to the substrates discussed above, the crystalline chroman **20** was not hydrolyzed by the enzyme without cosolvent. Suitable cosolvents are tetrahydrofuran, acetone, dimethyl sulfoxide, acetonitrile, dimethylformamide, and ethanol. In a typical experiment, the hydrolysis of the chroman ester **20** was allowed to proceed to 55% conversion in 10% v/v of tetrahydrofuran/aqueous buffer to afford the *R* ester **21** in 99% ee. The carboxylic acid **22** from this hydrolysis was 75% enantiomerically pure (Scheme III). It was observed during these hydrolyses that partial evaporation of the solvent promoted crystallization of unreacted ester and resulted in a decrease in the enantiomeric purity of the product. The preparation of the enantiomerically pure *S* ester **23** required, as in the case of (2*S*)-ethyl 2-fluorohexanoate (**15**), two successive lipase-catalyzed hydrolyses. This was accomplished by allowing the first hydrolysis to proceed to 40% conversion at which point the optically active *S* carboxylic acid **22** (~80–85% ee) was isolated, reesterified, and resubjected to enzymatic hydrolysis for enantiomeric purity enhancement. Depending on the enantiomeric purity of **22**, the second hydrolysis was allowed to proceed to 70–80% conversion to give the enantiomerically pure *S* acid **22**. This was finally reesterified to the enantiomerically pure *S* ester **23**. Each of the two enantiomerically pure esters **21** and **23** have been used in chiral syntheses of the biologically active compounds **4** and **5** following a previously described synthetic pathway for the corresponding racemic material.[24] No racemization was observed during the chiral synthesis. The enantiomeric excesses of the chroman esters **21** and **23** and those of the methyl esters of the compounds **4** and **5**, prepared via the chiral synthesis, were determined by HPLC analysis on a 25 cm × 4.6 mm covalently bonded (*R*)-phenylglycine column (Pirkle column)[25] eluted with 10% ethanol–heptane at 1 mL/min and monitored by UV absorption at 254 nm.

Discussion

The bacterial lipase ex. *Pseudomonas fluorescens* (P-30 Amano) has displayed excellent stereoselectivity in the hydrolysis of 2-substituted racemic esters. The substrates examined include alkanoates, arylalkanoates, and chromans. It is apparent that the enzyme tolerates a certain degree of steric bulk at its hydrophobic binding site where the alkyl and aralkyl groups fit. The substituents at the C-2 position accepted by the enzyme have been fluorine, chlorine,[4] bromine, hydroxy, cyclic ether, methyl,[9] and cyclic imide.[16] The excellent enantioselectivity observed with 2-fluorohexanoate suggests that the contributing factors at the binding site of the 2-substituent are other than steric since the van der Waal's radius of fluorine is comparable to that of hydrogen. Hydrogen bonding or dipole–dipole interactions are possible stabilizing forces. The steric requirements of the binding site of the 2-substituent are not very clear. The methyl group has been tolerated previously in the case of 3-(acetylthio)-2-methylpropanoate[9] with whole cells from *Pseudomonas fluorescens*, but the observed stereoselectivity was the reverse from that observed in this work. However, different isolates from *Pseudomonas* species previously have shown opposite stereoselectivity.[16] It is not clear, therefore, whether it was the same enzyme that catalyzed the hydrolysis of 3-(acetylthio)-2-methylpropanoate. The trifluoromethyl and ethyl groups at the C-2 position were not tolerated by lipase P-30 Amano.

The rate of hydrolysis of the esters studied in this work was very fast with the 2-fluorohexanoate exhibiting the fastest rate. A decrease in the rate of hydrolysis was observed as the van der Waal's radius of the 2-substituent increased. The pH at which these hydrolyses were conducted ranged between 7 and 8 and was not optimized. For liquid substrates no cosolvent was necessary to effect hydrolysis with lipase P-30 Amano. However, a cosolvent was required for the crystalline chroman **20**. The concentration of the substrate and the ratio of substrate to enzyme were very high. For example, the substrate concentration of ethyl 2-fluorohexanoate was approximately 500 g/L, and the substrate to enzyme ratio was 4000 g/g of enzyme. Under these unoptimized conditions the hydrolysis proceeded to 50% in approximately 4 h. The above results together with the availability and low cost of lipase ex. *Pseudomonas fluorescens* (P-30 Amano) indicate that enzyme-mediated kinetic resolutions can be practical, inexpensive, and adaptable to industrial-scale applications.

Experimental Section

Enantioselective Hydrolysis of Racemic Ethyl 2-Fluorohexanoate (6) with Lipase Enzyme: Preparation of Ethyl (2*R*)-2-Fluorohexanoate (9) and (2*S*)-2-Fluorohexanoic Acid (12). A 3-L, four-necked flask equipped with a mechanical stirrer, a glass baffle, an electrode connected to a pH control unit, and an addition tube connected to a peristaltic pump, was charged with 250 mL of 0.05 M aqueous phosphate buffer (pH 7.0), 250 mL of deionized water, and 162.0 g of racemic ethyl 2-fluorohexanoate (**6**). The resulting mixture was stirred for several minutes, and the pH was adjusted to 7.0 by adding a few drops of 0.1 N sodium hydroxide solution. Then 1.0 g of *Pseudomonas* lipase enzyme (P-30, Amano International Enzyme Co., Inc., Troy, VA)[27] was added, and the hydrolysis was allowed to proceed at room temperature, with stirring. The pH was kept constant at 7.0 by adding 1.0 N sodium hydroxide via the peristaltic pump, which was activated by the pH control unit. The hydrolysis was discontinued when 600 mL of 1.0 N sodium hydroxide solution had been added (60% conversion). The mixture was extracted with 4 × 500 mL of diethyl ether. The combined organic layers were dried (K_2CO_3), filtered, and concentrated at 40 °C/70 mm. Vacuum distillation at 85 °C/25 mm gave 60.7 g (37% yield, 93% of theory) of pure 2*R* ester **9**, which was 99% enantiomerically pure: $[\alpha]^{25}_D$ +13.18° (*c* 1.3, $CHCl_3$). The aqueous layer was

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(27) The activity of this lipase was 30 000 units/g as reported by the manufacturer and was determined using olive oil as the substrate. This enzyme is currently sold in more pure form [lipase P-80 Amano (80 000 units/g) and lipase P-120 Amano (120 000 units/g)].

acidified to pH 2 with 3 N hydrochloric acid and extracted with 3 × 500 mL of diethyl ether. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated at 40 °C/70 mm. The residue was then distilled at 79 °C/2 mm of vacuum to give 70.7 g (52% yield; 88% of theory) of 2*S* acid (**12**) (68.5% ee; $[\alpha]^{25}_D$ −8.68° (*c* 1.3, $CHCl_3$)).

Preparation of Optically Pure Ethyl (2*S*)-2-Fluorohexanoate (15). A 1302 g lot (52% yield; 86% of theory) of 2*S* acid **12** was prepared from 3052 g of racemic **6** by allowing the lipase-catalyzed hydrolysis to proceed to 40% conversion. This material was esterified directly by heating at reflux a solution of ethanol (6 L) containing 29 mL of concentrated H_2SO_4 for 2 h. The ethanol was then removed by atmospheric distillation, and the residue was dissolved in 3 L of dichloromethane. The resulting solution was partitioned with 2 L of saturated $NaHCO_3$, washed with 1 L of brine, and dried (K_2CO_3). The solution was filtered through a silica gel plug and concentrated at 40 °C/100 mm to provide 1176 g (73% yield) of 2*S* ester **15**: $[\alpha]^{25}_D$ −12.44° (*c* 1.0, $CHCl_3$); optical purity 90%; chemical purity 97%.

This ester was resubjected to enzymatic hydrolysis for optical purity enhancement. It was placed into a 22-L, three-necked flask equipped as described above which contained 2.74 L of deionized water and 303 mL of 0.05 M phosphate buffer pH 7.0. The pH was adjusted to 7.0 with a few drops of 1 N aqueous sodium hydroxide solution, and 0.31 g of *Pseudomonas* lipase enzyme (P-30, Amano International) was added to the mixture. The hydrolysis was allowed to proceed at room temperature. The pH was kept at 7.0 by adding 1 N aqueous sodium hydroxide solution via the peristaltic pump. The hydrolysis was discontinued when 5444 mL (75% uptake) of 1 N aqueous sodium hydroxide solution had been added (total reaction time: 4.5 h). The mixture was quickly extracted with 2 × 3.5 L of ethyl ether. The combined organic layers were dried (K_2CO_3) and concentrated at 35 °C/100 mm to provide 164 g of nearly racemic ester. The aqueous phase was acidified to pH 2.0 with approximately 400 mL of concentrated hydrochloric acid and extracted with 2 × 3.5 L of ethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated at 35 °C/100 mm to give 698 g (72% yield; 95% of theory) of 2*S* acid. This acid was distilled at 76–77 °C/3 mm to give 583 g of enantiomerically pure 2*S* acid as a colorless oil; $[\alpha]^{25}_D$ −13.63° (*c* 1.7, $CHCl_3$). The acid was esterified as described previously in this experiment to give 703 g (99% yield) of enantiomerically pure ethyl (2*S*)-2-fluorohexanoate (**15**) as a colorless oil: $[\alpha]^{25}_D$ −13.49° (*c* 1.0, $CHCl_3$), chemical purity 97%.

Enantioselective Hydrolysis of Racemic Ethyl 2-Hydroxyhexanoate (8) with Lipase P-30 Amano: Preparation of Ethyl (2*R*)-2-Hydroxyhexanoate (11) and (2*S*)-2-Hydroxyhexanoic Acid (14). The racemic ethyl 2-hydroxyhexanoate (**8**) (80.0 g) was hydrolyzed with 1.0 g of *Pseudomonas* lipase by the procedure described above to afford after 50% conversion 42.3 g of 2*R* ester **11**. Vacuum distillation at 96 °C/23 mm, gave 34.6 g (43% yield; 86% of theory) of pure 2*R* ester (**11**) ($[\alpha]^{25}_D$ +5.45° (neat); 95% ee). From the aqueous layer was isolated 31.7 g (24% yield; 48% of theory) of optically active 2*S* acid (**14**) (79% ee; $[\alpha]^{25}_D$ +4.78° (*c* 6.3, $CHCl_3$)).

Enantioselective Hydrolysis of Ethyl 2-Bromohexanoate (7) with Bacterial Lipase P-30 Amano: Preparation of Ethyl (2*R*)-2-Bromohexanoate (10) and (2*S*)-2-Bromohexanoic Acid (13). Similar hydrolysis of 3.00 g (0.0134 mol) of racemic ethyl 2-bromohexanoate (**7**) with 0.027 g of lipase P-30 Amano afforded after 50% conversion 1.19 g (40% yield; 80% of theory) of 2*R* ester **10** ($[\alpha]^{25}_D$ +24.4° (*c* 1.0, $CHCl_3$); 72.5% ee) and 1.24 g (47% yield, 94% of theory) of 2*S* acid **13** ($[\alpha]^{25}_D$ −23.2° (*c* 1.1, $CHCl_3$); 69.4% ee).

Enantioselective Hydrolysis of Racemic Ethyl 2-Hydroxy-4-phenylbutanoate (16) with Lipase P-30 Amano: Preparation of Ethyl (2*R*)-2-Hydroxy-4-phenylbutanoate (17) and (2*S*)-2-Hydroxy-4-phenylbutanoic Acid (18). A 3-L three-necked, round-bottomed flask equipped as described above was charged with 450 mL of deionized water, 50 mL of 0.05 M aqueous phosphate buffer (pH 7.0), 52.0 g of racemic ethyl 2-hydroxy-4-phenylbutanoate (**16**), and 0.67 g of lipase P-30 Amano. After 50% conversion, the reaction mixture was extracted with 3 × 200 mL of diethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated at 45 °C/70 mm to provide 25.3 g of 2*R* ester **17** (48.5% yield; 97% of theory; 91% ee; $[\alpha]^{25}_D$ −7.8° (*c* 1.0, EtOH)). The aqueous layer was acidified to pH 2.0 with 3 N HCl and extracted with 3 × 200 mL of diethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated at 45 °C/70 mm to give 20.8 g of 2*S* acid **18** (46% yield; 92% of theory; 90.8% ee; $[\alpha]^{25}_D$ +7.6° (*c* 1.0, EtOH); mp 109–110 °C).

A similar hydrolysis allowed to proceed to 55% conversion afforded 22.4 g of 2*R* ester **17** (43% yield; 96% of theory; 99% ee; $[\alpha]^{25}_D$ −8.4° (*c* 1.15, EtOH)) and 22.7 g of 2*S* acid **18** (50% yield; 91% of theory; 73% ee; $[\alpha]^{25}_D$ +5.7° (*c* 1.0, EtOH); mp 109–110 °C).

After 35% conversion, the following results were obtained:

	ethyl (2*R*)-2-hydroxy-4-phenylbutanoate (**17**)	(2*S*)-2-hydroxy-4-phenylbutanoic acid (**18**)
weight, g	32.5	14.6
% yield	63	33
% theory	96	93
$[\alpha]^{25}_D$	−4.6° (*c* 1.5, EtOH)	+7.8° (*c* 1.1, EtOH)
(% e.e.)	52	92

Kinetic Resolution of Racemic 3,4-Dihydro-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (20) with Lipase P-30 Amano. A 250-mL three-necked, round-bottomed flask equipped as described above was charged with 60 mL of deionized water, 15 mL of 0.05 M phosphate buffer (pH 7.0), and 2.2 g of racemic ester **20** dissolved in 7.5 mL of tetrahydrofuran. The pH was adjusted to 8.0 with 0.1 N aqueous hydroxide solution, and 0.4 g of lipase P-30 Amano was added while the mixture was stirred at a fast rate. The reaction flask was stoppered to avoid loss of the cosolvent by evaporation. The pH was maintained at 8.0 by automatic addition of 0.1 N aqueous sodium hydroxide solution. The reaction was discontinued when 55 mL of 0.1 N aqueous sodium hydroxide had been consumed and the tetrahydrofuran was removed by evaporation at 35 °C/10 mm. The mixture was extracted with 3 × 50 mL of ethyl acetate. The combined organic layers were washed with 50 mL of saturated $NaHCO_3$ dried (Na_2SO_4) and concentrated at 40 °C/10 mm to obtain 1.0 g (45% yield, 100% of theory) of *R* ester **21** as an off-white solid (mp 77–78 °C; $[\alpha]^{25}_D$ −20.2° (*c* 1.0, $CHCl_3$); 99.6% ee).

The aqueous layer was acidified to pH 1.0 with concentrated HCl and extracted twice with 50 mL of ethyl acetate. The combined organic layers were dried (Na_2SO_4) and concentrated at 40 °C/10 mm to give 0.96 g (50% yield, 90% of theory) of *S*-acid **22** as an off-white solid (mp 156.5–158.5 °C; $[\alpha]^{25}_D$ −9.2° (*c* 1.0, MeOH); 75% ee).

The *S* ester **23** was obtained in 95.5% ee after two successive hydrolysis cycles with lipase P-30 Amano in a way analogous to that for the hexanoic acid esters described above.

Registry No. **6**, 17841-31-5; **7**, 63927-44-6; **8**, 124439-28-7; **9**, 124439-29-8; **10**, 124439-30-1; **11**, 113747-69-6; **12**, 113776-26-4; **13**, 91423-84-6; **14**, 70267-26-4; **15**, 124439-31-2; (±)-**16**, 93921-85-8; **17**, 90315-82-5; **18**, 115016-95-0; (±)-**20**, 96566-14-2; **21**, 124439-98-1; **22**, 124356-21-4; **23**, 124439-32-3; P-30, 9001-62-1; (±)n-BuCH(Br)CO_2Me, 70288-61-8; (±)n-BuCH(Br)CO_2Bu, 124439-33-4; (2R)*n*-BuCh(Br)CO_2Me, 114438-79-8; (2R)*n*-BuCh(Br)CO_2Bu, 99113-77-6.